Structure–Activity Relationships of 3-Substituted Dihydrobenzothiadiazine Diuretics[1]

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The effect of change in the 3-substituent of 3R-6-chloro-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine 1,1-dioxides on natruretic activity in the rat and the dog was investigated. R represented diverse structural types. A wide range of activities was observed. Several compounds were found to be considerably more potent than hydrochlorothiazide. In particular, alkyl, haloalkyl and aralkyl groups were associated with a high level of activity. In general it was found that those substituents which had a favorable effect on activity were hydrophobic in character. When these were modified to groups of a more hydrophilic nature, natruretic activity was reduced. Substituents which lacked a hydrogen on the α-carbon atom resulted in compounds of a low order of activity, even in those classes where activity was otherwise good.

Following the observation on the marked diuretic potency of 6-chloro-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine 1,1-dioxide (hydrochlorothiazide, III),[2] several reports have been published on the synthesis of related compounds and their diuretic properties.[2-13]

(1) Portions of this work were presented at the 136th meeting of the American Chemical Society, Atlantic City, N. J., 1959, and at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Miami, Florida, September, 1959.

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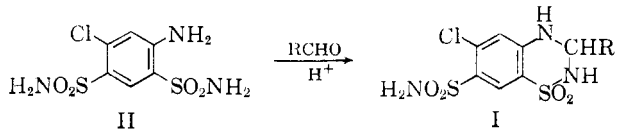
(11) F. C. Novello, S. C. Bell, E. L. A. Abrams, C. Ziegler, and J. M. Sprague, *ibid.*, **25**, 970 (1960).

(12) C. Pelayo, J. Iriarte, and H. J. Ringold, *ibid.*, **25**, 1067 (1960).

(13) B. Issekutz Sen., N. Jobbágyi, E. Osvald, and M. Székely, *Arch. Inter. Pharm. Thérap.*, **132**, 251 (1961).

Although a large body of information has been made available, little has been published in the realm of detailed studies of structure-activity relationships apart from the work of Lund and Kobinger.[14] Their studies covered a broad range of compounds including a series of substituted 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides (hydrochlorothiazide and analogs). In this paper we have considered the effect of changes in the group R in 3R-6-chloro-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine 1,1-dioxides (I) on natruretic activity in rats and dogs.

Synthesis.—3R-6-Chloro-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine 1,1-dioxides (I) were synthesized, with few exceptions, by the condensation of 5-chloro-2,4-disulfamylaniline (II) with an aldehyde, RCHO, or the corresponding acetal in a suitable solvent, usually in the presence of an acid catalyst. Full details of this work have been reported.[15]



II I

Pharmacological Methods

A. Bioassay in the Dog (Intravenous Route).—Healthy mongrel dogs of either sex were anesthetized with 50 mg./kg. of vinbarbital administered intravenously. Mammalian Ringer's solution was infused into the cephalic vein at a rate of 0.3 ml./min. to replace urine excreted and to facilitate administration of drugs. No additional fluid was given before or during the experiment. Both ureters were exposed and cannulated with polyethylene tubing. Urine flow was recorded on smoked paper by means of a Thorp impulse counter and a drop recording unit. Total urine samples were collected every 20 min. for measurement of both volume and sodium concentration. When a uniform rate of urine excretion was achieved, solutions of the compounds were administered intravenously through the infusion tube. Compounds were first dissolved in 0.5 ml. of 1.0 *N* NaOH and the pH of the solution adjusted to 7.5 to 8.0 by back titration with 1.0 *N* HCl.

The test for each dog was divided into four 20 min. intervals with the standard (hydrochlorothiazide) and test drug administered alternately. Drug response was defined as the difference in urine output (ml./20 min.) between the control period preceding treatment and the drug period. Relative natruretic activity of

(14) F. J. Lund and W. Kobinger, *Acta Pharm. Tox., Kbh.*, **16**, 297 (1960).

