Eur. J. Med. Chem. 33 (1998) 763–774

Synthesis and diuretic activity of 4,5-dihydro-6*H*-imidazo[4,5,1-ij]quinoline-6-one 6-oxime-*O*-sulfonic acid derivatives

Kazumi Nishijima[a]*, Tomoaki Shinkawa[a], Manabu Ito[a], Hidemitsu Nishida[a], Ichirou Yamamoto[a], Yoshiaki Onuki[a], Hitoshi Inaba[a], Soutarou Miyano[b]

[a]*Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., 722 Jinba-aza-Uenohara, Gotemba, Shizuoka 412-8524, Japan*
[b]*Department of Biochemistry and Engineering, Graduate School of Engineering, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai 980-8579, Japan*

(Received 10 December 1997; accepted 23 April 1998)

Abstract – Using our previously reported 7-chloro-2,3-dihydro-1-(2-methylbenzoyl)-4(1*H*)-quinolinone 4-oxime-*O*-sulfonic acid potassium salt **1a** (M17055) as a lead, a series of tricyclic (**2a–o**, **3**, **4**, **5**) and tetracyclic (**6**) quinolinone oxime *O*-sulfonic acid derivatives were synthesized by ring annulation of the 1-(2-methylbenzoyl) moiety to the quinolinone skeleton. They were compared with furosemide and compound **1a** for diuretic activity in dogs; some tricyclic 4,5-dihydro-6*H*-imidazo[4,5,1-ij]quinoline-6-one 6-oxime-*O*-sulfonic acid derivatives showed diuretic activity comparable (**2c,e**) or superior (**2m**) to the lead compound **1a**. These results are discussed on the basis of a comparison of the conformational and electronic characteristics of the relevant compounds with the aid of computer graphics. © Elsevier, Paris

M17055 / imidazo[4,5,1-ij]quinoline-6-one 6-oxime-*O*-sulfonic acid / diuretic activity / structure–activity relationship / computation chemistry

1. Introduction

Our previously reported quinolinone oxime sulfonic acid salts **1** are novel diuretics without any chemostructural similarity to the common diuretics such as furosemide family or ethacrynic acid family in that the salts **1** are lacking sulfonamide or carboxylic acid moiety in the molecule. Based on the studies on the structure–activity relationships of **1** by varying the R^1 and R^2 substituents [1], 7-chloro-2,3-dihydro-1-(2-methylbenzoyl)-4-(1H)-quinolinone 4-oxime-*O*-sulfonic acid potassium salt **1a** (M17055) has been selected as the candidate for further development for clinical use and now under the stage of phase III by Mochida Pharmaceutical Co. Ltd., Tokyo, Japan. It has been shown that principally acting site of compound **1a** is almost the same as that of the conventional loop diuretics represented by furosemide, which inhibit the Na^+–K^+–$2Cl^-$ cotransporter of the thick ascending limb of Henle's loop [2, 3]. However, it has also been shown that there are some differences in pharmacological properties between compound **1a** and the conventional loop diuretics, suggesting that different mechanisms may also be operating for diuretic action of compound **1a**. For example, the excretion of K^+ and Ca^{2+} into urine by compound **1a** is substantially less than that by furosemide [4–7]. In order to shed more light on the diuretic mechanism and to improve the potency of **1a** class compounds, we have been for some time engaged in the synthesis and the evaluation of the diuretic activities of a variety of quinolinone oxime sulfonic acid derivatives using **1a** as a lead. As reported previously, an X-ray structural analysis of compound **1a** showed that the carbonyl group locates in the vicinity of the benzene ring of the quinolinone skeleton while the 2-methyl group of the 1-(2-methylbenzoyl) moiety directs towards the piperidine part of the quinolinone unit [1]. Based on the X-ray crystal structural analysis as well as the relationship between the structure and diuretic activity of a series

*Correspondence and reprints

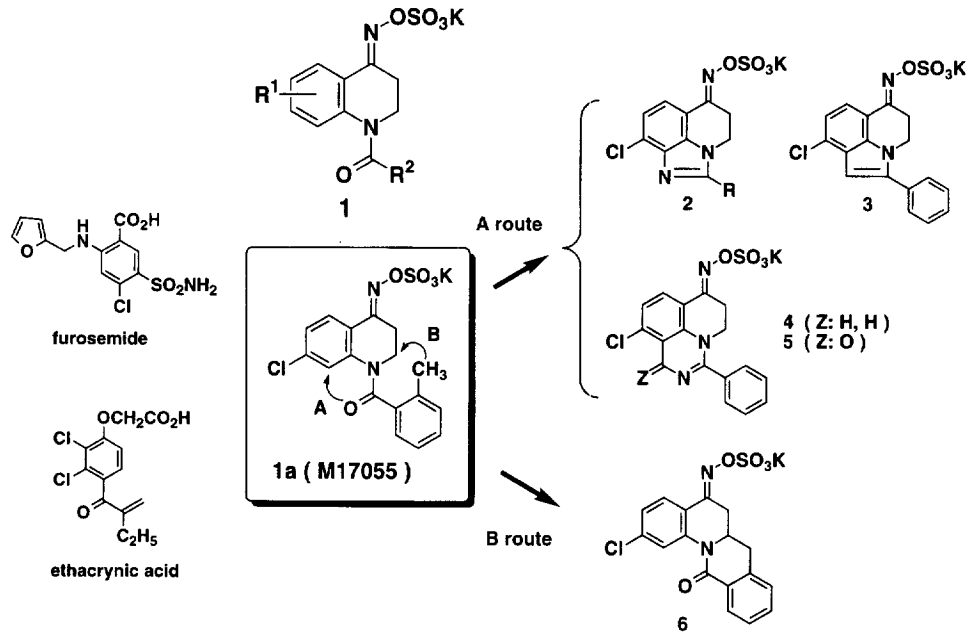


Figure 1. Synthetic plans for tricyclic and tetracyclic compounds.

of **1** class compounds, an active site model in the cotransporter has been presented with compound **1a** [1]. These results suggested the idea of examining the effect of annulation of the 1-(2-methylbenzoyl) moiety to the quinolinone system with the intention to fix the conformation still retaining the structural features of compound **1a** to improve the drug–cotransporter interaction and thus diuretic potency. Herein, we report the synthesis and diuretic activity of tricyclic (**2–5**) and tetracyclic analogues (**6**) by modulating compound **1a** (*figure 1*). The diuretic activities of the representative compounds are discussed on the basis of a comparison of the conformational and electronic characteristics with the aid of computer graphics.

