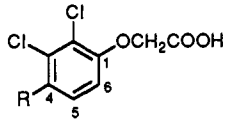
Studies on Uricosuric Diuretics. 4. Three-Dimensional Structure-Activity Relationships and Receptor Mapping of (Aryloxy)acetic Acid Diuretics[1]

Hiroshi Koga,* Haruhiko Sato, Takashi Dan, and Bunya Aoki

Fuji-gotemba Research Laboratories, Chugai Pharmaceutical Company, Ltd., 135, 1-Chome Komakado, Gotemba-shi, Shizuoka, 412, Japan. *Received February 12, 1991*

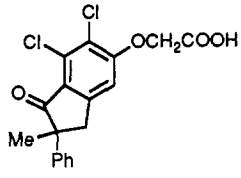
Attempts to develop new (aryloxy)acetic acids with a better profile of diuretic and uricosuric activities as well as with fewer side effects have produced a series of compounds in which the ring system has been varied. Diuretic screening of these analogues in rats indicated that the great difference in the activity between these compounds might be ascribed to a difference in the ring system rather than that in the substituent effect and that the annulation hypothesis described before is not necessarily applicable to all of these compounds. This prompted us to study the relationship between the structure and the diuretic activity of the (aryloxy)acetic acids. An active model (receptor model) was created with the indanone moiety of *R*-(−)-**3** and the dihydrobenzofuran-2-carboxylic acid moiety of *S*-(+)-**4**. The three-dimensional structure-activity study of known compounds **2**–**4**, and **5a** using the active model showed that the degree of fitting to the model is related to the diuretic activity of these compounds. This was also confirmed for compounds **6a**, **6b**, **9a**, **10a**, **11a**, **12a**, **13a**, **14a**, **15a**, and **16a**, and the relation between the structure and the diuretic activity was rationalized qualitatively. With these insights in mind, a modified receptor model was constructed. We believe that this model is useful for a prediction of the activity of compounds not yet synthesized as well as for designing new (aryloxy)acetic acid diuretics.

Since the discovery of ethacrynic acid (**1**), many (aryloxy)acetic acid derivatives have been synthesized and evaluated for their diuretic properties.[2] Further advance in this class of compounds has been made by the development of tienilic acid (**2**),[2] a diuretic with uricosuric activity. Unfortunately, however, this drug has been withdrawn from the market in most countries because of its liver toxicity.



1: R = COC(=CH$_2$)C$_2$H$_5$

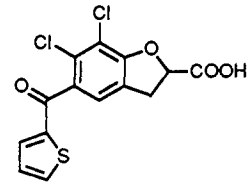
2: R = CO-(2-thienyl)



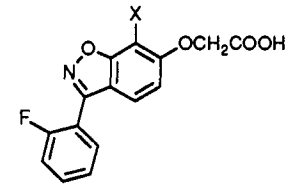
3

Attempts to develop this type of uricosuric diuretics led to the discovery of indacrinone (**3**),[2,3] a tienilic acid analogue **4**,[2,4] and HP-522 (**5**).[2,5] From these discoveries, it has been suggested that annulation of phenoxyacetic acids (as represented by **1** and **2**) leads to a high-ceiling uricosuric.[2] Also interesting is that the salidiuretic and uricosuric activities of compound **4** have been found to reside separately in the (+)- and (−)-enantiomers, respectively.[4]

We have studied how to obtain new (aryloxy)acetic acids with a better profile of diuretic and uricosuric activities as well as with fewer side effects. We have selected tienilic acid (**2**) as a lead. It is highly conceivable that the liver



4



5: X = Br

5a: X = Cl

toxicity of **2** might be due to the metabolic change of the thienyl moiety in the liver[6] and that the formation of metabolites might be reduced by changing the thienyl group to a phenyl group and constructing more hydrophilic ring systems. With these in mind, we designed and synthesized (aryloxy)acetic acids with a variety of ring systems (Scheme I),[7] but the substituents were generally limited to a halogen atom or methyl group from the reported results of structure-activity relationships studies of the (aryloxy)acetic acid diuretics.[2]

Annulation to position 3 or 5 of **2** affords (xanthonyloxy)acetic acids **6**, which are subsequently annulated to give dihydrofuroxanthone-2-carboxylic acids **9** and **10**.[7a] Dihydrofuro-1,2-benzisoxazoles **11** and **12** are derived by annulation to each ortho position of the oxyacetic acid group of HP-522 derivative **7**.[7b] Annulation of phenoxyacetic acids **8** leads to the dihydrobenzofurans **13**. The displacement of the methylene group of **11** and **12** by an oxygen atom affords the more hydrophilic compounds **14** and **15**, respectively.[7c] Dihydrofurobenzoxazoles **16**[7b] are the isomer of **12** and formally derived by Beckmann rearrangement and annulation of the oxime of **2**. The preparation of compounds **11** and **13** has been reported by Plattner et al.,[8] immediately after we completed the synthetic study.

(1) This work was presented at the 15th Symposium on Structure-Activity Relationships; Nov 6–8, 1987, Tokyo, Japan; Abstract of papers; pp 334–337.

(2) Cragoe, E. J., Jr. The (Aryloxy)acetic Acid Family of Diuretics. In *Diuretics. Chemistry, Pharmocology, and Medicine*; Cragoe, E. J., Jr., Ed.; Wiley-Interscience: New York, 1983; pp 201–266.