(15) J. G. Topliss, M. H. Sherlock, F. H. Clarke, M. C. Daly, B. W. Pettersen, J. Lipski, and N. Sperber, *J. Org. Chem.*, **26**, 3842 (1961).

the test drug in terms of the standard was calculated by a "constant standard" method described by Finney.[16] The results of this assay were used to provide estimates of dose levels used in the oral tests.

B. Bioassay in the Dog (Oral Route).—Healthy male mongrel dogs weighing 8 to 15 kg. were maintained on a diet of canned dog food and a bulk-forming agent. The dogs were offered the food once daily except on the treatment day when food and water were withheld until the experiment was completed. On treatment days bladders were emptied by catheterization and, after oral administration of drug in capsule form, the animals were placed in individual metabolism cages. After 5 hr. the dogs were again catheterized and total urine volume measured. Relative activity was determined in crossover experiments as described by Finney.[17]

C. Bioassay in the Rat (Oral Route).—Male Charles River rats (175 to 225 g.) were used in a modified Lipschitz procedure.[18] In the afternoon of the day preceding treatment (approximately 17 hr. prior to drug), 25 ml./kg. of water was administered by intubation to 60 rats which were then deprived of food and additional water. On the treatment day the animals were subdivided into 6 groups of 10 each. Three groups received hydrochlorothiazide *per os* at doses of 0.1, 0.4, and 1.6 mg./kg. in 25 ml./kg. of 0.9% saline and the remaining groups received the test drug at dose levels designed to produce similar natruretic effects. Following treatment, the animals were placed in metabolism cages for a 4 hr. urine collection period. Sodium concentrations were determined in two combined urine samples from each dosage group. Potency estimates were determined using six point assay methods described by Finney.[19]

Discussion

The chemical structure and relative natruretic activity of the compounds are presented in Table I. A discussion of these results is presented in the following paragraphs, arranged by substituent classes.

Alkyl. (a) Unbranched Chain.—The introduction of unbranched chain alkyl groups containing two to six carbons (V through IX) resulted in compounds which were more potent than hydrochlorothiazide (III). Natruretic activity increased with increasing chain length up to the *n*-butyl analog (VII) after which there was a gradual decline. These relationships were consistent in both the rat (oral route) and the dog (oral and intravenous route). Lund and Kobinger[14] also found a high degree of activity with the *n*-propyl and *n*-butyl derivatives.

(16) D. J. Finney, "Statistical Methods in Biological Assay," Hafner Publishing Co., New York, N. Y., 1952, p. 293.

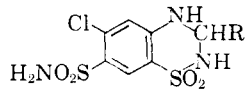
(17) D. J. Finney, *ibid.*, p. 267.

(18) W. L. Lipschitz, Z. Hadidian, and A. Kerpczar, *J. Pharmacol. Exptl. Therap.*, **79**, 97 (1943).

(19) D. J. Finney, "Statistical Methods in Biological Assay," Hafner Publishing Co., New York, N. Y. 1952, p. 139.

