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Original article

Synthesis and diuretic activity of bicyclic fused heterocycles containing oxime-*O*-sulfonic acid moiety

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Abstract – In order to investigate the origin of the loop-type diuretic activity of M17055 (**1**), several variants (**3**–**9**) were designed and synthesized by modifying the quinolinone skeleton, and their diuretic activities were compared with the lead **1** and furosemide in dogs. It was found that the negative charge distribution pattern afforded by the dispositional arrangement of the 4-oxime-*O*-sulfonic acid and 1-*N*-acyl carbonyl moiety attached to the tetrahydropyridine ring system is inevitable for the development of the activity, which strongly supports the previously proposed model for the active site of the Na^+-K^+-$2Cl^-$ cotransporter. Also reported is the first synthesis of the dihydrothieno[3,2-b]pyridine-7(4*H*)-one ring system required in the synthesis of compound **9**. © 2000 Éditions scientifiques et médicales Elsevier SAS

M17055 / oxime-O-sulfonic acid / diuretic activity / structure–activity relationship / computational chemistry

1. Introduction

7-Chloro-2,3-dihydro-1-(2-methylbenzoyl)-4-(1*H*)-quinolinone 4-oxime-*O*-sulfonic acid potassium salt **1** (M17055) is a novel diuretic of high potency, which is now under the last stage of phase III trials by Mochida Pharmaceutical Co. Ltd., Japan [1] (*figure 1*). Although the chemical structure of the oxime sulfonate **1** is quite different from those of the conventional diuretics, its dominant site of action has been shown to be the loop of the Henle like loop-type diuretics represented by furosemide, as evidenced by a wide range of pharmacological studies [2].

In the course of our efforts to clarify the origin of the diuretic activity induced by compound **1**, we recently carried out several computational analyses on these diuretic materials to show that compound **1** and furosemide are closely related to each other from the view point of electron charge distributions: both compounds show quite similar electrostatic potential maps, in which large and strong negative charges are located on the sulfonyl moiety (-SO_3-) of compound **1** and the carboxyl moiety (-CO_2-) of furosemide, while relatively small and weak ones reside on the acyl carbonyl (>N–CO-) group of the former and the sulfamoyl (-SO_2NH_2) group of the latter. Thus, it was suggested that the charge distribution, rather than the entity of the functional groups themselves, is essential for the development of the loop-diuresis. The structure–activity relationships derived from pharmacological, as well as computational, studies led us to propose a model for the active site of the Na^+-K^+-$2Cl^-$ cotransporter in which the -SO_3 function and the acyl carbonyl function are of particular importance in binding to the Cl^- binding site of the cotransporter and serving as a receptor for a hydrogen bonding, respectively, with the aid of the two lipophilic phenyl moieties of the quinolinone and the acyl residue assisting the interaction by fitting to the hydrophobic sites of the cotransporter.

In the next step, we designed tricyclic compound **2**, which, due to the restriction of the rotational freedom around the original >N–CO- bond by converting to a cyclic >N-C=N- linkage, while retaining the molecular features of the lead **1**, should fit more comfortably to the

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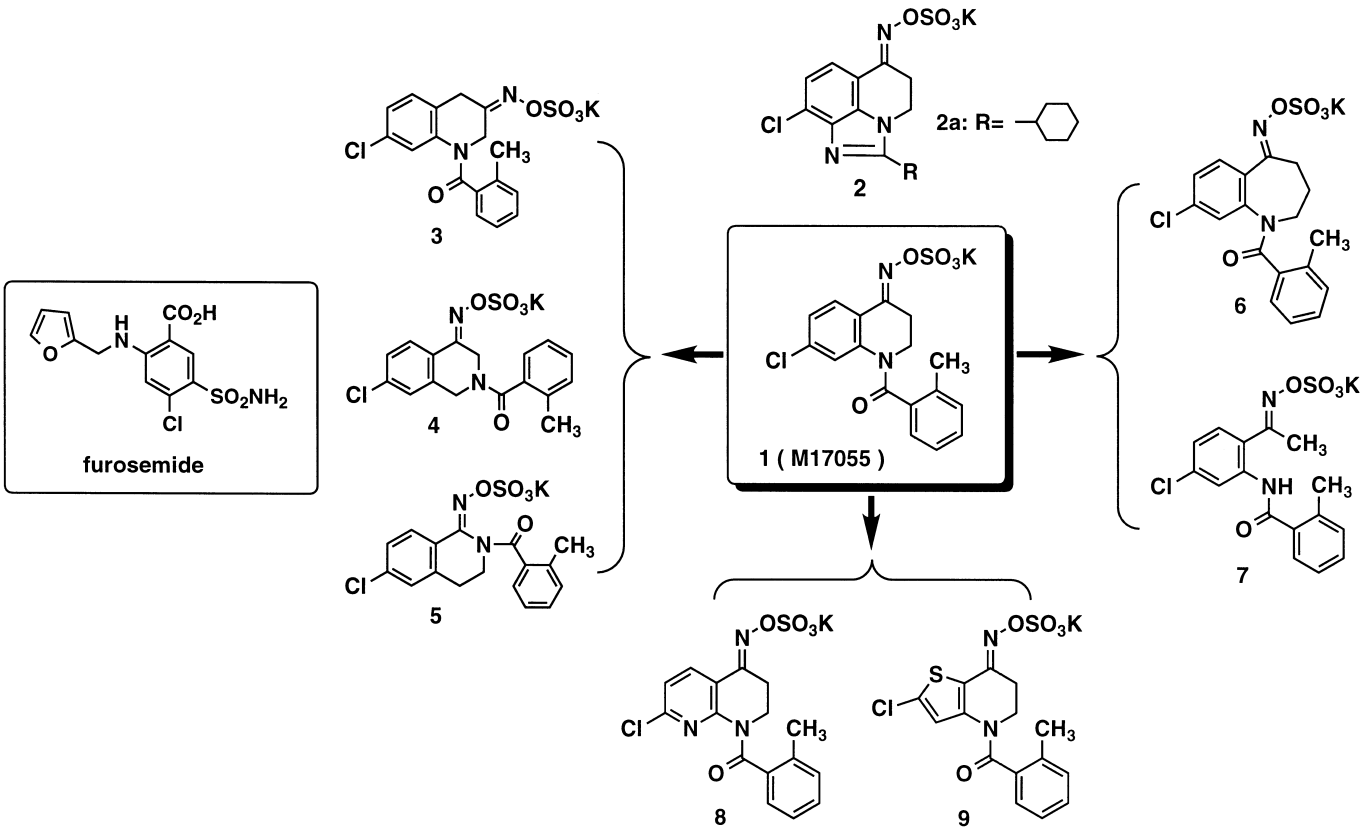

Figure 1. Synthetic plans for modification of the quinoline skeleton.

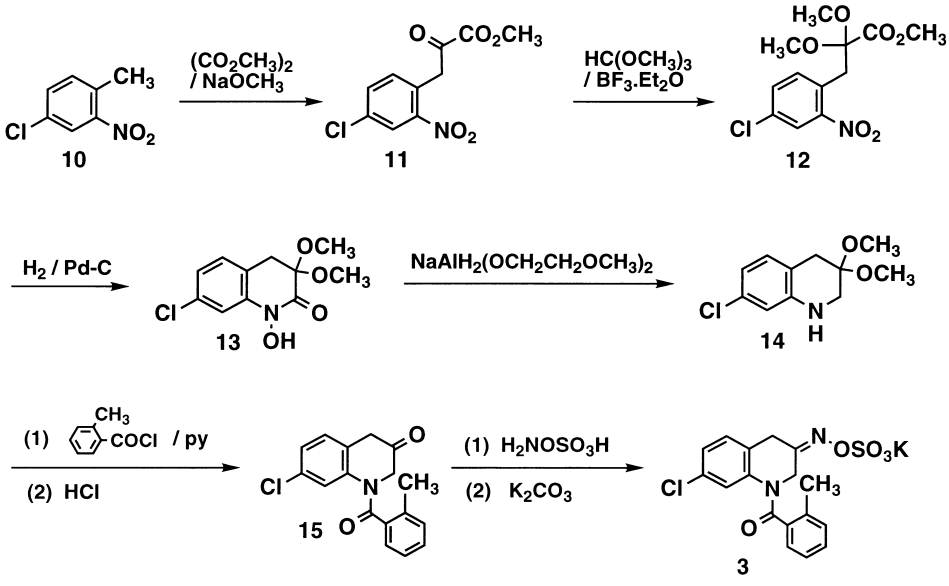

Figure 2. Synthetic pathway to **3**.