(3) deSolms, S. J.; Woltersdorf, O. W., Jr.; Cragoe, E. J., Jr.; Watson, L. S.; Fanelli, G. M., Jr. (Acylaryloxy)acetic Acid Diuretics. 2. (2-Alkyl-2-aryl-1-oxo-5-indanyloxy)acetic Acids. *J. Med. Chem.* **1978**, *21*, 437–443.

(4) Hoffman, W. F.; Woltersdorf, O. W., Jr.; Novello, F. C.; Cragoe, E. J., Jr.; Springer, J. P.; Watson, L. S.; Fanelli, G. M., Jr. (Acylaryloxy)acetic Acid Diuretics. 3. 2,3-Dihydro-5-acyl-2-benzofurancarboxylic Acids, a New Class of Uricosuric Diuretics. *J. Med. Chem.* **1981**, *24*, 865–873.

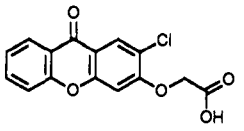
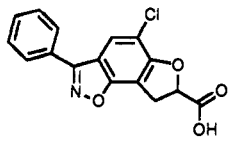
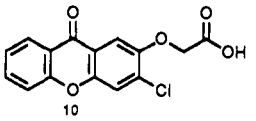
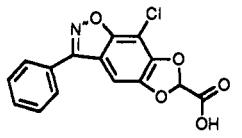
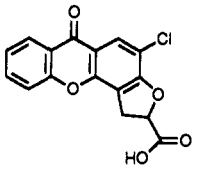
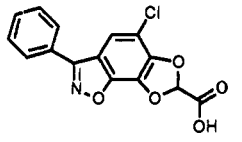
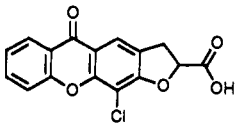
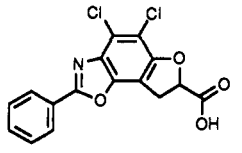
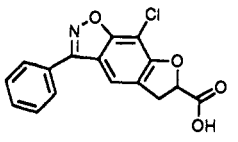
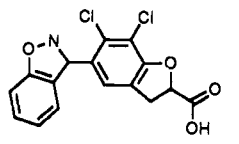
(5) Shutske, G. M.; Setescak, L. L.; Allen, R. C.; Davis, L.; Effland, R. C.; Ranbom, K.; Kitzen, J. M.; Wilker, J. C.; Novick, W. J., Jr. [(3-Aryl-1,2-benzisoxazol-6-yl)oxy]acetic Acids. A New Diuretic Series. *J. Med. Chem.* **1982**, *25*, 36–44.

(6) Nelson, S. D. Metabolic Activation and Drug Toxicity. *J. Med. Chem.* **1982**, *25*, 753–765.

(7) (a) Sato, H.; Dan, T.; Onuma, E.; Tanaka, H.; Koga, H. Studies on Uricosuric Diuretics. I. Syntheses and Activities of Xanthonyloxyacetic Acids and Dihydrofuroxanthone-2-carboxylic Acids. *Chem. Pharm. Bull.* **1990**, *38*, 1266–1277. (b) Sato. H.; Dan, T.; Onuma, E.; Tanaka, H.; Aoki, B.; Koga, H. Studies on Uircosuric Diuretics. II. Substituted 7,8-Dihydrofuro[2,3-*g*]-1,2-benzisoxazole-7-carboxylic Acids and 7,8-Dihydrofuro-[2,3-*g*]benzoxazole-7-carboxylic Acids. *Chem. Pharm. Bull.*, in press. (c) Sato, H.; Dan, T.; Onuma, E.; Tanaka, H.; Aoki, B.; Koga, H. Studies on Uricosuric Diuretics. III. Substituted 1,3-Dioxolo[4,5-*f*]-1,2-benzisoxazole-6-carboxylic Acids and 1,3-Dioxolo[4,5-*g*]-1,2-benzisoxazole-7-carboxylic Acids. *Chem. Pharm. Bull.*, in press.

0022-2623/91/1834-2702$02.50/0

Table I. Rat Diuretic Activity[a] for (Aryloxy)acetic Acids[7]

no.	structure	activity score[b]	no.	structure	activity score[b]
6a		2	12a		±
6b		2	14a		7
9a		4	15a		1
10a		2	16a		7
11a		4	13a		4

[a] Test compounds were administered at 100 mg/kg po to Wistar–Imamichi rats and the relative activity (%) to the control (100%) was calculated. See Experimental Section for details of test protocol. [b] To simplify the data, the diuretic results were scored according to the following criteria. The scores 0, ±, 1, 2, 3, 4, 5, 6, and 7 represent the relative activity of ≤100%, 110–120%, 130–160%, 170–200%, 210–250%, 260–300%, 310–350%, 360–400%, and 410–450% to the control (100%), respectively.

Table II. Rat Diuretic Activity[a] for (Aryloxy)acetic Acid Uricosuric Diuretics[2-5]

compound	activity score[b]	compound	activity score[b]
tienilic acid (2)	1	*S*-(+)-4	6
R-(−)-3	5	(±)-4	5
(±)-3	4	*R*-(−)-4	0
S-(+)-3	2	5a	3

[a] The conditions are the same as for Table I, footnote *a*. [b] The criteria are the same as for Table I, footnote *b*.

The diuretic activity in rats of typical compounds with these diverse ring systems is shown in Table I.[7] From these and related data,[7] it is observed that the great difference in the activity between these compounds might be ascribed to the ring system rather than the substituent effect and that the annulation hypothesis[8] described above is not necessarily the case for all of these compounds. This prompted us to study the three-dimensional structure–activity relationships and receptor mapping for the diuretic activity of the (aryloxy)acetic acids.