2. Chemistry

2.1. Synthesis of the tricyclic compounds 2a–o, 3–5

As a representative for the synthesis of type **2** compounds (**2a–o**), the preparation of 9-chloro-4,5-dihydro-2-phenyl-6H-imidazo[4,5,1-ij]quinoline-6-one oxime-*O*-sulfonic acid potassium salt **2b** is summarized in *figure 2*. Compound **2b** could be prepared formally by annulating the carbonyl oxygen of compound **1a** to the 8-position of the quinolinone ring and replacing nitrogen for

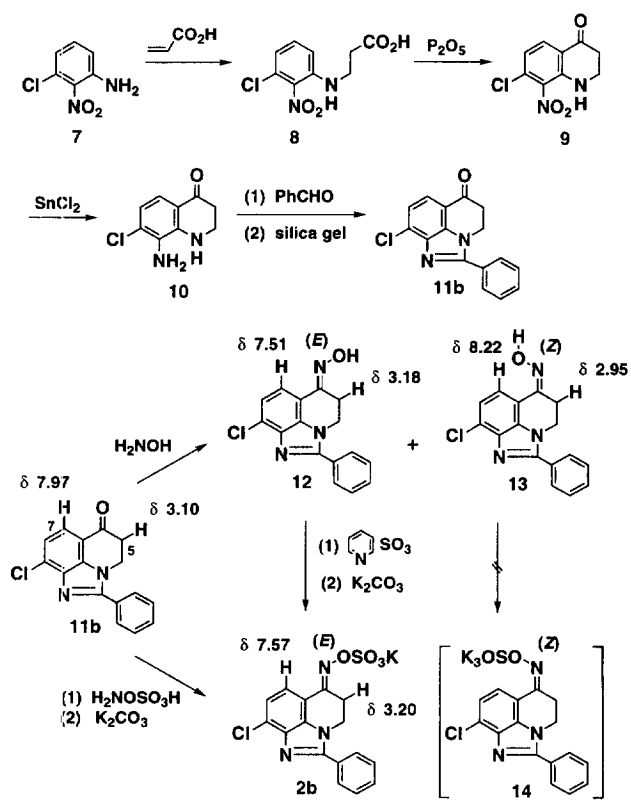


Figure 2. Synthetic pathways to **2b**, and ^{1}H-NMR spectral data of **11b, 12, 13** and **2b.**

the oxygen (*figure 1*, route A). The key intermediate 9-chloro-4,5-dihydro-2-phenyl-6H-imidazo[4,5,1-ij]quinoline-6-one **11b** was prepared by following the literature procedure for the synthesis of similar 2-alkyl-substituted 4,5-dihydro-6H-imidazo[4,5,1-ij]quinoline-6-one derivatives [8–10, 11]: Michael addition of 3-chloro-2-nitroaniline **7** to acrylic acid provided 3-(3-chloro-2-nitrophenylamino)propanoic acid **8**, which was then cyclized to 7-chloro-8-nitro-2,3-dihydro-4(1H)-quinolinone **9** by treatment with phosphorous pentoxide. After the nitro group of compound **9** was reduced to give 8-amino-7-chloro-2,3-dihydro-4(1H)-quinolinone **10**, it was condensed with benzaldehyde by heating in the presence of silica gel to give compound **11b**. Subsequently, compound **11b** was converted to the oxime-*O*-sulfonic acid potassium salt **2b** in two ways. First, oximation of compound **11b** afforded a mixture of the two oximes (**12**, **13**) in a ratio of 14:1. The stereochemistry of the oximes was determined based on the chemical shifts data of ^{1}H-NMR in the same way used for assigning that of compound **1a** [1]. The chemical shifts of the 5-position proton of the *E*-isomer **12** and the 7-position proton of the *Z*-isomer **13** should be decidedly lowfield from those of the parent ketone **11b** (δ 3.10 and δ 7.97, respectively) because of the adjacent oxime hydroxy substituent. Therefore, the major product should be assigned to be the *E*-isomer **12** on the basis of the chemical shift of the 5-protons (δ 3.18) while the minor product to be the *Z*-isomer **13** based on that of the 7-proton (δ 8.22). Sulfonation of oxime **12** with a pyridine–sulfur trioxide complex was followed by treatment with potassium carbonate to exchange the cation to provide compound **2b**. The *E*-stereochemistry of compound **2b** was safely assigned by comparing the proton chemical shifts of the 7-position (δ 7.57) and 5-position (δ 3.20) with those of the parent ketone **11b** and the oxime **12**. Similar sulfonation of the *Z*-isomer **13** did not proceed to afford the corresponding oxime-*O*-sulfonic acid **14** apparently due to steric hindrance caused by the peri 7-proton. Alternatively, treatment of ketone **11b** with hydroxylamine-*O*-sulfonic acid and then with potassium carbonate provided compound **2b** as the sole product.

Figure 3 illustrates the synthesis of 9-chloro-4,5-dihydro-2-phenyl-6H-pyrrolo[3,2,1-ij]quinoline-6-one oxime-*O*-sulfonic acid potassium salt **3** by referring to the literature [12]. Acid hydrolysis of the nitrile **17**, which was prepared from compound **16**, gave the carboxylic acid **18**. Friedel–Crafts acylation of benzene with the acid chloride of compound **18** provided (2-chloro-6-nitrophenyl)acetophenone **19**. Reductive cyclization of compound **19** with zinc provided 4-chloro-2-phenylindole **20**, Michael addition of which gave 4-chloro-1-(2-cyanoethyl)-2-phenylindole **21**. Acid hydrolysis of phenylindole **21** gave 3-(4-chloro-2-phenylindole-1-yl)propanoic acid **22**, which was then cyclized by treatment with phosphorous pentoxide to afford 9-chloro-4,5-dihydro-2-phenyl-6H-pyrrolo[3,2,1-ij]quinoline-6-one **23**. Compound **23** was converted to the oxime-*O*-sulfonic acid potassium salt **3** by the similar way as stated for the synthesis of compound **2b** (*figure 2*).

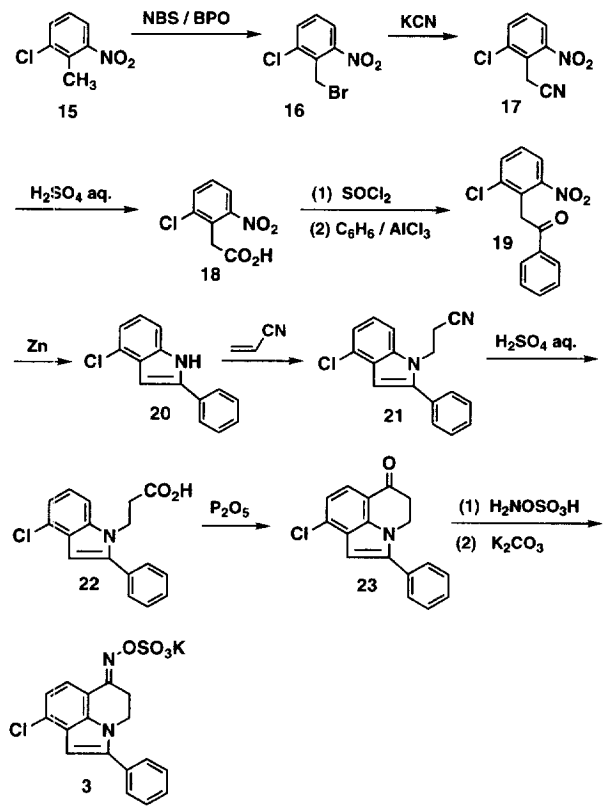


Figure 3. Synthetic pathway to **3**.