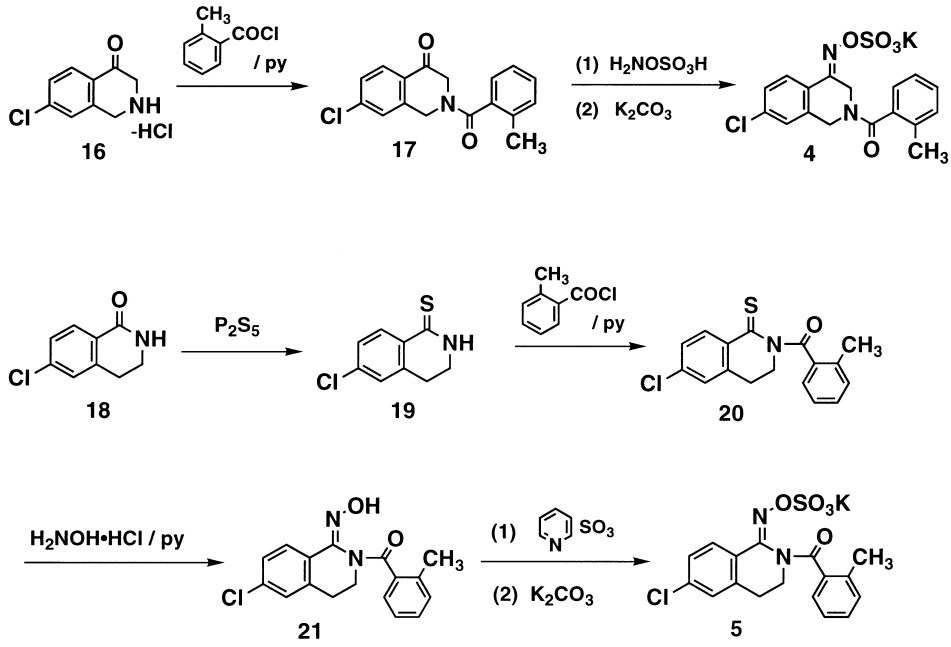

Figure 3. Synthetic pathway to **4** and **5**.

supposed active site, thereby inducing a larger diuretic potency than the parent [3]. To our satisfaction, many of the newly synthesized tricycles **2** actually showed a comparable or superior activity to that of the lead **1**, among which compound **2a** was the most powerful and has been adopted as the promising candidate to succeed **1** (M17055). Pharmacological and clinical studies on it are now underway.

With the above results in mind, we were tempted to further substantiate the active site model by demonstrating that the slight modification of the distribution of the electron charge density of compound **1** would reduce or eliminate the diuretic activity. Thus, we synthesized compounds **3**–**9**, where compounds **3**–**5** are different from each other in respect to the relative positions of the sulfonic acid and the acyl carbonyl function, compounds **6** and **7** are deformed in the three-dimensional structures from that of compound **1** by ring-enlargement (**6**) or by cleavage of the cyclic linkage (**7**), and compound **8** and **9** have either a pyridine (**8**) or thiophene ring (**9**) in place of the benzene ring of the quinolinone skeleton of compound **1**. Then, the diuretic activities of these newly synthesized compounds were tested in dogs and the structure–activity relationships discussed with the aid of computer graphics.

2. Chemistry

Figure 2 illustrates the synthetic pathway to compound **3**. By referring to a paper describing the synthesis of 1,4-dihydro-3(2*H*)-quinolinone derivatives [4], 7-chloro-1,4-dihydro-3(2*H*)-quinolinone dimethyl acetal **14** was synthesized from a commercially available 4-chloro-2-nitrotoluene **10** via the Claisen-type condensation with dimethyl oxalate to give compound **11** followed by acetallization to compound **12**, reductive cyclization to quinolinone **13** and finally, reduction of the carbonyl function with Selectlide. Compound **14** was *N*-acylated by 2-methylbenzoyl chloride/pyridine followed by acid-promoted deacetallization to compound **15**, which was then allowed to react successively with hydroxylamine-*O*-sulfonic acid and then with potassium carbonate to give the desired sulfonic acid salt **3**. Hereafter, the formation of pertinent sulfonate salts by treatment with (1) 2-methylbenzoyl chloride/pyridine, (2) H_2NOSO_3H, and (3) K_2CO_3 will be denoted as method A.

Target compounds **4** and **5** were obtained from the key intermediates **16** [5, 6] and **18** [7], respectively, which had been prepared according to procedures mentioned in the literature (*figure 3*). Compound **16** was readily acylated to compound **17** and then converted to the

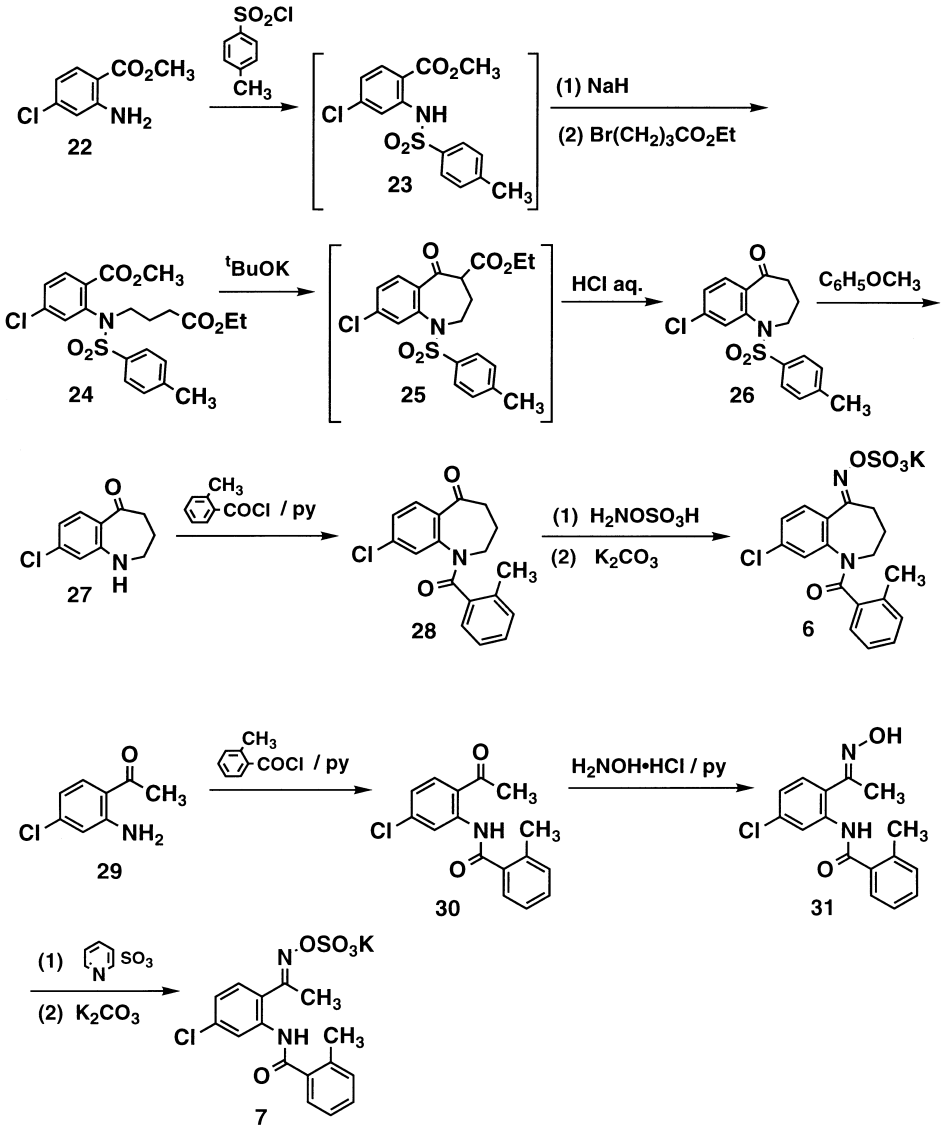

Figure 4. Synthetic pathway to **6** and **7**.