Compounds and Biological Activity

The compounds and diuretic activity analyzed are listed in Tables I and II.[2,7,8] The compounds were selected so that their substituent effects are similar to each other because we want to study the effect of the ring system rather than the substituent effect. The compounds were screened at 100 mg/kg po in rats where urine volumes were measured in comparison to controls. The activities were represented as relative activity (%) to the control (100%). For convenience, the compounds were scored according to the criterion of the footnote in Table I.

Molecular Modeling

A systematic search method was used for the analysis of conformational aspects of these compounds.[9] The three-dimensional structure of the (aryloxy)acetic acid was constructed from the X-ray data of tienilic acid (2).[10] The structure of the substituents of each compound was taken from the fragment library of a SYBYL system.[11] Original models of each compound were built with standard bond length and angles. The energy of each compound was then minimized with molecular mechanics with a Tripos force field. These structures were used as original coordinates for further energy optimization using quantum mechanics. Conformations were preliminarily examined by the SEARCH routine in the SYBYL system. Conditions for preliminary search were defined so that all rotatable bonds were rotated by an increment of 5°. Conformations were then analyzed by using the molecular orbital (MO) method (AM1).[12] In the MO analysis, all rotatable bonds were

(8) (a) Plattner, J. J.; Fung, A. K. L.; Parks, J. A.; Pariza, R. J.; Crowley, S. R.; Pernet, A. G.; Bunnell, P. R.; Dodge, P. W. Substituted 5,6-Dihydrofuro[3,2-*f*]-1,2-benzisoxazole-6-carboxylic Acids: High-Ceiling Diuretics with Uricosuric Activity. *J. Med. Chem.* **1984**, *27*, 1016–1026. (b) Plattner, J. J.; Fung, A. K. Benzisoxazole Carboxylic Acids. U. S. Patent 4 456 612, 1984.

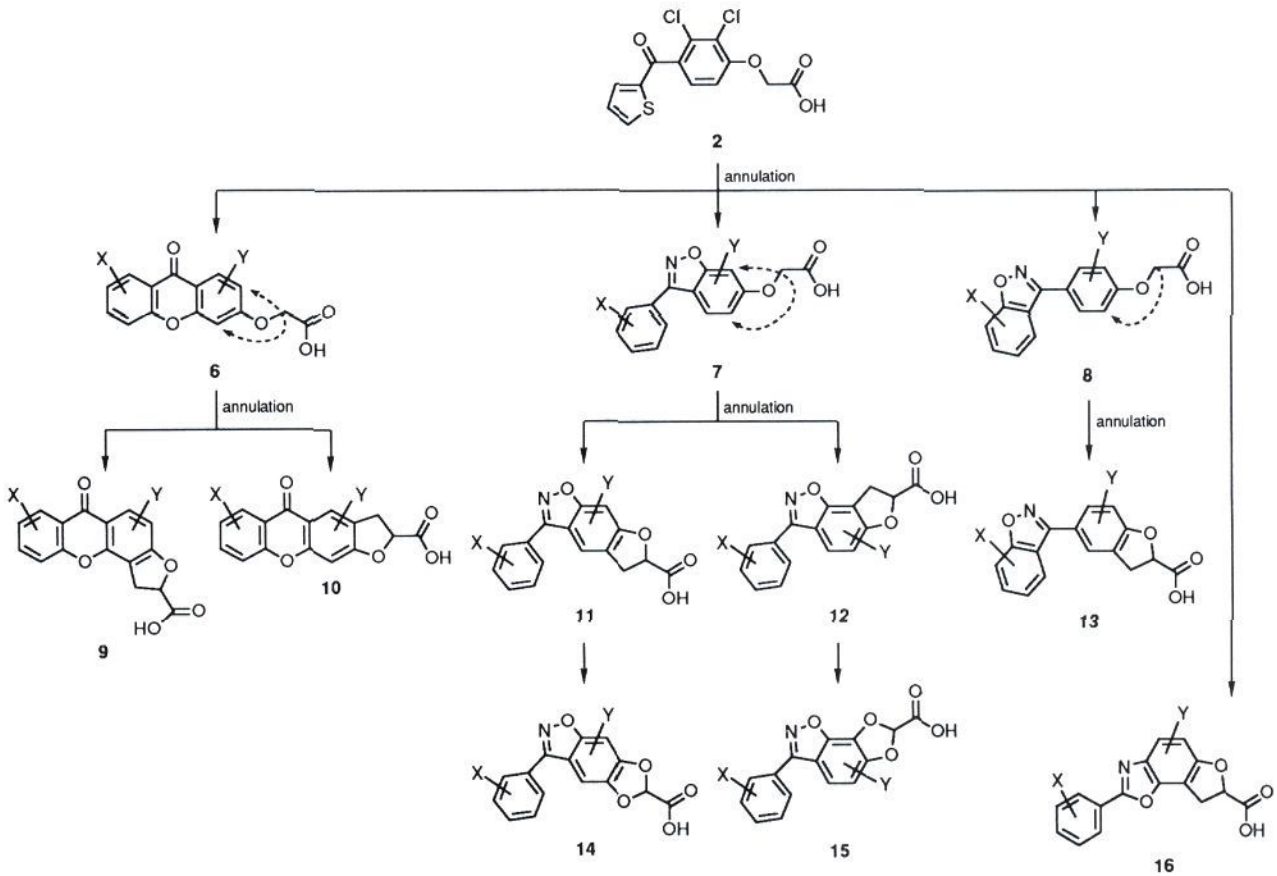
(9) Ohta, M.; Koga, H. Three-Dimensional Structure–Activity Relationships and Receptor Mapping of N_1-Substituents of Quinolone Antibacterials. *J. Med. Chem.*, **1991**, *34*, 131–139.

