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

^aFuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., 722 Jinba-aza-Uenohara, Gotemba, Shizuoka 412-8524, Japan ^bDepartment 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 R¹ and R² 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-Osulfonic 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 ¹H-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-2nitroaniline 7 to acrylic acid provided 3-(3-chloro-2nitrophenylamino)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-Osulfonic 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 ¹H-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 $(\delta 3.20)$ 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-4,5dihydro-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-cyano-



Figure 3. Synthetic pathway to 3.

ethyl)-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 4 illustrates the synthesis of 10-chloro-5,6-dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-7one oxime-O-sulfonic acid potassium salt 4 and -1,7dione 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-5,6dihydro-3-phenyl-1H,7H-pyrido[3,2-ij]quinazoline-1,7dione **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-Osulfonic 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-(3chlorophenyl)-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-5Hdibenzo[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-(2methylbenzoyl) 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 °	Ν	
4	N	Ν	
5	0.9	0.4	
6	Ν	Ν	
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 threedimensional structure-activity relationships and drugcotransporter 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 (21-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	Diuretic activity	
		i. r.a. ^a	i.v. ^b	
2a		3.5	2.3	
2b	\neg	3.9	2.6	
2c		5.7	4.1	
2d	cı → → cı	6.3	3.0	
2e	لير الر	4.5	4.2	
2f	رگر ا	5.4	2.0	
2g	-√ [−] N	2.7	0.8	
2h	CH ₃	0.6	0.1	
2i	$-CH(CH_3)_2$	5.8	1.6	
2ј	$-C(CH_3)_3$	1.4	0.7	
2k	$-CH(CH_2CH_3)_2$	2.1	2.9	
21		1.9	2.2	
2m	\rightarrow	5.1	6.2	
2n	$\overline{\bigcirc}$	3.9	2.8	
20	-	4.3	3.8	
1a (M17055) furosemide		4.2 1.0	4.2 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. ¹H-NMR spectra were taken on a JEOL FX-90A spectrometer with Me₄Si 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 $\pm 0.40\%$ of the calculated values. For column chromatography, silica gel (Kieselgel 60, 70–230 mesh, Merck) was used.