O-sulfonic acid salt **4** by method A. On the other hand, compound **18** failed to react with 2-methylbenzoyl chloride/pyridine due to the reduced reactivity of the amide. So, compound **18** was first converted to the thiocarbonyl derivative **19**, which was found to undergo a reaction with the acyl chloride to give compound **20**. Compound **20** was again not active enough to react with hydroxylamine-*O*-sulfonic acid, probably due to electronic as well as, at least in part, to steric reasons. Luckily, however, it was found that compound **20** reacted regioselectively with hydroxylamine hydrochloride on the thiocarbonyl rather than the acyl carbonyl function of the imide moiety to give compound **21**. This was then converted to sulfonic acid by treatment with sulfur trioxide/pyridine followed by cation exchange to give the desired product **5**. Hereafter, the formation of the pertinent sulfonate salts by treatment with (1) $H_2NOH{\cdot}HCl$/pyridine, (2) sulfur trioxide/pyridine and (3) K_2CO_3 will be denoted as method B.

The routes for preparing compounds **6** and **7** are shown in *figure 4*. Referring to a paper describing the synthesis of 1,2,3,4-tetrahydro-1-benzazepin-5(5*H*)-one [8], the

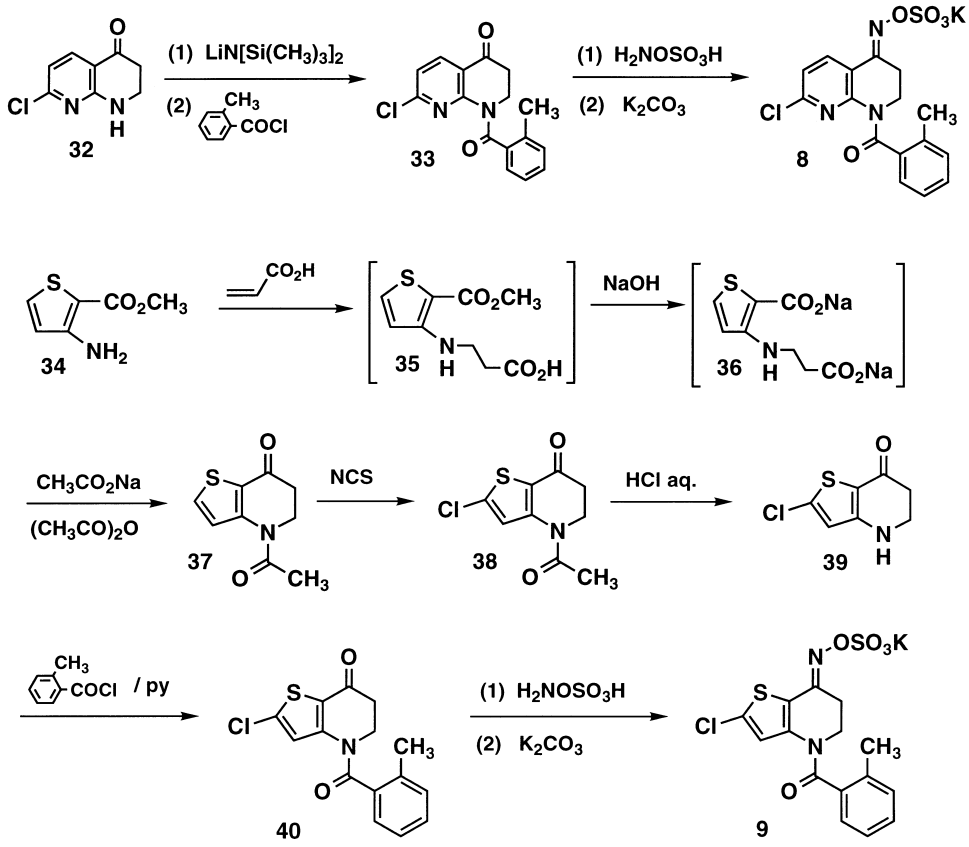

Figure 5. Synthetic pathway to **8** and **9**.

8-chloro counterpart **27** was formed from commercially available compound **22**. Compound **27** was then converted to the target compound **6** by method A. A known compound **29** [9] was acylated to compound **30**, which was then converted to the target compound **7** via an oxime **31** according to method B.

Figure 5 shows the synthesis of pyridine and thiophene derivatives **8** and **9**. 7-Chloro-2,3-dihydro-1,8-naphthyridine-4(1*H*)-one **32** was synthesized referring to a procedure found in the literature [10], and then it was converted to the desired compound **8** via *N*-acyl derivative **33** by using essentially method A, except that lithium bis(trimethylsilyl)amide was used instead of pyridine as the base in the acylation step.

The heteroaromatic ring system of compound **9**, dihydrothieno[3,2-b]pyridine-7(4*H*)-one nucleus, had seemed, to the best of our knowledge, to be unprecedented, although the [3,2-c] counterpart had been known. Several trials for the synthesis of compound **9** starting from 3-aminothiophene were unfruitful because of its incompatibility with the Michael reaction conditions. To our satisfaction, however, we eventually succeeded in the first synthesis of compound **37**, which should be the key intermediate for the syntheses of the [3,2-b]-ring class compounds, by finding that introduction of a 2-methoxycarbonyl substituent to 3-aminothiophene to form compound **34** substantially stabilized it under an alkaline medium, and thus compound **34** was readily subjected to the reaction with acrylic acid to give the Michael adduct **35**. After compound **35** had been treated with sodium hydroxide to give the disodium salt **36**, it was cyclized by heating at reflux in acetic anhydride in the presence of sodium acetate to give the Dieckmann-type ring-closure [11] product **37**. Chlorination [12] of compound **37** by treatment with *N*-chlorosuccinimide (NCS) to give the chloride **38** was followed by deacetylation to 2-chloro-5,6-dihydrothieno[3,2-b]pyridine-7(4*H*)-one **39**, which was eventually treated using method A to afford the desired compound **9**.

Figure 6 shows the main ^{1}H-NMR spectral data of the final sulfonic acid derivatives **3**–**9** along with those of parent **1**. Full details of the ^{1}H-NMR data for compounds

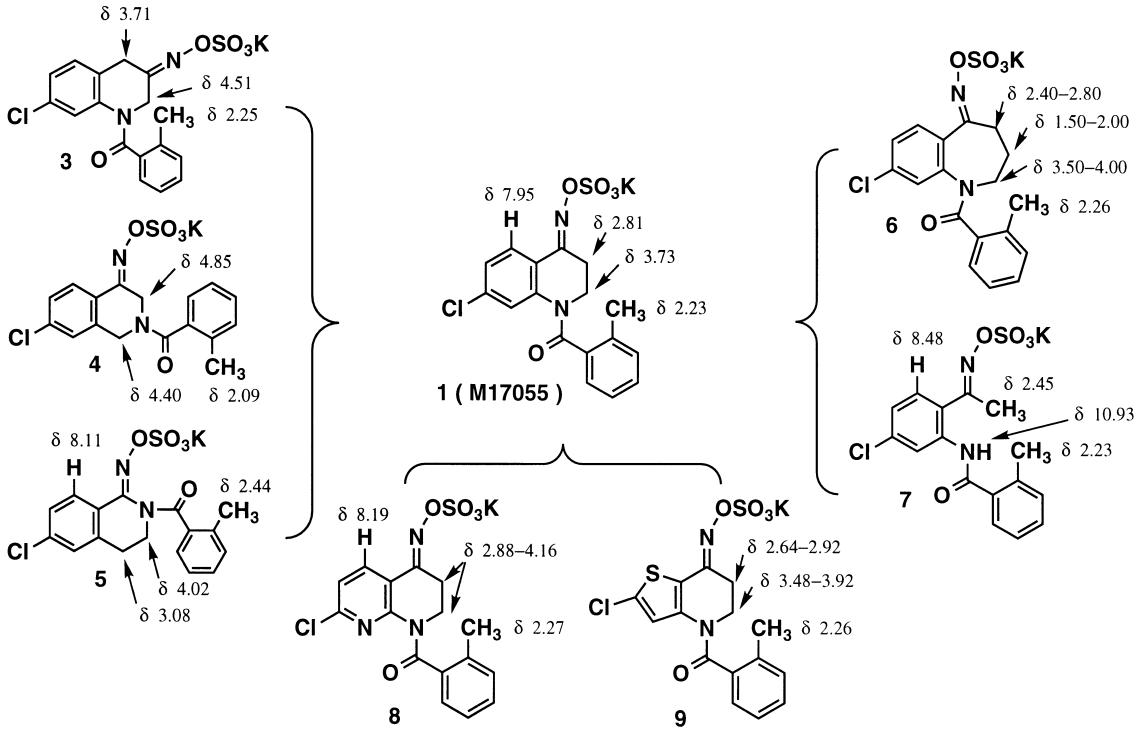

Figure 6. Main ^{1}H-NMR spectral data of variant compounds **3**–**9** and **1** (M17055).