(10) Carpy, P. A.; Goursolle, M.; Leger, J-M. *Acta Crystallogr.* **1980**, *B36*, 1706–1708.

(11) SYBYL Molecular Modeling System; Tripos Associates: St. Louis, MO.

(12) Dewer, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.

Scheme I



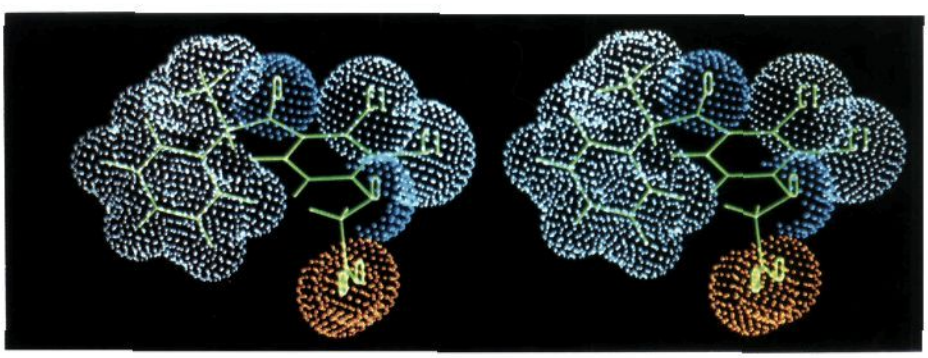


Figure 1. Stereoview of the active model (receptor model) for diuretic activity of (aryloxy)acetic acids.

rotated by a 15° increment. After the determination of the minimum-energy conformation, the conformation was optimized by using the AM1 program.

The total occupied volume of the compounds was calculated by superposing the van der Waals volumes using the MVOLUME routine in the SYBYL system.

Results and Discussion

Cragoe et al. reported that indacrinone (3) has two enantiomers in which the diuretic activity of the *R* isomer is higher than that of the *S* isomer.[2] They also revealed that all the diuretic activity of compound **4** resides in the *S* isomer, while the *R* isomer is inactive.[2] These were also confirmed in our studies (Table II). The fact that the *S* isomer is more active than the *R* isomer is also reported for the dihydrobenzofuran-2-carboxylic acid derivatives A56234 (**11**: X = 2-F, Y = 8-Cl)[8a] and S-8666 (5-(dimethylsulfamoyl)-6,7-dichloro-2,3-dihydrobenzofuran-2-carboxylic acid).[13]

From the data in Table II it is shown that the annulation of tienilic acid (**2**) leads to the more active compounds **3**, **4**, and **5a** and that the steric effect of these annulated compounds is critical in explaining the diuretic activity. From these observations, it is proposed that the indanone moiety of *R*-(−)-**3** and the dihydrobenzofuran-2-carboxylic acid moiety of *S*-(+)-**4** successfully mimic the receptor-bound conformations of the acyl and oxyacetate side chains, respectively, of **2** and the analogues. Indeed, it is because they are so active that they can assume the active conformations. Accordingly, the active model (receptor model) was created with these moieties (Figure 1). In this model, the carboxylate group (anionic site) is thought to interact with the cationic site of the receptor. The carbonyl

(13) Harada, H.; Yonetani, Y. 5-Sulfamoyl-2,3-dihydrobenzofuran-2-carboxylic Acid Derivatives: S-8666, a Favorable Candidate with Uricosuric and Diuretic Activities. *Drugs Future* **1988**, *13*, 257–264.

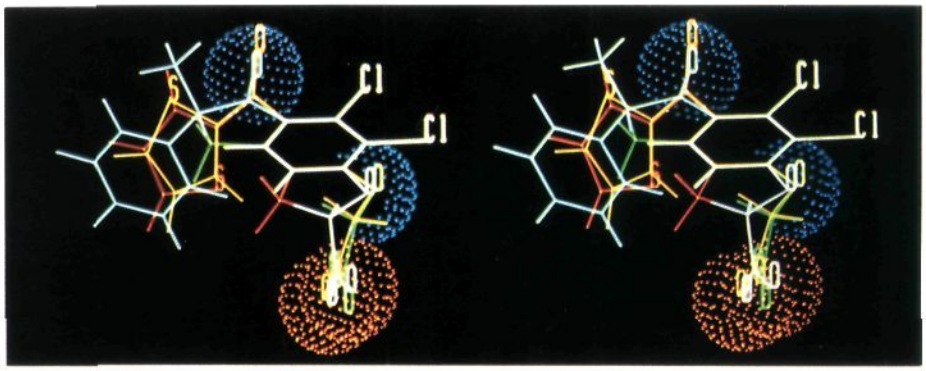


Figure 2. Stereoview of the superposition of the proposed active conformers of **2** (yellow). *R*-(−)-**3** (green), and *S*-(+)-**4** (red) and the active model (white).