Table I

Comparative Activity of 3-Substituted Dihydrobenzothiadiazines



Compound	R	Relative natruretic activity[a] Intravenous Dog	Oral Rat	Oral Dog
III	H	1.0	1.0	1.0
	Alkyl—Unbranched Chain			
IV	CH_3	0.8	0.4	0.5
V	C_2H_5	2.7	2.0	1.5
VI	$(CH_2)_2CH_3$	2.7	4.6[b]	3.6
VII	$(CH_2)_3CH_3$	7.1	7.8[b]	4.5
VIII	$(CH_2)_4CH_3$	4.7	4.5[b]	
IX	$(CH_2)_5CH_3$	3.6	2.0[b]	
	Alkyl—Branched Chain			
X	$CH(CH_3)_2$	2.2	1.7	
XI	$CH_2CH(CH_3)_2$		6.0[b]	
XII	$C(CH_3)_3$		0.4[b]	
	Monohaloalkyl			
XIII	CH_2Cl	1.9	1.8	2.5
XIV	CH_2Br	0.7	0.5	1.4
XV	CH_2I	1.4	0.9	0.9
XVI	$CH(Br)CH_3$	0.6	0.8	0.5
XVII	$CH(Br)C_2H_5$	0.4	1.1	
XVIII	$C(Br)(CH_3)C_2H_5$	0.1	0.1[b]	
	Dihaloalkyl			
XIX	CHF_2	1.2	0.5	
XX	$CHCi_2$	6.3	4.5[b]	13.6
XXI	$CHBr_2$	6.7	4.1[b]	
XXII	CH(Cl)Br	2.9	3.8[b]	
XXIII	$C(Cl)_2CH_3$	0.2		
	Trihaloalkyl			
XXIV	$C(Cl)_3$	0.6	1.0	0.4
	Oxygenated Alkyl			
XXV	$(CH_2)_2OH$	1.6		
XXVI	$\ulcorner\!\!-O-\!\!\urcorner$ CH—CH_2	1.0		
XXVII	$C(CH_3)_2CH_2OH$	0.1		
XXVIII	$CH_2OC_2H_5$	1.6	1.1	

TABLE I (*Continued*)

Compound	R	Relative natruretic activity[a] Intravenous Dog	Oral Rat	Oral Dog
XXIX	$CH_2OC_6H_5$	3.3	3.6[a]	
XXX	$CH(OC_2H_5)C_6H_5$	1.9		
XXXI	CH_2COCH_3	1.8	0.5	
XXXII	$CH_2COOC_2H_5$	1.5	0.2[b]	
XXXIII	$C_{10}H_{15}O_2$[c]	1.6		
	Thioalkyl			
XXXIV	CH_2SCH_3	3.5	2.7	
XXXV	$CH_2SC_6H_5$	1.2	5.0[b]	
XXXVI	$CH_2SCH_2C_6H_5$	2.4	3.0[b]	
XXXVII	$CH_2SO_2CH_3$	3.2	0.2[b]	
	Aminoalkyl			
XXXVIII	CH_2NH_2	0.2		
XXXIX	$CH_2NC_5H_{10}$	1.4	0.5	
	Aralkyl			
XL	$CH_2C_6H_5$	6.7	6.7[b]	2.4
XLI	$(CH_2)_2C_6H_5$		8.5[b]	11.3
XLII	$(CH_2)_3C_6H_5$		7.1[b]	
XLIII	$CH(CH_3)C_6H_5$	1.0	0.9	
XLIV	$CH_2C_6H_4$-*p*-Me	4.0	1.1	0.4
XLV	$CH_2C_6H_4$-*p*-*i*-C_3H_7	2.4	4.1[b]	4.4
XLVI	$CH_2C_6H_2$-2,4,6(Me)$_3$		0.4	
XLVII	$CH_2C_6H_4$-*p*-Cl	1.0	2.3[b]	
XLVIII	$CH_2C_6H_4$-*p*-OMe	4.2	4.3[b]	0.7
XLIX	$CH_2C_6H_3$-3,4(OMe)$_2$	5.2	<0.1[b]	
	Pyridylalkyl			
L	CH_2-3-C_5H_4N	9.4		
LI	$(CH_2)_2$-3-C_5H_4N	11.5	0.6	
LII	$(CH_2)_2$-2-C_5H_4N	16.7	0.9	
	Aryl			
LIII	C_6H_5	0.5	0.1[b]	
LIV	C_6H_3-2,4(Cl)$_2$	2.1	<0.1[b]	
LV	C_6H_4-*o*-Cl	0.7		
LVI	C_6H_4-*p*-CO_2H	0.2		
LVII	C_6H_4-*p*-CO_2Et	0.8		
LVIII	C_6H_4-*o*-OCH_3		<0.1[b]	
LIX	C_6H_3-2,4(OCH$_3$)$_2$	0.1		
LX	C_6H_2-3,4,5(OCH$_3$)$_3$		<0.1[b]	

TABLE I (*Continued*)

Compound	R	Relative natruretic activity[a] Intravenous Dog	Oral Rat	Oral Dog
	Heterocycles			
LXI	2-thienyl	0.4		
LXII	2-furyl	0.6		
LXIII	2-furyl-5-NO_2	0.3	0.2[b]	

[a] Mg. potency based on the activity of hydrochlorothiazide (III). [b] Significant difference between standard and test drug at p. 0.05 (listed for rat data only). [c] 1-(2,5-Endomethylene-3-ethoxycarbonylcyclohexyl).