3–**9** and synthesized intermediates are summarized in *tables I* and *II*.

3. Diuretic activity of compounds 3–9

The diuretic activities of the newly synthesized compounds **3**–**9** were determined as reported in the previous paper [1] based on the ratio of increase in urine volume after their administration to dogs, via the intrarenal artery (i.r.a.) or intravenously (i.v.), to that after furosemide administration to the same dogs at the same dose in the same manner, the results are summarized in *table III*. The diuretic activities of **1** (M17055) and furosemide are also included in the table for the convenience of comparison.

It can be seen that compounds **3**–**5** completely lost diuretic activity, showing that the 1,4-relationship of the *N*-(2-methylbenzoyl)amino group and the *O*-sulfonic acid group on the tetrahydropyridine ring system is crucial to induce potency. Seven-membered heterocycle **6** and ring-opened compound **7** did not show any activity, suggesting that a slight deformation of the three-dimensional conformation of the tetrahydopyridine ring structure of the lead **1** is detrimental.

On the other hand, pyridine derivative **8** retained substantial activity of the lead **1**, while the thiophene counterpart **9** was largely lost but still retained diuretic activity. These results show that here again, as long as the 1,4-relationship of the *N*-(2-methylbenzoyl)amino group and the *O*-sulfonic acid function is retained on the tetrahydropyridine ring system, the adjacent phenyl nucleus of the parent quinolinone skeleton may be substituted by another heteroaromatic ring.

4. Discussion

The structure–activity relationships of the newly synthesized compounds **3**–**9** were considered with the aid of computer graphics. As stated above, compounds **3**–**5** completely lost the diuretic activity of the lead **1**. *Figure 7* compares the computer-generated electrostatic potential map of **1** (M17055) with those of compounds **3**–**5**. It can be seen that the alternation of the relative disposition of the -SO_3^- and *N*-acyl function induced various changes in the negative charge distribution. Compounds **3** and **4** still retained the large negative charges and the small ones located on the relevant functional groups as in

Table I. Physical data of the intermediates.

Compound	M.p. (°C)	Formula [a] [recryst. solv.]	^{1}H-NMR (ppm) [Solvent]
11	111–113	$C_{10}H_8ClNO_5$ [CH_2Cl_2-Hexane]	3.94 (3H, s), 6.78 (1H, d, *J* = 14.2), 6.80 (1H, d, *J* = 14.2), 7.55 (1H, dd, *J* = 8.6, *J* = 2.3), 7.89 (1H, d, *J* = 2.3), 8.21 (1H, d, *J* = 8.6) [$CDCl_3$]
12	95–98	$C_{12}H_{14}ClNO_6$ [CH_2Cl_2-Hexane]	3.31 (6H, s), 3.52 (2H, s), 3.63 (3H, s), 7.20–7.60 (2H, m), 7.78 (1H, d, *J* = 1.7) [$CDCl_3$]
13	125–127	$C_{11}H_{12}ClNO_4$ [MeOH-Et_2O]	3.16 (2H, s), 3.36 (6H, s), 7.00–7.40 (3H, m), 8.70 (1H, br) [$CDCl_3$]
14	116–117	$C_{11}H_{14}ClNO_2$ [CH_2Cl_2-Hexane]	2.91 (2H, s), 3.30 (8H, s), 3.90 (1H, br), 6.50–6.70 (2H, m), 6.86 (1H, d, *J* = 7.9) [$CDCl_3$]
15	135–137	$C_{17}H_{14}ClNO_2$ [CH_2Cl_2-Hexane]	2.30 (3H, s), 3.66 (2H, s), 4.39 (2H, s), 7.10–7.30 (7H, m) [$CDCl_3$]
17	99–102	$C_{17}H_{14}ClNO_2$ [CH_2Cl_2-Hexane]	2.09 (3H, s), 4.40 (2H, s), 4.85 (2H, s), 6.86–7.68 (6H, m), 7.76–8.00 (1H, m) [DMSO-d_6]
20	120–122	$C_{17}H_{14}ClNOS$ [CH_2Cl_2-Hexane]	2.54 (3H, s), 3.20 (2H, t, *J* = 6.3), 4.13 (2H, t, *J* = 6.3), 6.92–7.40 (6H, m), 8.40 (1H, d, *J* = 9.2) [$CDCl_3$]
21	165–168	$C_{17}H_{15}ClN_2O_2$ [CH_2Cl_2-Hexane]	2.26 (3H, s), 3.04 (2H, t, *J* = 6.4), 4.14 (2H, t, *J* = 6.4), 6.80–7.36 (6H, m), 7.68 (1H, br), 8.31 (1H, d, *J* = 9.2) [$CDCl_3$]
24	134–135	$C_{21}H_{24}ClNO_6S$ [EtOH]	1.23 (3H, t, *J* = 7.2), 1.80–2.10 (2H, m), 2.42 (2H, t, *J* = 7.2), 2.43 (3H, s), 3.40–3.80 (2H, m), 3.82 (3H, s), 4.10 (2H, q, *J* = 7.2), 7.20–7.60 (6H, m), 7.83 (1H, d, *J* = 8.6) [$CDCl_3$]
26	83–84	$C_{17}H_{16}ClNO_3S$ [CH_2Cl_2-Hexane]	1.80–2.20 (2H, m), 2.43 (2H, t, *J* = 5.8), 2.44 (3H, s), 3.83 (2H, t, *J* = 6.4), 7.20–7.40 (3H, m), 7.50–7.80 (4H, m) [$CDCl_3$]
27	99–103	$C_{10}H_{10}ClNO$ [Et_2O-hexane]	1.90–2.40 (2H, m), 2.80 (2H, t, *J* = 6.3), 3.00–3.40 (2H, m), 4.69 (1H, br), 6.50–6.90 (2H, m), 7.63 (1H, d, *J* = 8.6) [$CDCl_3$]
28	138–139	$C_{18}H_{16}ClNO_2$ [EtOH]	1.90–2.30 (2H, m), 2.41 (3H, s), 2.90 (2H, t, *J* = 6.3), 3.80–4.20 (2H, m), 6.70–7.10 (6H, m), 7.84 (1H, d, *J* = 8.0) [$CDCl_3$]
30	121–123	$C_{16}H_{14}ClNO_2$ [CH_2Cl_2-Hexane]	2.55 (3H, s), 2.64 (3H, s), 7.00–8.00 (6H, m), 9.06 (1H, s), 12.19 (1H, s) [$CDCl_3$]
31	189–194	$C_{16}H_{15}ClN_2O_2$ [CH_2Cl_2-Hexane]	2.22 (3H, s), 2.44 (3H, s), 7.12–7.76 (6H, m), 8.54 (1H, d, *J* = 2.2), 11.32 (1H, s), 11.54 (1H, s) [DMSO-d_6]
33	174–177	$C_{16}H_{13}ClN_2O_2$ [CH_2Cl_2-Hexane]	2.38 (3H, s), 2.91 (2H, t, *J* = 6.5), 4.40 (2H, t, *J* = 6.5), 6.72–7.44 (5H, m), 8.12 (1H, d, *J* = 8.4) [$CDCl_3$]
37	97–98	$C_9H_9NO_2S$ [CH_2Cl_2-Hexane]	2.34 (3H, s), 2.71 (2H, t, *J* = 6.6), 4.15 (2H, t, *J* = 6.6), 7.76 (1H, d, *J* = 5.5), 8.00 (1H, d, *J* = 5.5) [DMSO-d_6]
38	65–68	$C_9H_8ClNO_2S$ [CH_2Cl_2-Hexane]	2.33 (3H, s), 2.72 (2H, t, *J* = 6.6), 4.15 (2H, t, *J* = 6.6), 7.80 (1H, s) [DMSO-d_6]
39	128–129	C_7H_6ClNOS [CH_2Cl_2-Hexane]	2.43 (2H, t, *J* = 7.1), 3.65 (2H, t, *J* = 7.1), 7.44 (1H, br), 7.00 (1H, s) [DMSO-d_6]
40	118–121	$C_{15}H_{12}ClNO_2S$ [CH_2Cl_2-Hexane]	2.30 (3H, s), 2.73 (2H, t, *J* = 6.5), 3.96 (2H, t, *J* = 6.5), 7.04–7.52 (5H, m) [DMSO-d_6]

[a]Compounds were analysed by HR-MS.

Table II. Physical date of compounds **3–9**.