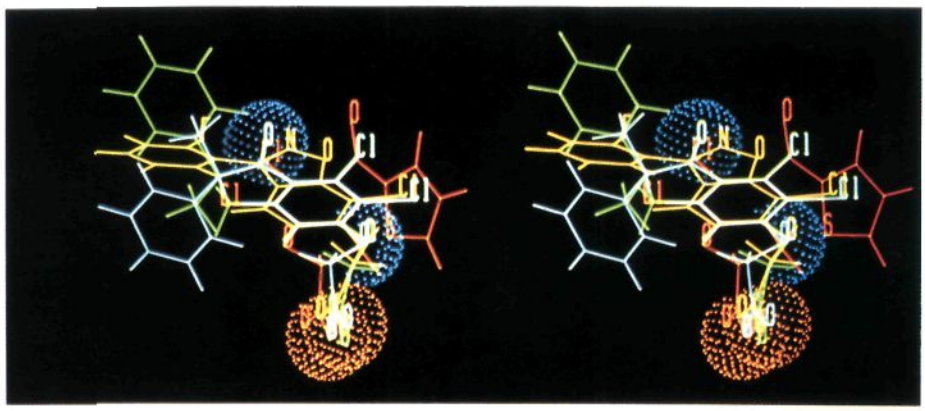


Figure 3. Stereoview of the superposition of the proposed active conformers of *S*-(+)-**3** (green), *R*-(−)-**4** (red), and **5a** (yellow) and the active model (white).

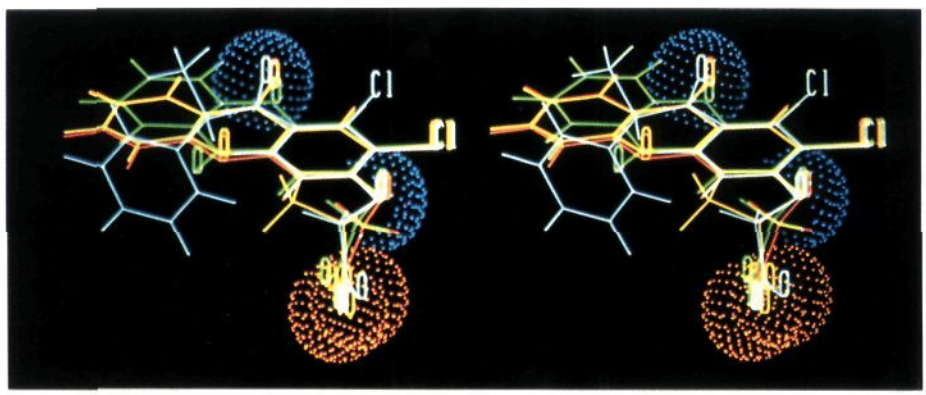


Figure 4. Stereoview of the superposition of the proposed active conformers of **6a** (red), **6b** (green), and **9a** (yellow) and the active model (white).

oxygen seems to function as a hydrogen-bond acceptor and is located so that it is coplanar with the dichlorobenzene ring and in the same side as the chlorine atoms. This configuration seems to be important to form the hydrogen bond with the receptor favorably. The ether oxygen moiety seems to work as a hydrogen-bond acceptor and/or as a bridge which is limited in size.[2] Other substituents (two chlorine atoms and methyl and phenyl groups) are estimated to serve as hydrophobic groups which are limited in size.[2] The bridging methylene of indanone and dihydrofuran moieties is removed in this model because it seems to be necessary only to fix the conformations of the moieties favorably. This model may be used for verification of the diuretic activity of (aryloxy)acetic acids and further for prediction of the activity of compounds yet to be synthesized. If a compound fits this model well, it is expected to have high activity.

The active conformation of compounds was estimated by using this active model. Thus it was defined so that among the lowest energy conformers, the conformer which resembles the active model was selected (Figures 2–7). The torsion angles of the active conformer are shown in Table III. As can be seen in Figure 2, it is difficult to make the carbonyl and oxyacetate moieties of tienilic acid (**2**) take the same conformations as those of the active model because of the intramolecular steric repulsion. This is also the case for such compounds with a similar side chain as **3**, **4**, **5a**, **6a**, **6b**, and **13a**.

The three-dimensional structure–activity study of known compounds **2**, **3**, **4**, and **5a** was made by using the active model. The active conformations of **2** and enantiomers of **3** and **4** were superposed on the active model by matching

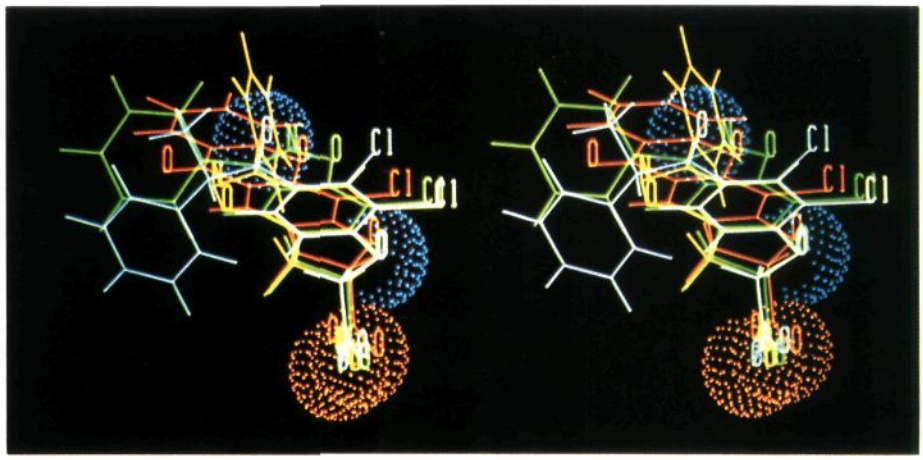


Figure 5. Stereoview of the superposition of the proposed active conformers of **10a** (red), **11a** (green), and **12a** (yellow) and the active model (white).