Alkyl. (b) Branched Chain.—In the small series of branched chain alkyl derivatives examined (X, XI, and XII), chain length again appeared to be critical for good activity. In the rat the isobutyl analog (XI) was more potent than the isopropyl (X) and showed the same order of activity as the *n*-butyl derivative (VII). Additional branching on the α-carbon resulted in diminished activity. Thus, if the α-hydrogen in X was replaced by methyl, as in XII, natruretic activity was markedly reduced.

Haloalkyl.—The monochloromethyl analog (XIII) was slightly more active than the standard but the other monohalomethyl compounds (XIV and XV) were approximately equal in activity to hydrochlorothiazide. Longer alkyl chains with monohalo substitution on the α-carbon definitely depressed activity (XVI and XVII). These agents were also considerably less active than their alkyl counterparts (V and VI). When the α-hydrogen was replaced by a methyl in the monohalogen series as in XVIII, activity was further reduced.

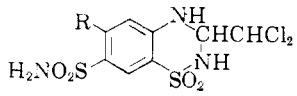
In contrast to the monohalo analogs, the introduction of dihalomethyl groups in the 3-position (XX, XXI, and XXII) resulted in compounds possessing a high order of activity. An exception to this finding was the difluoromethyl derivative (XIX) which was not significantly different from the standard. When the α-hydrogen in XX was replaced by a methyl (XXIII) or chloro group (XXIV), the resulting compounds were less active.

The dichloromethyl derivative (XX) was found to be 4 to 14 times more potent than hydrochlorothiazide depending on species and route of administration. The biological activity of this compound

has been reported in detail elsewhere.[20-23] In view of its pronounced activity, certain structural modifications of this agent were studied further. Table II shows the effect of change of substituent in the 6-position as determined orally in the rat. In this series, the chloro and bromo analogs (XX and LXIV) were equipotent whereas the 6-trifluoromethyl derivative (LXVI) was somewhat weaker. The other member of the series, the 6-fluoro (LXV), had only one-tenth the activity of the chloro compound.

TABLE II

COMPARATIVE ACTIVITY OF 6-SUBSTITUTED 3-DICHLOROMETHYL DIHYDROBENZOTHIADIAZINES



Compound No.	R	R.N.A.[a]
XX	Cl	1.0
LXIV	Br	1.0
LXV	F	0.1
LXVI	CF_3	0.4

[a] Relative natruretic activity based on compound XX in the rat, oral route.

Oxygenated Alkyl.—When compounds in this series were compared with related alkyl or aralkyl compounds, some reduction in natruretic activity was evident. This was true for the alcohols (XXV *vs.* V or VI and XXVII *vs.* XII), esters (XXXII *vs.* VIII), ethers (XXVIII *vs.* VI or VII and XXIX *vs.* XLI), ketones (XXXI *vs.* VI) and oxides (XXVI *vs.* V) where comparisons could be made.

Thioalkyl.—Compounds containing a thioalkyl group were generally more active than the standard (XXXIV through XXXVII). However, when compared to their alkyl counterparts, the potency was either the same (XXXIV *vs.* VI) or slightly less (XXXV *vs.* XLI and XXXVI *vs.* XLII). These results are in agreement with those reported by McManus, *et al.*[4] The sulfone compound (XXXVII)

(20) R. M. Taylor, J. S. Mershon, and M. M. Winbury, *Fed. Proc.*, **19**, 364 (1960).
(21) G. de Stevens, L. H. Werner, W. E. Barrett, J. J. Chart, and A. H. Renzi, *Experientia*, **16**, 113 (1960).
(22) M. H. Sherlock, N. Sperber, and J. Topliss, *ibid*, **16**, 184 (1960).
(23) R. M. Taylor and M. M. Winbury, *Nature*, **187**, 603 (1960).

was considerably less active by the oral route (rat) than by the intravenous route (dog), indicating poor oral absorption.