Compound	M.p. (°C)[a] (dec)	Formula[b]	^{1}H-NMR (DMSO-d_6, ppm)
3	138–140	$C_{17}H_{14}ClKN_2O_5S$	2.25 (3H, s), 3.71 (2H, s), 4.51 (2H, s), 7.20–7.80 (7H, m)
4	193–195	$C_{17}H_{14}ClKN_2O_5S$	2.09 (3H, s), 4.40 (2H, s), 4.85 (2H, s), 6.86–7.68 (6H, m), 7.76–8.00 (1H, m)
5	172–173	$C_{17}H_{14}ClKN_2O_5S$	2.44 (3H, s), 3.08 (2H, t, *J* = 6.4), 4.02 (2H, t, *J* = 6.4), 6.72–7.52 (6H, m), 8.11 (1H, d, *J* = 8.6)
6	114–115	$C_{18}H_{16}ClKN_2O_5S$	1.50–2.00 (2H, m), 2.26 (3H, s), 2.40–2.80 (2H, m), 3.50–4.00 (2H, m), 6.80–7.50 (7H, m)
7	171–174	$C_{16}H_{14}ClKN_2O_5S$	2.23 (3H, s), 2.45 (3H, s), 7.04–7.72 (6H, m), 8.48 (1H, d, *J* = 1.8), 10.93 (1H, s)
8	130–133	$C_{16}H_{13}ClKN_3O_5S$	2.27 (3H, s), 2.88–4.16 (4H, m), 6.80–7.36 (5H, m), 8.19 (1H, d, *J* = 7.9)
9	172–175	$C_{15}H_{12}ClKN_2O_5S_2$	2.26 (3H, s), 2.64–2.92 (2H, m), 3.48–3.92 (2H, m), 7.20–7.44 (5H, m)

[a]Recrystalisation solvent CH_2Cl_2-MeOH. [b]Compounds **3** and **5** were analysed for C, H and N. Analytical results obtained for these elements were within ± 0.4% of the calculated values for the formulae shown. Compounds **4** and **6–9** were analysed by HR-FAB-MS.

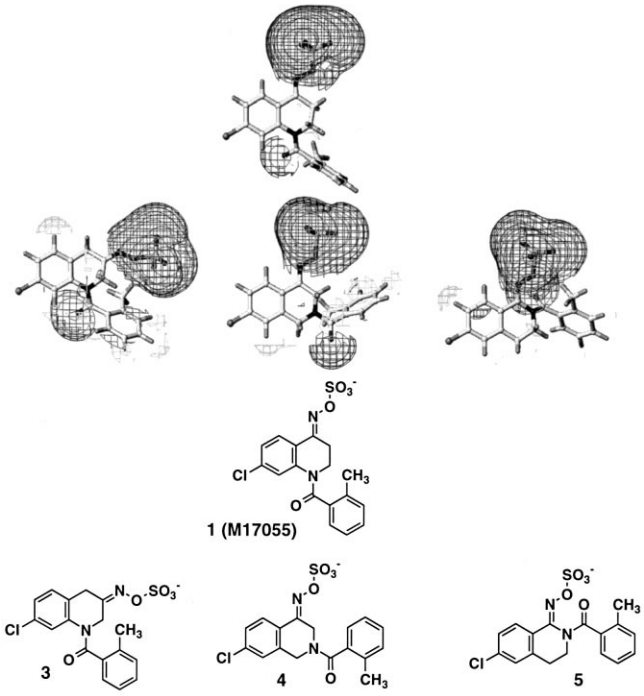

Figure 7. Electrostatic potential maps of **1** (M17055), **3**, **4** and **5**. The thick line is the contour at –30 kcal/mol and the thin line at 10 kcal/mol of the electrostatic potential energy.

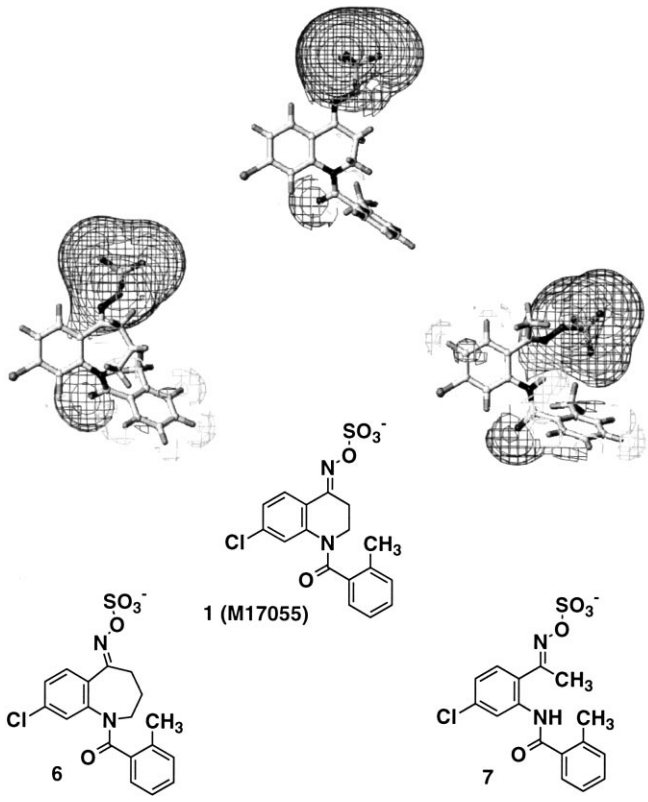

Figure 8. Electrostatic potential maps of **1** (M17055), **6** and **7**. The thick line is the contour at –30 kcal/mol and the thin line at 10 kcal/mol of the electrostatic potential energy.

the case of the lead **1**, but their disposition patterns apparently differ to some extent from that of the lead **1**. Furthermore, in the case of compound **5**, the disposition of the sulfonyl and acyl carbonyl function in close vicinity brought about the collapse of the two negative charges to form only one domain of a distinct negative charge. These results may support the assumption that the negative charge distribution on the tetrahydropyridine backbone is the main determinant for effective interaction with the Na^+-K^+-$2Cl^-$ cotransporter to induce diuretic activity.

Interestingly, it was found that ring-enlarged **6** and ring-opened compound **7** showed rather similar electrostatic potential maps to that of the lead **1**, as shown in *figure 8*, in spite of the fact that the two did not show any activity at all (*table III*). Consideration of the three-dimensional structures of these compounds seems to answer the apparent exceptions to the active site model hypothesis: *figure 9* shows the molecular graphics of **1** (M17055) (in black) and compounds **6** and **7** (both in grey), in which the main parts of the molecule containing the chlorobenzene ring and the oxime sulfonic acid moiety are superimposed on each other. The relevant pictures show clearly that the 2-methylbenzoyl moieties of both compounds **6** and **7** are shifted drastically from their original position in the lead **1** to disturb effective interaction with the active site of the cotransporter.

Figure 10 shows the electrostatic potential maps of compounds **8** and **9** as compared with **1** (M17055). It can be seen that the negative charge around the *N*-acyl carbonyl function of compound **8** is slightly strengthened, apparently due to the nearby nitrogen atom of the pyridine ring, but retains almost the same characteristic pattern of the negative charge distribution of the lead **1**. Thus, it may be reasonable to suppose that compound **8** retains ca. 70% of the activity of **1** (M17055).

In the case of compound **9**, however, it is seen that the charge distribution is somewhat disturbed from that of the lead **1**, which should result in the significant loss of the activity. The perturbation of the charge distribution seems to be brought about by the conformational change of the 2-methylbenzoyl moiety of compound **9** from that of the lead **1**, as also indicated by AM 1 calculation (*figure 11*).