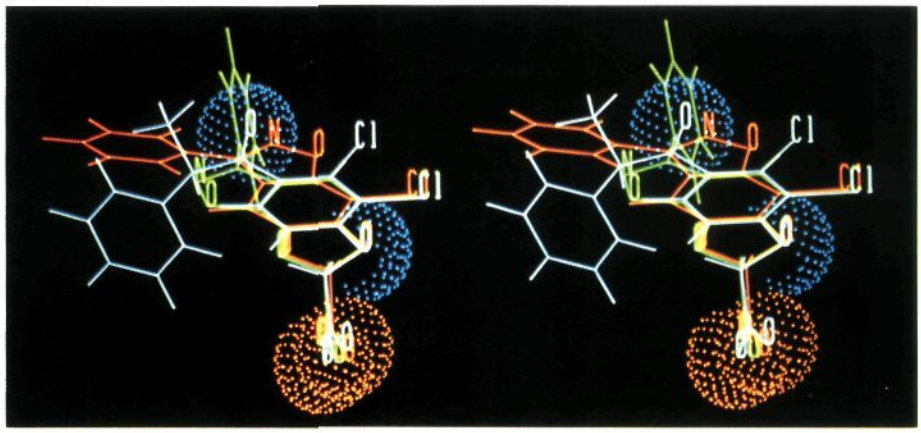


Figure 6. Stereoview of the superposition of the proposed active conformers of **14a** (red) and **15a** (green) and the active model (white).

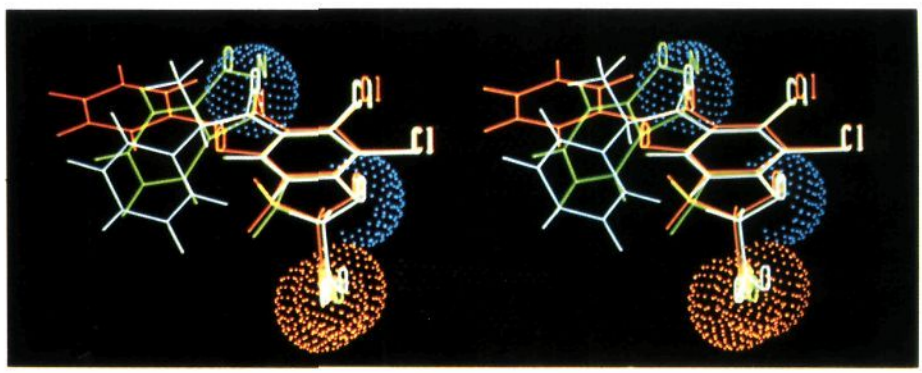
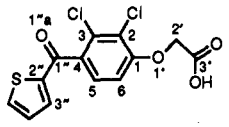
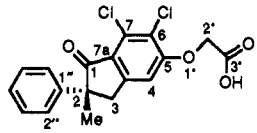
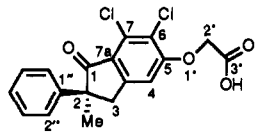
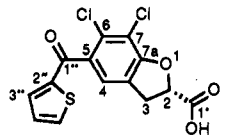
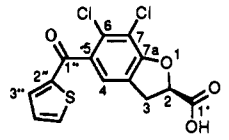
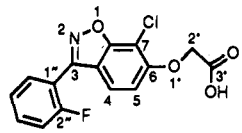
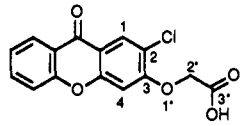
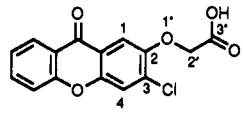
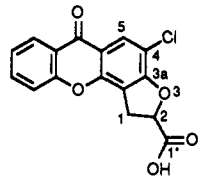
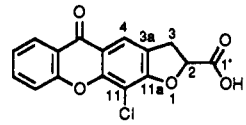
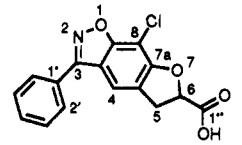
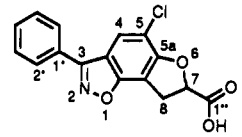
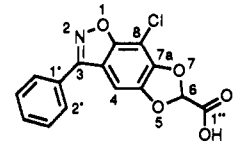
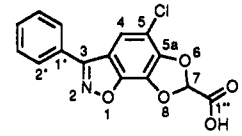
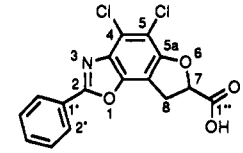
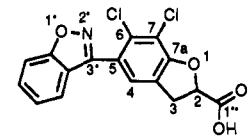


Figure 7. Stereoview of the superposition of the proposed active conformers of **16a** (red) and **13a** (green) and the active model (white).

the corresponding atoms in the benzene ring so that the carboxylate group occupies the same side of the benzene plane as that of the active model (Figures 2 and 3). Compound **5a** was superposed on the model by matching the nitrogen atom of the isoxazole ring with the carbonyl oxygen of the model, in addition to the same procedure as described above (Figure 3). Compounds *R*-(−)-**3** and *S*-(+)-**4** are very potent and have almost the same level of activity (Table II), suggesting that the indanone moiety with the *R* configuration and the dihydrofuran-2-carboxylic acid moiety with the *S* configuration contribute to the great increase of the activity to almost the same extent. The enantiomer *S*-(+)-**3** has the extra region occupied by the more bulky phenyl group in the region of the methyl group of the active model. This was thought to cause a reduction of activity for *S*-(+)-**3** compared to that of *R*(−)-**3** (Table II).[2] On the other hand, tienilic acid (**2**) does not fit the active model well and occupies a different region in space than does the model. This is thought to bring about a detrimental effect on the activity and to make the relevant binding with the receptor unfavorable. Enantiomer *R*-(−)-**4** does not have the corresponding regions of hydrogen bonding and hydrophobicity of the receptor model, and the thienyl group penetrates deeply into the region where the chlorine atoms of the model just interact with the receptor. Therefore, it is expected that it is inactive. In Figure 3 it is shown that HP-522 derivative **5a** has a nitrogen that can accept a hydrogen bond in the region of hydrogen bonding of the active model, but it does not possess a hydrophobic group in the region occupied