Aminoalkyl.—The introduction of a basic nitrogen atom in an alkyl side chain (XXXVIII and XXXIX) resulted in compounds with a relatively low order of activity.

Aralkyl.—Several compounds in this series showed a high order of activity. In particular, unsubstituted unbranched aralkyls (XL, XLI and XLII) markedly increased natruretic activity. The 3-β-phenethyl analog, the most potent compound in this class, was 11.3 times more active than hydrochlorothiazide orally in the dog. Lund and Kobinger[14] also found a high order of activity with the benzyl and phenethyl derivatives. In the benzyl series substitution in the phenyl nucleus depressed activity (XLIV through XLIX). Substitution in the alkyl chain also reduced activity in the one case examined (XLIII). It is noteworthy that some of the compounds in this series appeared to be considerably less active by the oral route than when administered intravenously in the dog (XL, XLIV and XLVIII).

Pyridylalkyl.—By the intravenous route in dogs these compounds were the most potent studied, the β-(2-pyridyl)-ethyl analog (LII) being 16.7 times more potent than hydrochlorothiazide. However, when administered orally in the rat they showed a much lower order of activity. Preliminary experiments in the dog also indicated poor oral activity. These findings are in sharp contrast to the results obtained with the related aralkyl compounds (XL and XLI) which showed good oral activity in the rat.

Aryl.—Irrespective of the type of substitution on the nucleus, aryl derivatives (LIII through LX) were generally less potent than hydrochlorothiazide. An exception appeared to be the *p*-chlorophenyl analog (LIV) which was about twice as active intravenously as the standard. A noteworthy feature of the aryl series was the almost complete lack of oral activity in the rat.

Heterocycles.—One thienyl (LXI) and two furyl (LXII and LXIII) analogs were the only members of this class investigated. These three compounds were less active than hydrochlorothiazide.

Conclusions

It is apparent from these results that the substituents which had the most favorable effect on activity were alkyl, haloalkyl, and aralkyl.

These groups may be classified as hydrophobic or hydrocarbon-like in character. When they were modified to more polar radicals, activity usually was reduced. For example, the introduction of oxygen, sulfur or nitrogen in the alkyl chain resulted in a lower level of activity. Obviously, such a broad generalization might not apply in certain cases where other operative factors take precedence (*e.g.*, chain length, steric hindrance, acidity, etc.).

The results have also demonstrated the importance of a hydrogen atom on the α-carbon of the 3-substituent. Thus, the trichloromethyl analog was far less active than the dichloromethyl, and the *tert*-butyl analog was less active than the isopropyl. Other pairs of compounds in which this relationship held were XVII *vs.* XVIII, XX *vs.* XXIII and XXV *vs.* XXVII. In addition, all compounds examined with aryl and heterocyclic groups attached at the 3-position exhibited a low order of activity.

Certain compounds in the aralkyl (XLIV and XLIX), pyridylalkyl (LI and LII) and aryl (LIII and LIV) series were less active by the oral than by the intravenous route. The most striking in this respect were the pyridylalkyl compounds which showed a very high degree of activity after intravenous administration but were weaker than hydrochlorothiazide when administered orally. This discrepancy is an indication that these compounds are poorly absorbed from the intestinal tract. With the possible exception of compound XLVIII, no appreciable differences between the response of the rat and the dog to the compounds were observed.

The present study has clearly shown the types of groups in the 3-position of dihydrobenzothiadiazine which enhance diuretic activity. Further studies would be required to determine the influence of different types of substituents at other positions in the nucleus.

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