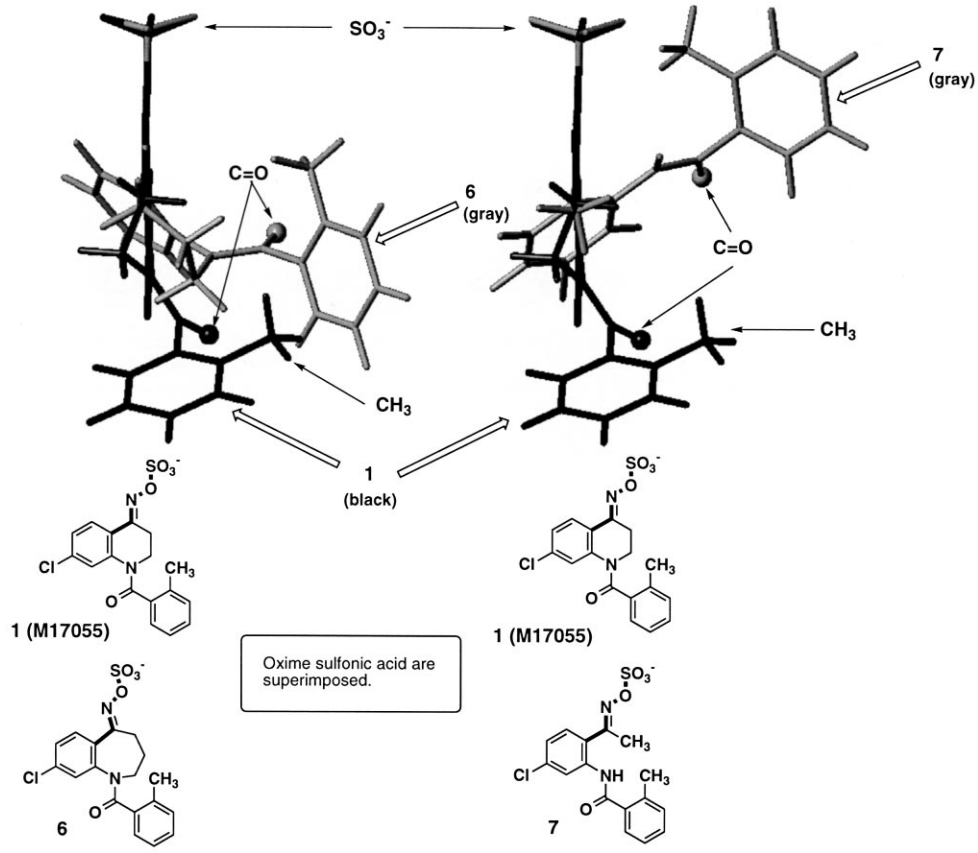

Figure 9. Superimposition of active compound **1** (M17055) and inactive compounds **6** and **7**.

The calculation indicated that the most stable conformation of compound **9** is the one in which the carbonyl function is orientated in the opposite direction to that of the lead **1**. Therefore, it may be said that, in spite of the appreciable change in the negative charge distribution, compound **9** still retains the activity because the 1,4-relationship of the *N*-acyl and the sulfonic acid moiety on the tetrahydropyridine ring system is retained.

5. Conclusion

In order to shed more light on the origin of the diuretic activity induced by **1** (M17055), several derivatives (**3**–**9**) were synthesized by modifying the quinolinone ring skeleton, and their activities in dogs were compared with the lead **1**. The pharmacological studies showed that the molecular structure provided by the 4-oxime-*O*-sulfonic acid and 1-*N*-acyl carbonyl moiety attached to a tetrahydropyridine ring system is the determinant for the development of the activity. With the aid of computer graphics it has been shown that the pattern of electron potential distributions afforded by the above molecular array is

Table III. Diuretic activities of compounds **1** and **3**–**9** and furosemide.

Compound	Diuretic activity	
	i.r.a.[a]	i.v.[b]
1 (M17055)	4.2	4.2
3	no activity	no activity
4	no activity	no activity
5	no activity	no activity
6	no activity	no activity
7	no activity	no activity
8	2.9	2.6
9	0.2	0.4
furosemide	1.0	1.0

[a]Test compounds were injected into the renal artery of dogs and activity was expressed relative to that of furosemide. [b]Test compounds were administered intravenously into dogs and activity was expressed relative to that of furosemide.

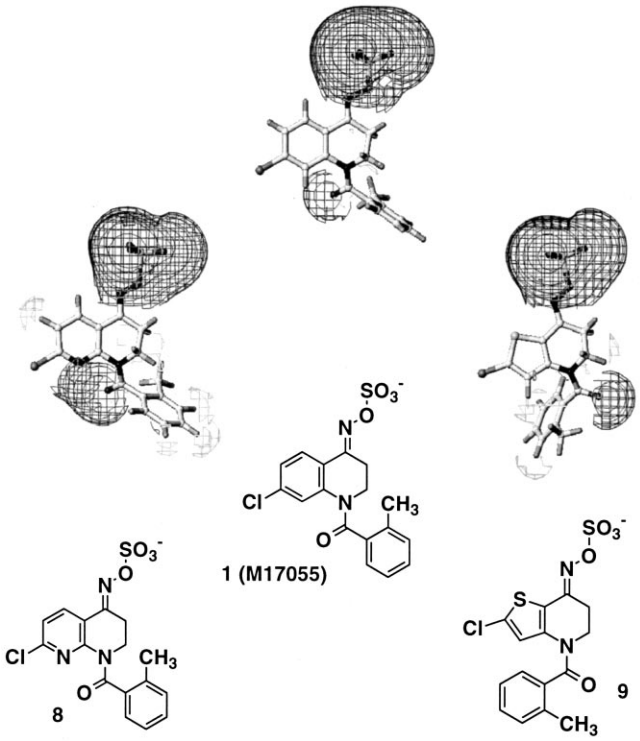

Figure 10. Electrostatic potential maps of **1** (M17055), **8** and **9**. The thick line is the contour at –30 kcal/mol and the thin line at 10 kcal/mol of the electrostatic potential energy.

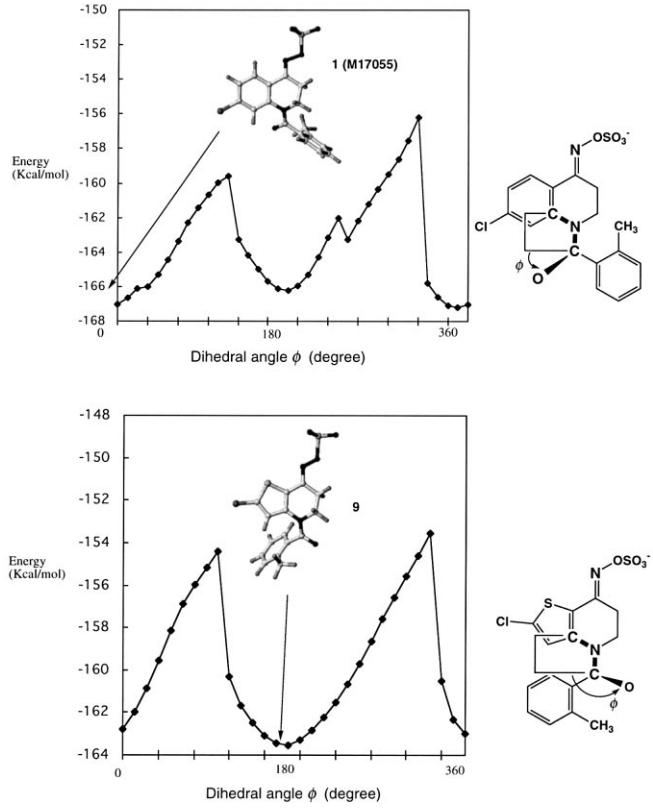

Figure 11. Dihedral angle/energy curve of **1** (M17055) and **9**.

crucial for the development of the activity, which substantiates the previously proposed active site model. Of special importance in a series of this study is the finding in the previous paper [3] that the C=N double bond of tricyclic compounds **2** plays the same role as the C=O function of compound **1**, and type **2** compounds can induce diuretic activity comparable to, or even better than, type **1** bicyclic compounds. These facts can reasonably be explained by assuming that coplanarity of type **2** compounds represents the most suitable conformation of **1** (M17055) to fit to the proposed Na^+-K^+-$2Cl^-$ cotransporter active site, having lead us to adopt compound **2a** as the promising candidate to succeed parent **1** (M17055) for pharmacological and clinical studies.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Mettler FP-800 hot stage melting point apparatus and are uncorrected. ^{1}H-NMR spectra were taken on a JEOL FX-90A spectrometer with Me_4Si as internal standard. Signal multiplicities are represented by s (singlet), d (doublet), dd (double doublet), t (triplet), brs (broad singlet), and m (multiplet). Chemical shifts were expressed in ppm and coupling constants (*J*) in hertz (Hz). Mass spectra (EI-MS) and high-resolution mass spectra (HRMS or HR-FAB-MS) were obtained on a JEOL DX-300 and a JEOL SX-102A mass spectrometer. Elemental analysis was carried out with a Carlo Erba model 1106 analyzer and the results were within ± 0.40% of the calculated values. For column chromatography, silica gel (Kieselgel 60, 70–230 mesh, Merck) was used.

Melting points, formulae and ^{1}H-NMR data for synthesized intermediates and final sulfonic acid derivatives are summarized in *tables I* and *II*.