Figure 4 illustrates the synthesis of 10-chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-7-one oxime-*O*-sulfonic acid potassium salt **4** and -1,7-dione 7-oxime-*O*-sulfonic acid potassium salt **5** using 7-chloro-8-cyano-2,3-dihydro-4(1H)-quinolinone **24** as the common starting material, which had been obtained by a similar method used for the synthesis of the 8-nitro analogue **9** (*figure 2*). First, compound **24** was converted to the ethylene ketal **25**, the 8-cyano group of which was reduced by Selectride to give the 8-aminomethyl derivative **26**. This was treated with N-(ethoxycarbonyl)thiobenzamide and then with hydrochloric acid to give 10-chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]qui-

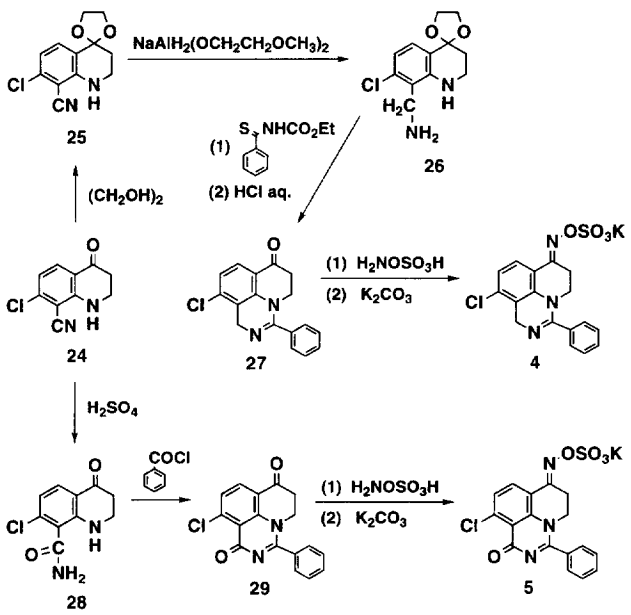


Figure 4. Synthetic pathways to **4** and **5**.

nazoline-7-one **27** by referring the method described in the literature [13]. This was eventually converted to the oxime-*O*-sulfonic acid potassium salt **4** by subsequent treatment with hydroxylamine sulfonic acid and potassium carbonate in the same way as before. Secondly, compound **24** was hydrolyzed to acid amide **28**, which was cyclized with benzoyl chloride to 10-chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-1,7-dione **29** by referring the method described in the literature [14]. Compound **29** was also converted to the oxime-*O*-sulfonic acid potassium salt **5** in the same way as before.

2.2. *Synthesis of the tetracyclic compound 6*

Figure 5 shows the synthesis of 2-chloro-6a,7-dihydro-5H-dibenzo[b,f]quinolizine-5,12(6H)-dione 5-oxime-*O*-sulfonic acid potassium salt **6** by referring to the literature [15]. Reaction of 3-chloroaniline **30** with homophthalic acid gave N-(3-chlorophenyl)homophthalimide **31**, which was reduced with sodium borohydride to 2-(3-chlorophenyl)-3-hydroxy-3,4-dihydroisocarbostyril **32**. Upon reaction of compound **32** with ethyl diethylphosphonoacetate in the presence of sodium hydride, the resulting product was hydrolyzed to give 3-carboxymethyl-2-(3-chlorophenyl)-3,4-dihydroisocarbostyril **33**, and this was cyclized to 2-chloro-6a,7-dihydro-5H-dibenzo[b,f]quinolizine-5,12(6H)-dione **34**. Compound **34** was converted to compound **6** in the same way as before.

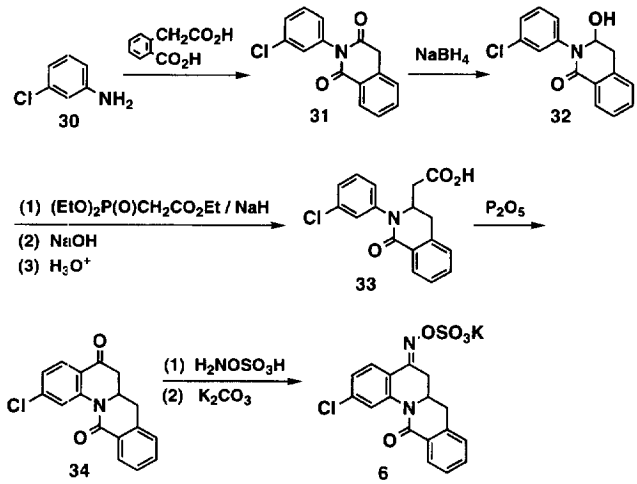


Figure 5. Synthetic pathway to **6**.

3. Diuretic activity of the tri- and tetracyclic compounds

The diuretic activities of the newly synthesized compounds were determined based on the ratio of increase in urine volume after their administration to dogs via renal 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 as reported in the previous paper [1] (see Experimental protocols for details).

Table I lists the diuretic activities of the tricycles (**2b**, **3–5**) and the tetracycle **6** as compared with furosemide; it also contains the data of compound **1a** for comparison. It should be noted that compound **2b** exerted significantly higher activity than furosemide, which prompted us to further search for this type of tricyclic compounds of an elevated activity (see below). Compound **3**, in which the 1-nitrogen atom of compound **2b** was replaced by a methine carbon, showed no activity. Insertion of a methylene unit into the 1–10 nitrogen–carbon bond of compound **2b** provided the six-membered homologue **4** (*figure 1*), and this also lost the activity. On the other hand, compound **5**, in which the methylene linkage of compound **4** is replaced by a carbonyl, resumed an appreciable, but slightly reduced activity as compared to furosemide. These results seem to suggest the importance of the presence of a polar double bond joined directly to the quinolinone ring system to exert diuretic activity in the tricyclic compounds.

The tetracyclic compound **6** was constructed formally by annulating the 2-methyl substituent of the 1-(2-methylbenzoyl) moiety of compound **1a** to the quinoli-

Table I. Diuretic activities of **2b**, **3–6**, **1a** (M17055) and furosemide.

Compound	Diuretic activity	
	i.r.a. [a]	i.v. [b]
2b	3.9	2.6
3	N [c]	N
4	N	N
5	0.9	0.4
6	N	N
1a (M17055)	4.2	4.2
furosemide	1.0	1.0

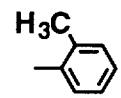
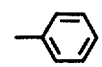
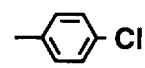
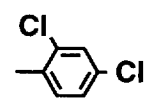
[a] Text compounds were injected into the renal artery of dogs and activity was expressed relative to that of furosemide.
[b] Text compounds were administered intravenously to dogs and activity was expressed relative to that of furosemide.
[c] No activity.

none residue (*figure 1*, route B), the molecular structure of which is apparently quite similar to that of the stable conformer of the parent **1a**. Therefore, at the beginning of this project, we assumed that compound **6** might possibly expert an improved diuretic activity. On the contrary, however, compound **6** showed no potency, and the reasons will be discussed later based on the three-dimensional structure–activity relationships and drug-cotransporter interaction with the aid of computer graphics.

Table II lists the results of the diuretic screening of type **2** compounds. In the case of type **1** compounds, it has been shown that the presence of 1-arylcarbonyl substituent, especially 1-(2-substituted phenyl)carbonyl substituent as exemplified by compound **1a**, is essential to exert a meaningful diuretic activity. The aryl moiety has been concluded to endow suitable lipophilicity with proper steric bulk to fit an active site in the cotransporter, the 2-substituent on the aryl ring being required to prevent metabolic hydrolysis of the amide linkage especially when dosed intravenously. However, the results in *table II* show that in the case of type **2** family, the corresponding 2-R substituent could be an aryl- (**2a–d**), heteroaryl- (**2e–g**) or alkyl (**2h–k**) or preferably cycloalkyl group (**2l–o**). All the 2-aryl or 2-heteroaryl substituted tricycles **2a–f** but pyridine derivative **2g** induced significantly higher diuretic activity than furosemide, among which 2-(4-chlorophenyl) (**2c**) and 2-(furane-3-yl) derivatives (**2e**) showed comparable to or even higher activity than the lead **1a** by either of the dose methods.

Among the 2-alkyl analogues **2h–k**, 2-methyl derivative **2h** induced only slight activity. Interestingly, 2-isopropyl derivative **2i** showed ca. 6 times as potent as furosemide

Table II. Diuretic activities of **2**, **1a** (M17055) and furosemide.