Table III. Calculated Dihedral Angles of (Aryloxy)acetic Acids

no.	structure	definition of dihedral angle	dihedral angle, deg
2		2-1-1′-2′	−175
		1-1′-2′-3′	75
		3-4-1″-2″	123
		3-4-1″-1″a	−59
		4-1″-2″-3″	−14
R-(−)-**3**		6-5-1′-2′	−172
		5-1′-2′-3′	78
		7-7a-1-2	−179
		7a-1-2-1″	−119
		1-2-1″-2″	−128
S-(+)-**3**		6-5-1′-2′	−172
		5-1′-2′-3′	78
		7-7a-1-2	179
		7a-1-2-1″	119
		1-2-1″-2″	−52
S-(+)-**4**		7-7a-1-2	−175
		7a-1-2-1′	115
		4-5-1″-2″	−55
		5-1″-2″-3″	161
R-(−)-**4**		7-7a-1-2	175
		7a-1-2-1′	−115
		4-5-1″-2″	55
		5-1″-2″-3″	−161
5a		7-6-1′-2′	178
		6-1′-2′-3′	76
		2-3-1″-2″	−136
6a		2-3-1′-2′	−178
		3-1′-2′-3′	74
6b		1-2-1′-2′	4
		2-1′-2′-3′	71
9a		4-3a-3-2	−170
		3a-3-2-1′	107
10a		3a-11a-1-2	8
		11a-1-2-1′	110
11a		2-3-1′-2′	104
		8-7a-7-6	−174
		7a-7-6-1″	112
12a		2-3-1′-2′	71
		5-5a-6-7	−174
		5a-6-7-1″	112
14a		2-3-1′-2′	−137
		8-7a-7-6	−172
		7a-7-6-1″	108
15a		2-3-1′-2′	76
		5-5a-6-7	−172
		5a-6-7-1″	108
16a		1-2-1′-2′	2
		5-5a-6-7	−175
		5a-6-7-1″	114
13a		4-5-3′-2′	142
		7-7a-1-2	−175
		7a-1-2-1″	116

by the chlorine atom at position 3 of the model. Thus it was possible to rationalize the relation between the structure and the activity of the known compounds **2**, **3**, **4**, and **5a** by using this active model qualitatively.

We then attempted to relate the diuretic activity of compounds in Table I to the three-dimensional structure using the receptor model. The (aryloxy)acetic acids were superposed on the model so that the carbon atom of the carboxylate group and then the hydrogen-bonding and hydrophobic regions matched with those of the model, without the aryl ring plane deviating from the dichlorobenzene ring plane of the model (Figures 4–7).

Xanthones **6a** and **6b** possessed the same activity (Table I). The carbonyl oxygen of xanthone **6a** fits the carbonyl oxygen region of the active model well, whereas in the case of xanthone **6b**, the oxygen at position 10 appears to work as a hydrogen-bond acceptor (Figure 4). Figure 4 shows both xanthones **6a** and **6b** are predicted to possess activity comparable to that of compound **5a**. Dihydrofuroxanthone **9a** (*S* isomer) fits the model better than xanthones **6a** and **6b**. Consequently, the activity of **9a** is expected to be superior to these xanthones **6a** and **6b** (Figure 4 and Table I). In contrast, isomer **10a** (*S* isomer) does not appreciably fit the model, as shown in Figure 5, and the activity as the racemate is estimated to be less than that of **9a**.

In the series of the benzisoxazoles, compound **11a** (*S* isomer) fits the model well, whereas the isoxazole moiety of compound **12a** does not occupy the favorable regions for increasing the activity and the phenyl group stands out from the receptor model. It seems that the difference in the activity between **11a** and **12a** shown in Table I is ascribable to the degree of the fitting. Similarly, the difference in activity between compounds **14a** and **15a** seems to be attributable to this same reason as described above (Figure 6). The dioxole derivatives **14a** and **15a** displayed diuretic activity of a level higher than that of the corresponding dihydrofuran derivatives **11a** and **12a**, respectively. As shown in Figures 5 and 6, compounds **14a** and **15a** have an oxygen atom in the region of the methylene group of compounds **11a** and **12a**. This difference

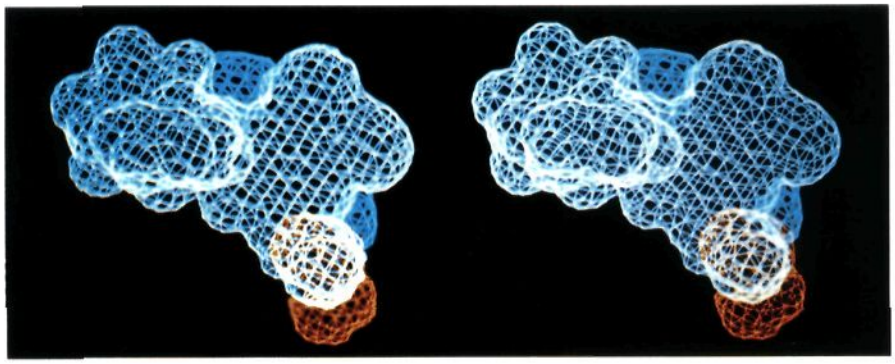


Figure 8. Stereoview of the modified receptor model for diuretic activity of (aryloxy)acetic acids. The red region represents the proposed anionic site; the blue, the hydrogen bonding sites and/or areas that are limited in size; and the white, hydrophobic sites that are limited in size.

seems to make the binding of **14a** and **15a** to the receptor or the transport into the active site more favorable when compared to that of **11a** and **12a**.