6.1.1. Methyl-4-chloro-2-nitrophenylpyruvate **11**

A mixture of 4-chloro-2-nitrotoluene **10** (171.6 g, 1 mol), dimethyl oxalate (118.1 g, 1 mol) and sodium methoxide (1 mol) in methanol (350 mL) was refluxed for 2 h. The reaction mixture was poured into H_2O,

acidified with 2 N HCl (aq.) and extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt-hexane (1:5) as an eluent to give compound **11** (103.2 g, 40%).

6.1.2. Methyl 4-chloro-2-nitrophenylpyruvate dimethyl acetal **12**

To a mixture of compound **11** (95 g, 0.369 mol), trimethyl orthoformate (413 g, 3.89 mol) and MeOH (700 mL) was added boron trifluoride etherate (140 g, 0.98 mol) at room temperature. The reaction mixture was refluxed for 10 h, and then the solvent was removed. The residue was poured into H_2O and extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt-hexane (1:4) as an eluent to give compound **12** (58.5 g, 52%).

6.1.3. 7-Chloro-3,4-dihydro-3,3-dimethoxy-1-hydroxy-2(1H)-quinolinone **13**

A solution of compound **12** (55.5 g, 0.183 mol) in MeOH (200 mL) was stirred with 5% Pd-C (2.2 g) in an atmosphere of H_2 at room temperature for 20 h. The catalyst was filtered off and the filtrate was evaporated. Recrystallization from MeOH/Et_2O gave compound **13** (29.5 g, 62%).

6.1.4. 7-Chloro-1,4-dihydro-3(2H)-quinolinone dimethyl acetal **14**

To a solution of compound **13** (27.5 g, 0.107 mol) in benzene (200 mL) was added sodium bis(2-methoxyethoxy)aluminum hydride (ca. 3.4 M in toluene: 80 mL, 0.272 mol) in benzene (400 mL) at 0 °C with stirring. After the mixture had been stirred at room temperature for 22 h, 1 N NaOH (aq.) was added. An insoluble material was filtered off and the filtrate was extracted with Et_2O. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt/hexane (1:10) as an eluent to give compound **14** (4.1 g, 17%).

6.1.5. 7-Chloro-1,4-dihydro-1-(2-methylbenzoyl)-3(2H)-quinolinone **15**

To a mixture of compound **14** (1.0 g, 4.40 mmol), pyridine (0.83 g, 10.5 mmol) and CH_2Cl_2 (10 mL) was added 2-methylbenzoyl chloride (0.82 g, 5.29 mmol) at room temperature with stirring. The mixture was refluxed for 4 h. The mixture was poured into H_2O and extracted with CH_2Cl_2. The extract was washed with 1 N HCl (aq.) and water, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt/hexane (1:5) as an eluent to give 7-chloro-1,4-dihydro-1-(2-methylbenzoyl)-3(2*H*)-quinolinone dimethyl acetal (1.5 g, 98%). To a solution of the dimethyl acetal (1.5 g, 4.34 mmol) in THF (40 mL) was added 1 N HCl (aq.) (20 mL) with stirring. The mixture was stirred at room temperature for 20 h, poured into H_2O and extracted with Et_2O. The extract was washed with $NaHCO_3$ (aq.) and water, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt/hexane (1:5) as an eluent to give compound **15** (0.6 g, 45%).

6.1.6. 7-Chloro-1,4-dihydro-1-(2-methylbenzoyl)-3(2H)-quinolinone 3-oxime-O-sulfonic acid potassium salt **3**

To a mixture of compound **15** (1.0 g, 3.34 mmol), MeOH (20 mL) and CH_2Cl_2 (10 mL) was added hydroxylamine-*O*-sulfonic acid (0.6 g, 5.31 mmol) at room temperature. The mixture was stirred at room temperature for 30 min, and an aqueous solution of K_2CO_3 (0.73 g in 1 mL of H_2O, 5.31 mmol) was added. The reaction mixture was stirred at room temperature for 5 h, and then the solvent was removed. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (5:1) as an eluent to give a white solid, which was recrystallized from MeOH/CH_2Cl_2 to give compound **3** (0.38 g, 26%).

6.1.7. 7-Chloro-2,3-dihydro-2-(2-methylbenzoyl)-4(1H)-isoquinolinone **17**

This compound (85%) was prepared from compound **16** by the same procedure as for compound **15**.

6.1.8. 7-Chloro-2,3-dihydro-2-(2-methylbenzoyl)-4(1H)-isoquinolinone 4-oxime-O-sulfonic acid potassium salt **4**

This compound (22%) was prepared from compound **17** by the same procedure as for compound **3**.

6.1.9. 6-Chloro-3,4-dihydro-2-(2-methylbenzoyl)-1(2H)-isoquinolinethione **20**

To a solution of 6-chloro-3,4-dihydro-1(2*H*)-isoquinoline **18** (4.65 g, 25.6 mmol) in $CHCl_3$ (60 mL) was added phosphorous pentasulfide (7.4 g, 33.3 mmol). The reaction mixture was stirred at room temperature for 13 h and refluxed for 2 h. An insoluble matter was filtered off and the solvent was removed. The residue was purified by silica gel column chromatography with CH_2Cl_2 as an eluent to give 6-chloro-3,4-dihydro-1(2*H*)-isoquinolinethione **19** (4.35 g, 86%). Compound **20** (83%) was prepared from compound **19** by the same procedure as for compound **15**.

6.1.10. 6-Chloro-3,4-dihydro-2-(2-methylbenzoyl)-1(2H)-isoquinolinone 1-oxime **21**

To a mixture of compound **20** (3.4 g, 10.8 mmol), pyridine (2.60 g, 32.4 mmol) and EtOH (150 mL) was added hydroxylamine hydrochloride (1.13 g, 16.3 mmol), and the mixture was refluxed for 2 h. The mixture was poured into 1 N HCl (aq.) and extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (1:19) as an eluent to give compound **21** (0.58 g, 17%).

6.1.11. 6-Chloro-3,4-dihydro-2-(2-methylbenzoyl)-1(2H)-isoquinolinone 1-oxime-O-sulfonic acid potassium salt **5**

To a solution of compound **21** (12.0 g, 38.1 mmol) in CH_2Cl_2 (250 mL) was added pyridine-sulfur trioxide complex (7.1 g, 44.6 mmol). The reaction mixture was stirred at room temperature for 24 h, and the solvent was removed. To the residue were added MeOH (200 mL) and then an aqueous solution of K_2CO_3 (6.2 g in 10 mL of H_2O, 45.0 mmol). The reaction mixture was stirred at room temperature for 5 h, and then the solvent was removed. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (5:1) as an eluent to give a white solid, which was recrystallized from MeOH/CH_2Cl_2 to give compound **5** (6.1 g, 37%).

6.1.12. Ethyl 4-[N-(5-chloro-2-methoxycarbonylphenyl)-N-p-toluenesulfonylamino]butyrate **24**

A mixture of NaH (60% dispersion in mineral oil; 16 g, 0.4 mol), dry DMF (800 mL) and methyl 4-chloro-2-(p-toluenesulfonylamino)benzoate **23** (135 g, 0.398 mol), which had been prepared from methyl 2-amino-4-chlorobenzoate **22**, was stirred at room temperature for 0.5 h. To the mixture was added ethyl 4-bromobutyrate (93 g, 0.475 mol) and the mixture was stirred at 90 °C for 5 h. The mixture was poured into H_2O and extracted with CH_2Cl_2. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. Recrystallization from EtOH gave compound **24** (115 g, 64%).

6.1.13. 8-Chloro-1-(p-toluenesulfonyl)-1,2,3,4-tetrahydro-1-benzazepin-5(5H)-one **26**

To a refluxed solution of potassium *tert*-butoxide (12.4 g, 110 mmol) in toluene (500 mL) was added a toluene (300 mL) solution of compound **24** (24 g, 52.9 mmol), and stirring was continued for 1 h. The reaction mixture was poured into H_2O, acidified with 1 N HCl (aq.) and extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. To a mixture of the crude product **25** in AcOH (180 mL) and EtOH (60 mL) was added 6 N HCl (aq.) (60 mL), and the mixture was refluxed for 16 h. The reaction mixture was poured into H_2O and extracted with AcOEt. The extract was washed with water and $NaHCO_3$ (aq.), dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt/hexane (1:4) as an eluent to give compound **26** (7.3 g, 40%).