Compound	R	Diuretic activity	
		i.r.a. [a]	i.v. [b]
2a		3.5	2.3
2b		3.9	2.6
2c		5.7	4.1
2d		6.3	3.0
2e		4.5	4.2
2f		5.4	2.0
2g		2.7	0.8
2h	$-CH_3$	0.6	0.1
2i	$-CH(CH_3)_2$	5.8	1.6
2j	$-C(CH_3)_3$	1.4	0.7
2k	$-CH(CH_2CH_3)_2$	2.1	2.9
2l		1.9	2.2
2m		5.1	6.2
2n		3.9	2.8
2o		4.3	3.8
1a (M17055)		4.2	4.2
furosemide		1.0	1.0

[a, b] See footnotes to *table I*.

via i.r.a. but only 1.6 times via i.v. Although the activity via i.v. was substantially improved by replacing 3-pentyl group (**2k**) for the isopropyl substituent (**2i**), this in turn caused reduction in the activity via i.r.a. A tert-butyl group (**2j**) seemed to be too large for the 2-substituent, while 2-cycloalkyl derivatives **2l–o** induced significant activities. These results seem to indicate that the 2-substituents serve as hydrophobic groups which are limited in size to fit

to an active site in the cotransporter. It should be noted that the activity of 2-cyclohexyl derivative **2m** substantially supersedes that of the lead **1a**, being more than 5 times as potent as furosemide. Therefore, compound **2m** is a promising candidate for further development which will advantageously succeed compound **1a** and further therapeutic investigations are now underway.

4. Discussion

In the previous paper [1], a cotransporter active sites model has been presented with compound **1a**, in which the negative charge distributions provided by the sulfonate and carbonyl function are supposed to be of particular importance; the sulfonate group (anionic site) is thought to interact with the cationic site of the cotransporter, and the carbonyl oxygen to function as a hydrogen-bond receptor.

With the proposed model in mind, electrostatic potentials of typical compounds synthesized in this work were calculated in the same manner as stated in the previous report [1], and the electrostatic potential maps of compounds **2a**, **3** and **6** were graphically shown in *figure 6* together with that of compound **1a**. It can be seen that the potential distributions of compounds **1a** and **2a** are quite similar to each other, having strong negative charge distributions on the sulfonic acid functions and comparatively small negative charge distributions on the C=O double bond of compound **1a** and on the C=N bond of compound **2a**, respectively. This shows that the intracyclic C=N double bond of compound **2a** plays the same role of the C=O function of compound **1a**. However, the negative charge associated with the C=N bond of the former is to some extent higher than that associated with the C=O bond of the latter, presumably because of the increased coplanarity of the molecular shape of the former. This may explain that type **2** compounds induce diuretic activity comparable to or even better than type **1** compounds. The importance of the negative charge distribution provided by the C=N bond is confirmed by the fact that compound **3**, which does not have the corresponding charge as clearly shown in *figure 2*, has no diuretic activity at all.

Compound **6** apparently retains nearly all of the structural features of compound **1a** as mentioned above, and its electrostatic potential is actually quite similar to that of compound **1a** (*figure 6*). Never the less, compound **6** lost

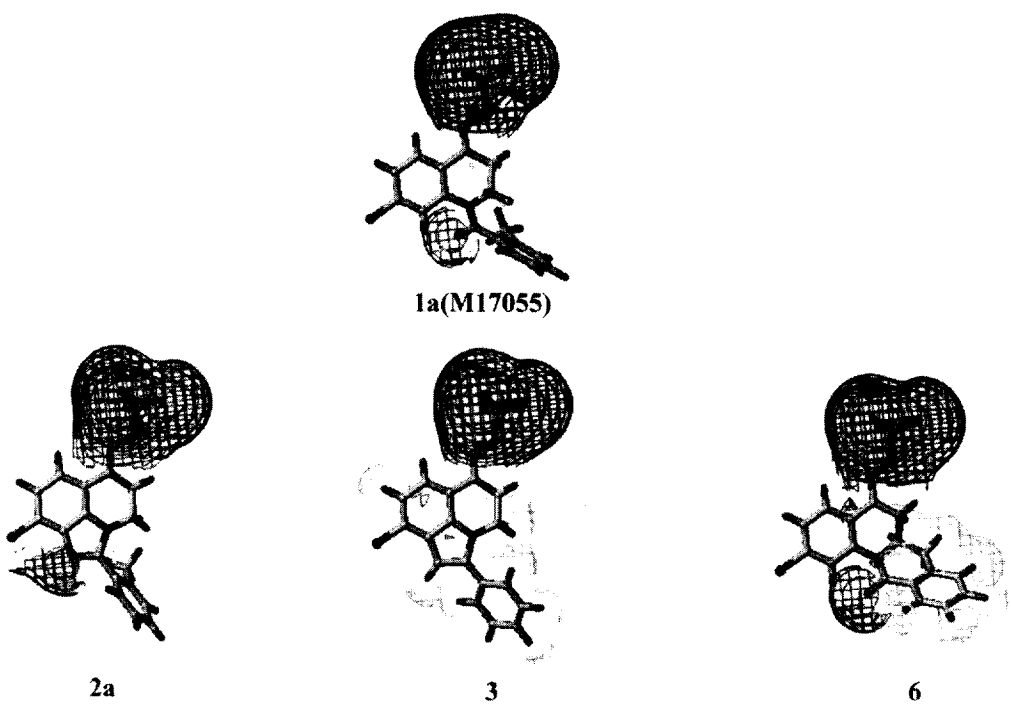


Figure 6. Electrostatic potential energy contour maps of **1a** (M17055), **2a**, **3** and **6**. The thick line is the contour at −30 kcal/mol and the thin line at 10 kcal/mol of the electrostatic potential energy.

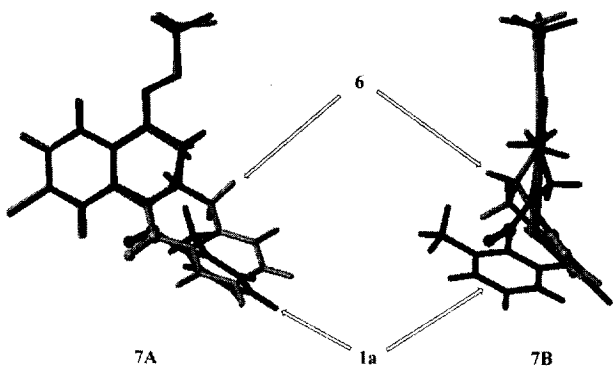


Figure 7. Superimpose of **1a** (M17055) and **6**.

the diuretic activity at all (*table I*). Detailed inspections of the three-dimensional geometries of compounds **1a** and **6** with the aid of the molecular modeling software SYBYL 6.0 indicated that they could not sufficiently be superposed each other as shown in *figure 7*. *Figure 7A* is a front view of compound **6** and **1a** superposed each other by matching the quinolinone rings and the sulfonic acid moieties, looking at the quinolinone planes. *Figure 7B* is a side view of *figure 7A*, looking along the quinolinone rings from the sides of the chlorophenyl parts down to the piperidine parts. It can be seen that the annulated phenylcarbonyl moiety of compound **6** deviates significantly upwards from that of compound **1a**, thus forcing the carbonyl function to locate in the opposite side of the quinolinone plane compared to that of compound **1a**. This conformational change seems to reduce compound **6** inactive because of a steric as well as electrostatic incompatibility with active sites in the cotransporter.