Melting points, formulae and ¹H-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-2nitroaniline **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 Na₂SO₄ and evaporated. The residue was washed with Et₂O 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 CH₂Cl₂ (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 CH₂Cl₂ 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 H₂O and extracted with AcOEt. The extract was washed successively with water and brine, dried over Na₂SO₄ 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 ₂ Cl 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	$C_{16}\tilde{H}_{11}\tilde{C}IN_2O$ [Et ₂ O-hexane]	3.10 (2H, t, <i>J</i> = 6.4), 4.80 (2H, t, <i>J</i> = 6.4), 7.41 (1H, d, <i>J</i> = 8.8), 7.55–8.01 (5H, m), 7.97 (1H, d, <i>J</i> = 8.8) [DMSO- <i>d</i> ₆]
12	251-253	$C_{16}H_{12}ClN_{3}O$ [CH ₂ Cl 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	$C_{16}\tilde{H}_{12}\tilde{C}IN_{3}O$ [CH ₂ Cl 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 ₂ Cl 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 ₂ Cl 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	$C_8H_6CINO_4$ [CH ₂ Cl 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	$C_{14}\tilde{H}_{10}\tilde{C}INO_3$ [CH ₂ Cl ₂ -hexane]	4.89 (2H, s), 7.26–7.86 (5H, m), 7.86–8.16 (3H, m) [CDCl ₃]
20	74–77	$C_{14}\overline{H}_{10}\overline{C}IN$ [CH ₂ Cl ₂ -hexane]	6.84-7.80 (9H, m), 8.43 (1H, s) [CDCl ₃]
21	92–93	C ₁₇ H ₁₃ ClN ₂ [Et ₂ O]	2.56 (2H, t, <i>J</i> = 7.4), 4.48 (2H, t, <i>J</i> = 7.4), 6.68 (1H, s), 7.10–7.49 (8H, m) [CDCl ₃]
22	159–161	$C_{17}H_{14}CINO_2$ [CH ₂ Cl ₂ -hexane]	2.56 (2H, t, <i>J</i> = 7.6), 4.56 (2H, t, <i>J</i> = 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	$C_{17}\overline{H}_{12}\overline{C}INO$ [Et ₂ O-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 ₂ Cl 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	$C_{17}\overline{H}_{13}\overline{C}IN_2O$ [Et ₂ O-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 ₂ Cl ₂ -hexane]	2.51 (2H, t, <i>J</i> = 7.3), 3.48 (2H, t, <i>J</i> = 7.3), 6.63 (1H, d, <i>J</i> = 8.7), 7.59 (1H, d, <i>J</i> = 8.7), 7.70 (1H, brs), 7.95 (2H, brs) [DMSO- <i>d</i> ₆]
29	281-282	$C_{17}H_{11}CIN_2O_2$ [Et ₂ O-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	$\begin{array}{c} C_{15}H_{12}CINO_2\\ [Et_2O] \end{array}$	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	$C_{17}H_{14}CINO_3$ [CH ₂ Cl 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	$\begin{array}{c} C_{17}\tilde{H}_{12}\tilde{C}INO_2\\ [Et_2O-hexane] \end{array}$	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 CH₂Cl₂–MeOH (4:1) as an eluent to give a white solid, which was recrystallized from MeOH–CH₂Cl₂ 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- <i>d</i> ₆ , ppm)
2a	222-223	C ₁₇ H ₁₃ ClKN ₃ O ₄ S	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, J) 7.00 (1H, J, L, R, R)
•	255 256		$ \begin{array}{c} \text{m}, \ 7.90 \ (1\text{H}, 0, J = 8.5) \\ 2.20 \ (2\text{H}, L, 6.2) \ 4.54 \ (2\text{H}, L = 6.2) \ 7.14 \ (1\text{H}, d = L = 2.0) \ 7.20 \ (1\text{H}, d = 1.56) \\ \end{array} $
ze	233-230	$C_{14}H_9CIKN_3O_5S$	J = 8.3 7.52 (1H d $J = 8.3$) 7.92 (1H d $J = 2.0$), 7.50 (1H, u, $J = 2.0$), 7.50 (1H, u, $J = 8.3$) 7.52 (1H d $J = 8.3$) 7.92 (1H d $J = 2.0$) 8.54 (1H s)
ĴÊ	246 247	C H CIKN O S	3 = 0.5, 7.52 (III, $0.5 = 0.5$), 7.52 (III, $0.5 = 2.5$), $0.5 = (III, 3)3 = 0.5$, 7.52 (III, $0.5 = 0.5$), 7.55 (IIII, $0.5 = 0.5$), 7.55 (III, $0.5 = 0.5$), 7.55
<i>2</i> 1	240-247	C14119CIA 30402	I = 8.21 (211, 1, $3 = 0.5$), 4.01 (211, 1, $3 = 0.5$), 7.52 (111, $0, 5 = 0.2$), 7.55 (111,
20	219-221	C14H10ClKN4O4S	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,
-9		-1310	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 ₉ ClKN ₃ O ₄ S	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	$C_{13}H_{13}CIKN_3O_4S$	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, $J = 8.6$)
2ј	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
A 1	001 002		(IH, d, J = 8.6) 0.82 ((II + 1 - 7.5) 1.50 1.05 (4II - m) 2.68 2.04 (1II - m) 2.14 (2II + 1 - 6.2)
2K	201-203	$C_{15}H_{17}CIKN_3U_4S$	0.82 (0H, I, J = 7.3), 1.39 - 1.95 (4H, M), 2.08 - 5.04 (1H, M), 5.14 (2H, I, J = 0.3), 4.22 (2H + 1 - 6.2), 7.27 (1H + 1 + 8.2), 7.46 (1H + 1 - 8.2)
21	227 228	CHCIKNOS	$1.03_{1} = 0.03, 7.27(11, u, J = 0.3), 7.40(11, u, J = 0.3)$ $1.03_{1} = 0.04$ m) $2.01_{-2} = 2.0(11, u, J = 0.3), 7.40(111, u, J = 0.3)$
21	257-256	$C_{13}\Pi_{11}CIRV_{3}O_{4}O$	720 (111, 111, 120, 120, 120, 120, 120, 120,
2m	240-241	C ₁ ,H ₁ ,ClKN ₂ O ₄ S	1.17-2.07 (10H, m), $2.81-3.20$ (1H, m), 3.12 (2H, t, $J = 6.3$), 4.33 (2H, t,
	210 211	01611/0111 (3040	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 ₃ O ₄ S	1.58-1.93 (4H, m), $2.30-2.60$ (4H, m), 3.12 (2H, t, $J = 6.3$), 4.43 (2H, $J = 6.3$),
		10 10 5 1	6.45-6.55 (1H, m), 7.27 (1H, d, $J = 8.3$), 7.48 (1H, d, $J = 8.3$)
20	250-251	C ₁₆ H ₁₅ ClKN ₃ O ₄ S	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	$C_{17}H_{12}CIKN_2O_4S$	3.17 (2H, t, $J = 6.5$), 4.28 (2H, t, $J = 6.5$), 6.66 (1H, s), 7.18 (1H, d, $J = 7.8$),
	AAA AA1		7.35 - 7.81 (6H, m)
4	220-221	$C_{17}H_{13}CIKN_3O_4S$	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, c), 7.78 (1H, d, J = 8.6), 7.49 (2H, s), 7.78 (1H, d, J = 8.6), 7.49 (2H, s), 7.78 (1H, d, J = 8.6), 7.49 (2H, s), 7.78 (1H, d, J = 8.6), 7.49 (2H, s), 7.78 (1H, d, J = 8.6), 7.49 (2H, s), 7.78 (1H, d, J = 8.6), 7.49 (2H, s), 7.78 (2H, s), 7.7
5	248 240	C H CIKN O S	$(J\Pi, 8), 1.70$ (III, $u, J = 0.0$) 3.03 (21) + $J = 6.0$) (21) + $J = 6.0$) 7.46 7.82 (61) m) 8.25 (11) 4
3	240-249	$U_{17}\Pi_{11}UKN_{3}U_{5}S$	J = 8.6
6	200-203	CHCIKN-O-S	2.72 - 3.82 (4H m) $4.02 - 4.42$ (1H m) $7.25 - 7.70$ (4H m) $7.70 - 8.10$ (3H m)
U	200 203	01711201112055	2.72 5.52 (11, m), 1.52 -7.72 (11, m), 1.25 7.76 (11, m), 7.70-0.10 (51, m)