6.1.14. 8-Chloro-1,2,3,4-tetrahydro-1-benzazepin-5(5H)-one **27**

To a mixture of compound **26** (17 g, 48.6 mmol), anisole (10 mL) and trifluoroacetic acid (30 mL) was added trifluoromethanesulfonic acid (5 mL), and stirring was continued for 2 h at room temperature. The precipitated crystals were separated by filtration. To a mixture of the product and AcOEt (50 mL) was added $NaHCO_3$ (aq.), and then extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. Recrystallization from Et_2O/hexane gave compound **27** (7.3 g, 77%).

6.1.15 8-Chloro-1-(2-methylbenzoyl)-1,2,3,4-tetrahydro-1-benzazepin-5(5H)-one **28**

This compound (2.9 g, 90%) was prepared from compound **27** by the same procedure as for compound **15**.

6.1.16. 8-Chloro-1-(2-methylbenzoyl)-1,2,3,4-tetrahydro-1-benzazepin-5(5H)-one 5-oxime-O-sulfonic acid potassium salt **6**

This compound (44%) was prepared from compound **28** by the same procedure as for compound **3**.

6.1.17. 1-[4-Chloro-2-(2-methylbenzoyl)aminophenyl]-ethanone **30**

This compound (62%) was prepared from 1-[2-amino-4-chlorophenyl]ethanone **29** by the same procedure as for compound **15**.

6.1.18. 1-[4-Chloro-2-(2-methylbenzoyl)aminophenyl]-ethanone oxime **31**

This compound (58%) was prepared from compound **30** by the same procedure as for compound **21**.

6.1.19. 1-[4-Chloro-2-(2-methylbenzoyl)aminophenyl]-ethanone oxime-O-sulfonic acid potassium salt **7**

This compound (24%) was prepared from compound **31** by the same procedure as for compound **5**.

6.1.20. 7-Chloro-2,3-dihydro-1-(2-methylbenzoyl)-1,8-naphthyridine-4(1H)-one **33**

To a cooled (–70 to –75 °C) solution of 7-chloro-2,3-dihydro-1,8-naphthyridine-4(1*H*)-one **32** (0.6 g, 3.29 mmol) in dry THF (30 mL) was added lithium

bis(trimethylsilyl)amide (ca. 1.0 M in hexane: 3.3 mL, 3.30 mmol), and stirring was continued for 0.5 h. A solution of 2-methylbenzoyl chloride (0.6 g, 3.83 mmol) in THF (5 mL) was added over a 0.5 h period. The reaction mixture was then warmed to –25 °C, poured into H_2O and extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. Recrystallization from CH_2Cl_2/hexane gave compound **33** (0.11 g, 10%).

6.1.21. 7-Chloro-2,3-dihydro-1-(2-methylbenzoyl)-1,8-naphthyridine-4(1H)-one 4-oxime-O-sulfonic acid potassium salt **8**

This compound (23%) was prepared from compound **33** by the same procedure as for compound **3**.

6.1.22. 4-Acetyl-5,6-dihydro thieno[3,2-b]-pyridine-7(4H)-one **37**

A mixture of an aqueous solution of NaOH (12.0 g in 50 mL of H_2O, 0.3 mol) and 3-(2-methoxycarbonyl-3-thienylamino)propionic acid **35** (35 g, 0.153 mol), which had been prepared from methyl 3-amino-2-thiophenecarboxylate **34** and acrylic acid, was refluxed for 1 h, and then the water was removed. To a solution of the crude compound **36** in Ac_2O (150 mL) was added AcONa (25 g, 0.305 mol), and the mixture was refluxed for 1 h. The reaction mixture was poured into H_2O and extracted with Et_2O. The extract was washed with water and $NaHCO_3$ (aq.), dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with CH_2Cl_2/hexane (3:2) as an eluent to give compound **37** (11.0 g, 37%).

6.1.23. 2-Chloro-4-acetyl-5,6-dihydrothieno-[3,2-b]pyridine-7(4H)-one **38**

To a solution of compound **37** (7.7 g, 39.5 mmol) in AcOH (60 mL) was added *N*-chlorosuccinimide (6.3 g, 47.2 mmol). The reaction mixture was refluxed for 1 h and the solvent was removed. To the residue was added $NaHCO_3$ (aq.) which was then extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with CH_2Cl_2/hexane (2:3) as an eluent to give compound **38** (3.1 g, 34%).

6.1.24. 2-Chloro-5,6-dihydrothieno[3,2-b]-pyridine-7(4H)-one **39**

A mixture of compound **38** (2.8 g, 12.2 mmol), 2 N HCl (aq.) (6 mL) and AcOH (40 mL) was refluxed for 2 h and then the solvent was removed. To the residue was added $NaHCO_3$ (aq.), which was then extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with CH_2Cl_2/hexane (3:1) as an eluent to give compound **39** (1.3 g, 58%).

6.1.25. 2-Chloro-5,6-dihydro-4-(2-methylbenzoyl)-thieno[3,2-b]pyridine-7(4H)-one **40**

This compound (32%) was prepared from compound **39** by the same procedure as for compound **15**.

6.1.26. 2-Chloro-5,6-dihydro-4-(2-methylbenzoyl)-thieno[3,2-b]pyridine-7(4H)-one 7-oxime-O-sulfonic acid potassium salt **9**

This compound (29%) was prepared from compound **40** by the same procedure as for compound **3**.

6.2. Methods of computational chemistry

6.2.1 Computer programs

The ab initio molecular orbital calculation program GAUSSIAN 92 (GAUSSIAN Inc.), semi-empirical molecular orbital calculation program MOPAC 6.0 (JCPE), and molecular modelling package software SYBYL 6.0 (TRIPOS Inc.) were run on a Indigo 2 work station (Silicon Graphics Inc.).

6.2.2. Molecular modelling

The starting geometries of compounds **3–9** were constructed from the X-ray crystal structure of compound **1** (M17055) and modified where necessary using the fragment library of SYBYL 6.0. Those geometries were optimized by the semi-empirical molecular orbital AM 1 method in MOPAC 6.0. The molecular geometry of furosemide was provided by the Cambridge Structural Database (CSD).

6.2.3. Electrostatic potential contour map preparation

Electrostatic potential was calculated using the classical Coulomb's equation, charges were estimated by the CHELP method using the 3-21G* basis set provided with GAUSSIAN 92. Contour maps of electrostatic potential were graphically represented by the isosurface of specific energy (–30 or 10 kcal/mol).

6.2.4. Dihedral angle/energy curve preparation (coordinate driving method)

The energy (heat of formation; kcal/mol) was calculated against the dihedral angle ϕ (C–N–C=O) by rotating the dihedral angle in 0–360 degrees in 10 steps by using the AM 1 semi-empirical molecular orbital method at every point.

6.3. Pharmacology

6.3.1. Injection via renal artery (i.r.a.)

Mongrel dogs weighing 7–15 kg were used after overnight fasting with free access to H_2O. They were anaesthetized with pentobarbital (30 mg/kg, i.v.) and ventilated. Following a left flank incision, the left ureter was cannulated for urine collection and an L-shaped needle connected to polyethylene tubing was inserted into the left renal artery for drug administration. The drug injection route was maintained by infusing 0.9% aq. NaCl (saline) at 0.05 mL/kg/min. Following the operation, prime 3 mL/kg saline was given initially and saline was continuously infused at 0.1 mL/kg/min from a catheter in the femoral vein. After an equilibration period of 1–2 h, urine was collected every 5 min. All compounds were dissolved in alkaline solution prior to left renal artery injection at 0.01 mg/kg. Administrations were conducted at appropriate intervals.

Increase in urine output in 20 min (ΔUV_{20}) was computed as follows:

ΔUV_{20} = (urine output in 20 min after the drug injection) – (urine output in 20 min before the drug injection).

Diuretic activity was expressed as the ratio of ΔUV_{20} to that for furosemide injected in the same dog at the same dose.

6.3.2. Injection intravenously (i.v.)

Experimental procedures were essentially as for i.r.a. The few exceptions are as follows:

– No needle was attached to the renal artery.

– Infusion rate of saline into a femoral vein was always 0.15 mL/kg/min.

– Urine was collected every 10 min.

– Compound dosage into the femoral vein was 0.1 mg/kg.

Increase in urine output in 90 min (ΔUV_{90}) was determined as follows:

ΔUV_{90} = (urine output in 90 min after the drug injection) – [(urine output in 30 min before the drug injection) × 3]

Diuretic activity was expressed as the ratio of ΔUV_{90} to that for furosemide administered at the same dose to the same dog.

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