5. Conclusion

M17055 (**1a**) has been reported to belong to a novel family of diuretics, the quinolinone oxime sulfonic acid salts [1, 4–7]. As a continuation of our efforts to optimize the diuretic activity of this class of compounds, several tricyclic and tetracyclic compounds (**2–6**), which had structural features of compound **1a** by possessing the quinolinone structure and oxime-*O*-sulfonic acid function, were synthesized and their diuretic activities were evaluated in dogs. It has been found that several 4,5-dihydro-6H-imidazo[4,5,1-ij]quinoline-6-one-*O*-sulfonic acid derivatives **2** showed very potent, i.e. 4 to 6 times stronger activity than furosemide. They are promising candidates to succeed M17055 (**1a**) and the pharmacological studies on them are now underway.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Metzler FP-800 hot stage melting point apparatus and 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). Low-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 were summarized in *table III* and *table IV*.

6.1.1. 7-Chloro-2,3-dihydro-8-nitro-4(1H)-quinolinone **9**

A mixture of 3-(3-chloro-2-nitrophenylamino)propionic acid **8** (21.5 g, 87.9 mmol), which had been prepared from 3-chloro-2-nitroaniline **7** according to the similar method of previous report [1], phosphorus pentoxide (37.0 g, 0.261 mol) and toluene (160 mL) was heated at reflux for 2 h. Toluene was removed under reduced pressure and the residue was washed with ether to give compound **9** (10.0 g, 50%).

6.1.2. 9-Chloro-4,5-dihydro-2-phenyl-6H-imidazo[4,5,1-ij]quinoline-6-one **11b**

A mixture of compound **9** (10.0 g, 44.2 mmol), tin (II) chloride dihydrate (20.0 g, 88.0 mmol) and concentrated HCl (120 mL) was stirred for 2 h at 30 °C. To the reaction mixture was added 20% aqueous NaOH to be slight alkaline under cooling condition and products were extracted with AcOEt. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. The residue was washed with Et_2O to give compound **10** (5.2 g, 60%). A mixture of compound **10** (15.0 g, 76.3 mmol), benzaldehyde (9.5 g, 89.5 mmol), MeOH (150 mL) and 1 M aqueous HCl (0.2 mL) was stirred for 1 h at room temperature. To the reaction mixture were added CH_2Cl_2 (100 mL) and silica gel (150 mL), and then the solvent was removed. The residue was heated for 4 h at 90 °C. After cooling, the residue was purified by silica gel column chromatography with CH_2Cl_2 as an eluent to give compound **11b** (13.0 g, 60%).

6.1.3. 9-Chloro-4,5-dihydro-2-phenyl-6H-imidazo[4,5,1-ij]quinoline-6-one 6-oxime **12** *(E-isomer) and* **13** *(Z-isomer)*

To a mixture of compound **11b** (18.0 g, 63.7 mmol), pyridine (13.0 g, 0.165) and EtOH (200 mL) was added hydroxylamine hydrochloride (8.8 g, 0.127 mol), and the mixture was heated at reflux for 2 h. The mixture was poured into H_2O and extracted with AcOEt. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with hexane–AcOEt (3:1) as an eluent to give compound **12** (13.0 g, 69%) and compound **13** (0.9 g, 5%).

Table III. Physical data of the intermediates.

Compound	M.p. (°C)	Formula [recryst. solv.] [a]	^{1}H-NMR (ppm) [solvent]
9	197–199	$C_9H_7ClN_2O_3$ [CH_2Cl_2–hexane]	2.75 (2H, t, J = 6.0), 3.73 (2H, t, J = 6.0), 6.33 (1H, brs), 6.83 (1H, d, J = 9.0), 7.75 (1H, d, J = 9.0) [$CDCl_3$]
10	105–109	$C_9H_9ClN_2O$ [CH_2Cl_2–hexane]	2.68 (2H, t, J = 6.5), 3.58–3.75 (4H, m), 4.47 (1H, brs), 6.76(1H, d, J = 9.5), 7.41 (1H, d, J = 9.5) [$CDCl_3$]
11b	206–209	$C_{16}H_{11}ClN_2O$ [Et_2O–hexane]	3.10 (2H, t, J = 6.4), 4.80 (2H, t, J = 6.4), 7.41 (1H, d, J = 8.8), 7.55–8.01 (5H, m), 7.97 (1H, d, J = 8.8) [DMSO-d_6]
12	251–253	$C_{16}H_{12}ClN_3O$ [CH_2Cl_2–hexane]	3.18 (2H, t, J = 6.0), 4.54 (2H, t, J = 6.0), 7.30 (1H, d, J = 8.3), 7.51 (1H, d, J = 8.3), 7.50–8.04 (5H, m), 11.60 (1H, s) [DMSO-d_6]
13	246–248	$C_{16}H_{12}ClN_3O$ [CH_2Cl_2–hexane]	2.95 (2H, t, J = 5.9), 4.59 (2H, t, J = 5.9), 7.34 (1H, d, J = 8.2), 8.22 (1H, d, J = 8.2), 7.46–8.06 (5H, m), 11.57 (1H, s) [DMSO-d_6]
16	246–247	$C_7H_5BrClNO_2$ [CH_2Cl_2–hexane]	4.87 (2H, s), 7.42 (1H, t, J = 8.0), 7.78 (1H, dd, J = 8.0, J = 1.7), 7.86 (1H, dd, J = 8.0, J = 1.7) [$CDCl_3$]
17	120–122	$C_8H_5ClN_2O_2$ [CH_2Cl_2–hexane]	4.16 (2H, s), 7.52 (1H, t, J = 8.1), 7.79 (1H, dd, J = 8.1, J = 1.7), 8.01 (1H, dd, J = 8.1, J = 1.7) [$CDCl_3$]
18	134–137	$C_8H_6ClNO_4$ [CH_2Cl_2–hexane]	4.01 (2H, s), 7.57 (1H, t, J = 8.1), 7.80–8.12 (2H, m), 12.69 (1H, s) [$CDCl_3$]
19	70–73	$C_{14}H_{10}ClNO_3$ [CH_2Cl_2–hexane]	4.89 (2H, s), 7.26–7.86 (5H, m), 7.86–8.16 (3H, m) [$CDCl_3$]
20	74–77	$C_{14}H_{10}ClN$ [CH_2Cl_2–hexane]	6.84–7.80 (9H, m), 8.43 (1H, s) [$CDCl_3$]
21	92–93	$C_{17}H_{13}ClN_2$ [Et_2O]	2.56 (2H, t, J = 7.4), 4.48 (2H, t, J = 7.4), 6.68 (1H, s), 7.10–7.49 (8H, m) [$CDCl_3$]
22	159–161	$C_{17}H_{14}ClNO_2$ [CH_2Cl_2–hexane]	2.56 (2H, t, J = 7.6), 4.56 (2H, t, J = 7.6), 6.55 (1H, s), 7.11–7.30 (2H, m), 7.42–7.80 (6H, m), 11.30 (1H, s) [$CDCl_3$]
23	220–221	$C_{17}H_{12}ClNO$ [Et_2O–hexane]	3.08 (2H, t, J = 6.9), 4.54 (2H, t, J = 6.9), 6.76 (1H, s), 7.24 (1H, d, J = 7.9), 7.43–7.82 (6H, m) [$CDCl_3$]
26	167–173	$C_{12}H_{15}ClN_2O_2$ [CH_2Cl_2–hexane]	2.00 (2H, t, J = 6.2), 3.48 (2H, t, J = 6.2), 4.04 (2H, s), 3.96–4.32 (4H, m), 6.65 (1H, d, J = 8.2), 7.20 (1H, d, J = 8.2) [$CDCl_3$]
27	185–188	$C_{17}H_{13}ClN_2O$ [Et_2O–hexane]	2.69 (2H, t, J = 6.8), 3.78 (2H, t, J = 6.8), 4.94 (2H, s), 7.07 (1H, d, J = 8.6), 7.40–7.50 (5H, m), 7.75 (1H, d, J = 8.6) [$CDCl_3$]
28	196–198	$C_{10}H_9ClN_2O_2$ [CH_2Cl_2–hexane]	2.51 (2H, t, J = 7.3), 3.48 (2H, t, J = 7.3), 6.63 (1H, d, J = 8.7), 7.59 (1H, d, J = 8.7), 7.70 (1H, brs), 7.95 (2H, brs) [DMSO-d_6]
29	281–282	$C_{17}H_{11}ClN_2O_2$ [Et_2O–hexane]	3.02 (2H, t, J = 6.5), 4.26 (2H, t, J = 6.5), 7.34–7.72 (6H, m), 8.18 (1H, d, J = 8.2) [DMSO-d_6]
31	160–161	$C_{15}H_{10}ClNO_2$ [EtOH]	4.22 (2H, s), 7.02–7.80 (7H, m), 8.24 (1H, dd, J = 1.7, J = 7.3) [$CDCl_3$]
32	121–124	$C_{15}H_{12}ClNO_2$ [Et_2O]	2.93 (1H, d, J = 8.6), 3.13 (1H, dd, J = 2.3, J = 6.5), 3.58 (1H, dd, J = 2.3, J = 6.5), 5.23–5.35 (1H, m), 7.22–7.68 (7H, m), 8.17 (1H, dd, J = 2.3, J = 8.1) [$CDCl_3$]
33	209–211	$C_{17}H_{14}ClNO_3$ [CH_2Cl_2–hexane]	2.80–3.92 (4H, m), 4.12–4.64 (1H, m), 7.32–7.70 (7H, m), 7.91 (1H, d, J = 7.3), 12.30 (1H, brs) [DMSO-d_6]
34	157–158	$C_{17}H_{12}ClNO_2$ [Et_2O–hexane]	2.53–3.58 (4H, m), 4.32–4.77 (1H, m), 7.14–7.68 (4H, m), 7.96 (1H, d, J = 8.2), 8.06–8.24.(2H, m) [$CDCl_3$]