^a Recrystallisation solvent: CH₂Cl₂–MeOH.

^b Compounds 2 were analyzed for C, H and N. Analytical results obtained for these elements were within $\pm 0.4\%$ 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 H₂O, 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 chroma-

tography 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 H₂O (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 Na₂SO₄ 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-6nitrophenyl)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 AlCl₃ (3 g, 22.5 mmol). After the reaction mixture was stirred at 60 °C for 10 min, it was poured into cold H₂O. The solution was acidified with concentrated HCl, and then extracted with CH₂Cl₂. The extract was washed successively with water and brine, dried over Na₂SO₄ 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 Na₂SO₄ and evaporated. Recrystallization from Et₂O 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 CH₂Cl₂ (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 H₂O, 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(2methoxyethoxy)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 Na₂SO₄ and evaporated. The residue was purified by silica gel column chromatography with CH₂Cl₂-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-(ethoxycarboryl)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 Et₂O. The extract was washed successively with water and brine, dried over Na₂SO₄ and evaporated. Recrystallization from Et₂O–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), CH₂Cl₂ (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 CH₂Cl₂. The extract was washed successively with water and brine, dried over Na₂SO₄ 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 H₂O and washed with Et₂O. 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:

 $\Delta UV_{90} = (\text{urine output in 90 min after drug injection}) \\ - [(\text{urine output in 30 min before drug injection}) \times 3]$

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.

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