Benzoxazole derivative **16a** showed a high order of potency and the *S* isomer exhibited a high degree of fitting to the active model as illustrated in Figure 7. Finally, in the case of compound **13a**, the nitrogen of the benzisoxazole moiety appears to have the same function as does the carbonyl oxygen of the model. As depicted in Figure 7, the region of the nitrogen does not match that of the carbonyl oxygen of the model well. Therefore, the level of activity of **13a** is predicted to be similar to that of compound **4**.

These analyses bring new and important insights into three-dimensional structure–activity relationships for (aryloxy)acetic acid diuretics. We propose some structural requirements for increasing the diuretic activity. These include the regions occupied by the carboxylate group below the plane of the chlorobenzene ring, the (two) hydrogen-bonding group(s) in the same plane as that of the chlorobenzene ring, and the hydrophobic groups which are limited in size. From these analyses, it has also become apparent that the annulation hypothesis of (aryloxy)acetic acids can be applied only when the annulated compound satisfies the structural requirements for the activity.

With these insights in mind, a modified receptor model was constructed as shown in Figure 8. The model was created with the total volume occupied by the active model shown in Figure 1, the dioxole moiety in **14a**, and the 2-phenyloxazole moiety in **16a**. It should be corrected or refined whenever new information about the receptor of the (aryloxy)acetic acid diuretics or structural requirements for the activity is acquired. This model can be used for verification of diuretic activity of (aryloxy)acetic acids. Furthermore, it may permit one to design compounds with the desired degree of diuretic activity.

It has been acknowledged from the study of drug action that transport, as well as interaction with the receptor, play an important role in controlling biological response. The dominant factor governing all aspects of drug transport is hydrophobicity, modeled by log *P* or related parameters.[14] Since in our analysis compounds have a common phenoxyacetic acid structure and similar hydrophobicity (the maximum difference in the calculatd log *P* value between these compounds is approximately 1.5), it is assumed that the pharmacokinetic behaviors resemble each other. This seems to be one of the reasons for which the activity was able to be successfully rationalized only with the drug–receptor interaction model despite the use of the in vivo data.

Recently, Plattner et al.[15] reported the receptor mapping of [4-[3-(aminomethyl)-4-hydroxybenzoyl]-2,3-dichlorophenoxy]acetate diuretics which structurally contain the essential pharmacophoric elements of two separate classes of diuretics: the (dichlorophenoxy)acetic acids[2] and the 2-(aminomethyl)phenols.[16] This model appears to be somewhat different in the spatial requirements of the (4-acyl-2,3-dichlorophenoxy)acetate moiety for the activity from ours.

Experimental Section

The molecular models were built with the program SYBYL and a SYBYL standard fragment library.[11] The original coordinates of the models were taken from crystallographic data of tienilic acid.[10] The calculations of molecular mechanics and AM1[12] were made on a VAX. The conformations were examined on an Evans & Sutherland PS 330 computer terminal by using the program SYBYL. The figures were photographed directly from the screen with Kodak Ektachrome 100 HC film.

Diuretic Activity in Rats.[7] Seven-week-old Wistar–Imamichi rats that had been fasted for 24 h were divided into groups of five so that the animals in each group would excrete almost the same amount of urine. After forced urination, the rats were orally administered the test compounds that were suspended in physiological saline containing 3% gum arabic in a dose volume of 25 mL per kg of body weight. The compounds were administered in amounts of 100 mg/kg. The control rats were given only physiological saline containing 3% gum arabic. The animals were housed in separate metabolic cages, and the urine excreted from each animal was collected over a period of 6 h following the administration of the test compounds or physiological saline after complete starvation. The urine volume was directly read on a measuring cylinder after forced urination thereinto, and the amount of urine per kg of body weight was calculated.

Acknowledgment. We are grateful to Dr. S. Hata, Dr. I. Matsunaga, and Dr. T. Mori for their support and encouragement during the course of this investigation.

(14) Dearden, J. C. Molecular Structure and Drug Transport. In *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: Oxford, 1990; Vol. 4, pp 375–411 and references therein.

(15) Plattner, J. J.; Martin, Y. C.; Smital, J. R.; Lee, C-M.; Fung, A. K. L.; Horrom, B. W.; Crowley, S. R.; Pernet, A. G.; Bunnell, P. R.; Kim, K. H. [(Aminomethyl)aryloxy]acetic Acid Esters. A New Class of High-Ceiling Diuretics. 3. Variation in the Bridge between the Aromatic Rings to Complete Mapping of the Receptor. *J. Med. Chem.* **1985**, *28*, 79–93.

(16) Smith, R. L. 2-Aminomethylphenols: A New Class of Saluretic Agents. In *Diuretics. Chemistry, Pharmacology, and Medicine*; Cragoe, E. J., Jr., Ed.; Wiley-Interscience: New York, 1983; pp 267–302.