[a] Compounds were analyzed by HR-MS.

6.1.4. 9-Chloro-4,5-dihydro-2-phenyl-6H-imidazo[4,5,1-ij]quinoline-6-one 6-oxime-O-sulfonic acid potassium salt **2b**

Method A: To a solution of compound **12** (13.0 g, 43.7 mmol) in CH_2Cl_2 (100 mL) and DMF (20 mL) was added pyridine–sulfur trioxide complex (14.0 g, 87.9 mmol). The reaction mixture was stirred at room temperature for 24 h and the solvent was removed by evaporation. To the residue was added MeOH (200 mL), followed by addition of aqueous K_2CO_3 (7.2 g in 10 mL of H_2O, 52.0 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 (4:1) as an eluent to give a white solid, which was recrystallized from MeOH–CH_2Cl_2 to give compound **2b** (8.0 g, 44%).

Method B: To a mixture of compound **11b** (12.0 g, 42.5 mmol), MeOH (150 mL) and CH_2Cl_2 (150 mL) was added hydroxylamine-*O*-sulfonic acid (7.2 g, 63.7 mmol) at room temperature. The

Table IV. Physical data of compounds **2–6**.

Compound	M.p. (°C) (dec) [a]	Formula [b]	^{1}H-NMR (DMSO-d_6, ppm)
2a	222–223	$C_{17}H_{13}ClKN_3O_4S$	2.35 (3H, s), 3.15 (2H, t, J = 6.2), 4.18 (2H, t, J = 6.2), 7.30–7.61 (6H, m)
2b	230–233	$C_{16}H_{11}ClKN_3O_4S$	3.20 (2H, t, J = 6.3), 4.56 (2H, t, J = 6.3), 7.35 (1H, d, J = 8.2), 7.57 (1H, d, J = 8.2), 7.40–8.10 (5H, m)
2c	250–251	$C_{16}H_{10}Cl_2KN_3O_4S$	3.19 (2H, t, J = 6.3), 4.61 (2H, t, J = 6.3), 7.35 (1H, d, J = 8.5), 7.57 (1H, d, J = 8.5), 7.66 (2H, d, J = 8.0), 8.01 (2H, d, J = 8.0)
2d	250–251	$C_{16}H_9Cl_3KN_3O_4S$	3.16 (2H, t, J = 6.3), 4.20 (2H, t, J = 6.3), 7.39 (1H, d, J = 8.3), 7.57–7.83 (3H, m), 7.90 (1H, d, J = 8.3)
2e	255–256	$C_{14}H_9ClKN_3O_5S$	3.20 (2H, t, J = 6.3), 4.54 (2H, t, J = 6.3), 7.14 (1H, d, J = 2.0), 7.30 (1H, d, J = 8.3), 7.52 (1H, d, J = 8.3), 7.92 (1H, d, J = 2.0), 8.54 (1H, s)
2f	246–247	$C_{14}H_9ClKN_3O_4S_2$	3.21 (2H, t, J = 6.5), 4.61 (2H, t, J = 6.5), 7.32 (1H, d, J = 8.2), 7.55 (1H, d, J = 8.2), 7.76–8.32 (3H, m)
2g	219–221	$C_{15}H_{10}ClKN_4O_4S$	3.21 (2H, t, J = 6.3), 4.59 (2H, t, J = 6.3), 7.38 (1H, d, J = 8.3), 7.59 (1H, d, J = 8.3), 7.46–7.72 (1H, m), 8.32–8.41 (1H, m), 8.44–8.95 (2H, m)
2h	250–252	$C_{11}H_9ClKN_3O_4S$	2.55 (3H, s), 3.13 (2H, t, J = 6.3), 4.29 (2H, t, J = 6.3), 7.23 (1H, d, J = 8.5), 7.45 (1H, d, J = 8.5)
2i	216–218	$C_{13}H_{13}ClKN_3O_4S$	1.36 (6H, d, J = 7.8), 3.15 (2H, t, J = 6.2), 3.18–3.49 (1H, m), 4.34 (2H, t, J = 6.2), 7.25 (1H, d, J = 8.6), 7.48 (1H, d, J = 8.6)
2j	229–230	$C_{14}H_{15}ClKN_3O_4S$	1.48 (9H, s), 3.14 (2H, t, J = 6.2), 4.50 (2H, t, J = 6.2), 7.24 (1H, d, J = 8.6), 7.49 (1H, d, J = 8.6)
2k	201–203	$C_{15}H_{17}ClKN_3O_4S$	0.82 (6H, t, J = 7.5), 1.59–1.95 (4H, m), 2.68–3.04 (1H, m), 3.14 (2H, t, J = 6.3), 4.33 (2H, t, J = 6.3), 7.27(1H, d, J = 8.3), 7.46 (1H, d, J = 8.3)
2l	237–238	$C_{13}H_{11}ClKN_3O_4S$	1.03–1.20 (4H, m), 2.01–2.40 (1H, m), 3.16 (2H, t, J = 6.2), 4.41 (2H, t, J = 6.2), 7.20 (1H, d, J = 8.6), 7.41 (1H, d, J = 8.6)
2m	240–241	$C_{16}H_{17}ClKN_3O_4S$	1.17–2.07 (10H, m), 2.81–3.20 (1H, m), 3.12 (2H, t, J = 6.3), 4.33 (2H, t, J = 6.3), 7.22 (1H, d, J = 8.2), 7.45 (1H, d, J = 8.2)
2n	250–251	$C_{16}H_{15}ClKN_3O_4S$	1.58–1.93 (4H, m), 2.30–2.60 (4H, m), 3.12 (2H, t, J = 6.3), 4.43 (2H, J = 6.3), 6.45–6.55 (1H, m), 7.27 (1H, d, J = 8.3), 7.48 (1H, d, J = 8.3)
2o	250–251	$C_{16}H_{15}ClKN_3O_4S$	1.67–2.44 (6H, m), 3.00–3.30 (1H, m), 3.15 (2H, t, J = 6.3), 4.36 (2H, J = 6.3), 5.70–5.80 (2H, m), 7.26 (1H, d, J = 8.3), 7.50 (1H, d, J = 8.3)
3	251–253	$C_{17}H_{12}ClKN_2O_4S$	3.17 (2H, t, J = 6.5), 4.28 (2H, t, J = 6.5), 6.66 (1H, s), 7.18 (1H, d, J = 7.8), 7.35–7.81 (6H, m)
4	220–221	$C_{17}H_{13}ClKN_3O_4S$	2.82 (2H, t, J = 6.3), 3.46 (2H, t, J = 6.3), 4.76 (2H, s), 7.12 (1H, d, J = 8.6), 7.49 (5H, s), 7.78 (1H, d, J = 8.6)
5	248–249	$C_{17}H_{11}ClKN_3O_5S$	3.03 (2H, t, J = 6.9), 4.00 (2H, t, J = 6.9), 7.46–7.82 (6H, m), 8.25 (1H, d, J = 8.6)
6	200–203	$C_{17}H_{12}ClKN_2O_5S$	2.72–3.82 (4H, m), 4.02–4.42 (1H, m), 7.25–7.70 (4H, m), 7.70–8.10 (3H, m)

[a] Recrystallisation solvent: CH_2Cl_2–MeOH.
[b] Compounds **2** were analyzed for C, H and N. Analytical results obtained for these elements were within ± 0.4% of the calculated values for the formulae shown. Compounds **3–6** were analyzed by HR-FAB-MS.

mixture was stirred at room temperature for 30 min, and then an aqueous K_2CO_3 (6.9 g in 10 mL of H_2O, 50.0 mmol) was added. The reaction mixture was treated in a similar manner as described in method A to give compound **2b** (13.0 g, 74%).

The other compounds **2** were prepared in a similar manner.

6.1.5. (2-Chloro-6-nitrophenyl)acetonitrile 17

To a solution of 2-chloro-6-nitrotoluene **15** (75 g, 0.436 mol) was added N-bromosuccinimide (85.6 g, 0.481 mol) and benzoyl peroxide (1.75 g, 7.2 mmol). The mixture was heated at reflux for 20 h. After the precipitate was filtered off, the solvent was removed. The residue was purified by silica gel column chromatography with AcOEt–hexane (3:97) as an eluent to give 2-chloro-6-nitrobenzyl bromide **16** (70 g, 64%). To a solution of compound **16** (70 g, 0.279 mol) in EtOH (300 mL) was added KCN (27 g, 0.415 mol) in H_2O (40 mL). The reaction mixture was stirred at 25 °C for 2 h. After the solvent was removed, the residue was dissolved in AcOEt and the solution was washed successively with water and brine, dried over Na_2SO_4 and evaporated to give compound **17** (35 g, 64%).

6.1.6. (2-Chloro-6-nitrophenyl)acetophenone 19

A mixture of compound **17** (35 g, 0.178 mol) and 50% aqueous H_2SO_4 was stirred at 110 °C for 3 h. The mixture was poured into H_2O and precipitated crystals were separated by filtration. The

product was washed with water and dried to give (2-chloro-6-nitrophenyl)acetic acid **18** (36 g, 94%). To a solution of compound **18** (10 g, 46.4 mmol) in dichloroethane (50 mL) was added thionyl chloride (11 g, 92.4 mmol). The mixture was heated at 70 °C for 2 h. To the cooled solution was added benzene (5.9 g, 75.6 mmol) and $AlCl_3$ (3 g, 22.5 mmol). After the reaction mixture was stirred at 60 °C for 10 min, it was poured into cold H_2O. The solution was acidified with concentrated HCl, and then extracted with CH_2Cl_2. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:9) as an eluent to give compound **19** (10 g, 78%).

*6.1.7. 4-Chloro-2-phenylindole **20***

To a solution of compound **19** (10 g, 36.3 mmol) in 80% aqueous AcOH (150 mL) was added zinc powder (15 g, 0.229 mol) at 70 °C. The reaction mixture was stirred at 90 °C for 1 h. After the unchanged zinc was filtered off, the solvent was removed. The residue was dissolved in AcOEt and the solution was washed successively 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 **20** (7.0 g, 85%).

*6.1.8. 3-(4-Chloro-2-phenylindole-1-yl)propionic acid **22***

A mixture of compound **20** (6.5 g, 28.6 mmol), acrylonitrile (3.0 g, 56.5 mmol), Triton B (10 drops) and dioxane (50 mL) was stirred at 70 °C for 2 h. The reaction mixture was poured into H_2O and acidified with 1 M aqueous HCl, and then extracted with AcOEt. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. Recrystallization from Et_2O gave 4-chloro-1-(2-cyanoethyl)-2-phenylindole **21** (6.5 g, 81%). Compound **21** (6.5 g, 23.1 mmol) gave compound **22** (5.2 g, 75%) by the same procedure as for compound **18**.

*6.1.9. 9-Chloro-4,5-dihydro-2-phenyl-6H-pyrrolo[3,2,1-ij]quinoline-6-one **23***

A mixture of compound **22** (5.2 g, 17.3 mmol), phosphorus pentoxide (8.0 g, 56.3 mmol) and xylene (30 mL) was heated by reflux for 1 h. The organic phase was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:9) as an eluent to give compound **23** (2.9 g, 59%).

*6.1.10. 9-Chloro-4,5-dihydro-2-phenyl-6H-pyrrolo[3,2,1-ij]quinoline-6-one 6-oxime-O-sulfonic acid potassium salt **3***

To a mixture of compound **23** (420 mg, 1.49 mmol), MeOH (5 mL) and CH_2Cl_2 (5 mL) was added hydroxylamine-*O*-sulfonic acid (250 mg, 2.21 mmol) at room temperature. The mixture was stirred at room temperature for 30 min, and then aqueous K_2CO_3 (310 mg in 1 mL of H_2O, 2.24 mmol) was added. The reaction mixture was treated in a similar manner as described for compound **2b** to give compound **3** (250 mg, 40%).

*6.1.11. 8-Aminomethyl-7-chloro-2,3-dihydro-4(1H)-quinolinone ethylene ketal **26***

To a cooled (0 °C to 5 °C) solution of sodium bis(2-methoxyethoxy)aluminum hydride (70% solution in toluene; 27 mL, 0.1 mol) in benzene (30 mL) was added a benzene solution (250 mL) of 7-chloro-8-cyano-2,3-dihydro-4(1H)-quinolinone ethylene ketal **25** (6.5 g, 31.5 mmol), which had been prepared from 7-chloro-8-cyano-2,3-dihydro-4(1H)-quinolinone **24** and ethylene glycol by the usual method. The reaction mixture was poured into 1 M aqueous NaOH and extracted with benzene. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with CH_2Cl_2–MeOH (95:5) as an eluent to give compound **26** (3.5 g, 70%).

*6.1.12. 10-Chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-7-one **27***

A mixture of compound **26** (3.4 g, 13.3 mmol), N-(ethoxycarbonyl)thiobenzamide (3.0 g, 14.4 mmol) and benzene (150 mL) was heated at reflux for 5 h, and then the solvent was removed. To a cooled (0–5 °C) solution of the residue (4.1 g, 12.0 mmol) in THF (80 mL) was added 1 M aqueous HCl (40 mL), and stirring was continued for 13 h at room temperature. The reaction mixture was poured into 0.5 M aqueous NaOH and extracted with Et_2O. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. Recrystallization from Et_2O–hexane gave compound **27** (3.1 g, 88%).

*6.1.13. 10-Chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-7-one 7-oxime-O-sulfonic acid potassium salt **4***

Compound **4** (35%) was prepared from compound **27** by the same procedure as for compound **2b**.

*6.1.14. 7-Chloro-2,3-dihydro-4(1H)-quinolinone-8-carboxamide **28***

A mixture of compound **24** (3.6 g, 17.4 mmol) and concentrated H_2SO_4 (7.2 mL) was stirred at 70–80 °C for 4 h. The mixture was poured into H_2O and the solution was made basic with 10% aqueous NaOH. The precipitated crystals were separated by filtration. The product was washed with water and dried to give compound **28** (2.6 g, 66%).

*6.1.15. 10-Chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-1,7-dione **29***

To a solution of compound **28** (3.1 g, 13.8 mmol) in DMA (20 mL) was added benzoyl chloride (5.8 g, 41.3 mmol), and stirring was continued for 5 h at 70 °C. The reaction mixture was poured into H_2O and extracted with CH_2Cl_2. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with CH_2Cl_2 as an eluent to give compound **29** (2.4 g, 55%).

*6.1.16. 10-Chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-1,7-dione 7-oxime-O-sulfonic acid potassium salt **5***

Compound **5** (40%) was prepared from compound **29** by the same procedure as for compound **2b**.

*6.1.17. 2-(3-Chlorophenyl)-3-hydroxy-3,4-dihydroisocarbostyril **32***

A mixture of 3-chloroaniline **30** (17.7 g, 0.139 mol) and homophthalic acid (25 g, 0.139 mol) was stirred at 180 °C for 6 h. Recrystallization from EtOH gave N-(3-chlorophenyl) homophthalimide (**31**) (23.0 g, 60%). To a mixture of compound **31** (11.5 g, 42.3 mmol), CH_2Cl_2 (100 mL) and MeOH (100 mL) was added sodium borohydride (1.2 g, 31.7 mmol) at 0 °C with

stirring. The mixture was stirred at 0 °C for 30 min, poured into ice-cold water and extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4 and evaporated. Recrystallization from Et_2O gave compound **32** (8.0 g, 69%).

6.1.18. 3-Carboxymethyl-2-(3-chlorophenyl)-3,4-dihydroisocarbostyril ***33***

To a mixture of NaH (60% in mineral oil; 0.53 g, 13.3 mmol) and THF (20 mL) was added ethyl diethylphosphonoacetate (1.39 g, 6.21 mmol) in THF (2 mL), and stirring was continued at room temperature for 15 min. To the cooled (0–5 °C) solution was added a solution of compound **32** (1.0 g, 3.65 mmol) in THF (10 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into 1 M aqueous HCl and extracted with CH_2Cl_2. The extract was washed successively with water and brine, dried over Na_2SO_4 and evaporated. To the residue was added EtOH (10 mL) and then 10% aqueous NaOH (2.5 mL), and stirring was continued at room temperature for 1 h. The reaction mixture was poured into H_2O and washed with Et_2O. The water layer was acidified with 3 M aqueous HCl and precipitated crystals were separated by filtration. The product was washed with water and dried to give compound **33** (0.9 g, 94%).

6.1.19. 2-Chloro-6a,7-dihydro-5H-dibenzo[b,f]quinolizine-5,12 (6H)-dione ***34***

Compound **34** (54%) was prepared from compound **33** by the same procedure as for compound **9**.

6.1.20. 2-Chloro-6a,7-dihydro-5H-dibenzo[b,f]quinolizine-5,12 (6H)-dione 5-oxime-O-sulfonic acid potassium salt ***6***

Compound **6** (32%) was prepared from compound **34** by the same procedure as for compound **2b**.

6.2. Computational chemistry methods

6.2.1. Computer programs

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

6.2.2. Molecular modeling

The starting geometries of compound **2a**, **3** and **6** were constructed from the X-ray crystal structure of compound **1a** (M17055) and modified where necessary using the fragment library of SYBYL 6.0. Those geometries were optimized by the semiempirical molecular orbital AM 1 method in MOPAC 6.0. The molecular geometry of furosemide was provided by the Cambridge Structural Databases (CSD).

6.2.3. Electrostatic potential contour map preparation

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

6.3. Pharmacology

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

Mongrel dogs weighing 7 to 15 kg were used after overnight fasting with free access to H_2O. They were anesthetized 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% aqueous 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 drug injection) – (urine output in 20 min before 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.

(1) No needle was attached to the renal artery.

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

(3) Urine was collected every 10 min.

(4) Compound dosage into 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 drug injection) – [(urine output in 30 min before 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.

Acknowledgements

The authors express their gratitude to the Mochida diuretic project team.

References

[1] Nishijima K., Shinkawa T., Kato K., Sato N., Nishida H., Yamashita Y., Onuki Y., Mizota M., Ohtomo K., Miyano S., Eur. J. Med. Chem. 33 (1998) 267–277.

[2] Cragoe E.J. (Ed.), Chemistry and Pharmacology of Drugs, Vol. 2: Diuretics – Chemistry, Pharmacology and Medicine, Wiley, New York, 1983.

[3] Puschett J.B., Greenberg A. (Ed.), Diuretics: Chemistry, Pharmacology and Clinical Applications, Elsevier, New York, 1984.

[4] Shinkawa T., Yamasaki F., Kikuchi A., Nakakuki M., Nishijima K., Uemura A., Mizota M., Orita Y., Arzneim. Forsch. Drug Res. 42 (1992) 1466–1472.

[5] Shinkawa T., Yamasaki F., Notsu T., Nakakuki M., Nishijima K., Yoshitomi K., Imai M., Eur. J. Pharmacol. 238 (1993) 317–325.

[6] Yamasaki F., Yoshitomi K., Shinkawa T., Imai M., J. Pharmacol. Exp. Ther. 266 (1993) 1581–1588.

[7] Yasoshima K., Yamasaki F., Shinkawa T., Yoshitomi K., Imai M., J. Pharmacol. Exp. Ther. 266 (1993) 1589–1593.

[8] Kamenka J.M., Alam M.N., J. Heterocycl. Chem. 10 (1973) 459–462.

[9] Chothia D.S., Dike S.Y., Engineer A.B., Merchant J.R., Indian J. Chem. 14B (1976) 323–325.

[10] Geneste P., Kamenka J.M., Vidal Y., Fauquet J.P., Morel E., Muller P., Warolin C., Eur. J. Med. Chem. Chim. Ther. 13 (1978) 53–59.

[11] Geneste P., Kamenka J.M., Vidal Y., Besancon M., Garcet S., Muller P., Warolin C., Eur. J. Med. Chem. Chim. Ther. 12 (1977) 471–476.

[12] Merchant J.R., Shankarnarayan V., Curr. Sci. 48 (1979) 585–586.

[13] Papadopoulos E.P., George B., J. Org. Chem. 42 (1977) 2530–2532.

[14] Ozaki K., Yamada Y., Oine T., Chem. Pharm. Bull. 31 (1983) 2234–2243.

[15] Ishihara Y., Kiyota Y., Goto G., Chem. Pharm. Bull. 38 (1990) 3024–3030.