85390-18-7; **67**, 85390-19-8; **68**, 32160-68-2; **69**, 85390-20-1; **70**, 31478-68-9; **71**, 31478-71-4; **72**, 31431-44-4; **73**, 31545-14-9; **74**, 31431-53-5; **75**, 31478-70-3; **76**, 31431-66-0; **77**, 31545-21-8; **78**, 31545-23-0; **79**, 31478-72-5; **80**, 31850-03-0; **81**, 31850-04-1; **82**, 31850-05-2; **83**·2HCl, 85390-21-2; **84**·2HCl, 85390-22-3; **85**, 85390-23-4; **86**·2HCl, 85390-24-5; **87**, 85390-25-6; **88**, 85390-26-7; **89**·2HCl, 85390-27-8; **90**, 85390-28-9; **91**·2HCl, 85390-29-0; **92**, 85390-30-3; $NH_2CH_2CH_2NH_2$, 107-15-3; ClCN, 506-77-4; chloroacetonitrile, 107-14-2; ethylthioacetic acid, 627-04-3; cyanomethyl ethylmercaptoacetate, 85390-31-4.

Sulfonyliminoimidazolidines. A New Class of Oral Hypoglycemic Agents. 2. Mode of Action and X-ray Structure of 1-[[*p*-[2-(Crotonylamino)ethyl]phenyl]sulfonyl]-3-cyclohexyl-2-iminoimidazolidine†

Ernst H. Schweizer,‡ Fritz Märki,*‡ and Grety Rihs§

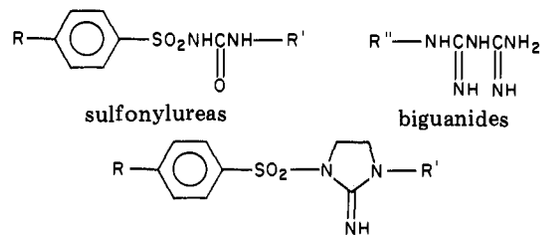
Research Department, Pharmaceuticals Division, and Central Research Services, Ciba-Geigy Limited, Basle, Switzerland.
Received June 4, 1982

Hypoglycemic sulfonyliminoimidazolidines were shown to stimulate insulin release in vitro (rabbit pancreas) and in vivo (normal rats) comparable to tolbutamide and to inhibit glucose oxidation in isolated rat fat cells in vitro similar to phenformin. These results support the hypothesis that the hypoglycemic effect of the compounds in normal and in diabetic animals may be due to a combination of mechanisms operative in sulfonylureas and biguanides. Determination of the three-dimensional structure of the potent analogue 1 by X-ray crystallography enabled us to identify specific regions of the molecule presumed to be involved in the molecular mode of action of sulfonyliminoimidazolidines.

Sulfonyliminoimidazolidines have recently been presented as a new class of oral hypoglycemic agents.[1] By combining structural elements of sulfonylureas and biguanides within one molecule (Chart I), these compounds display hypoglycemic activity in normal and in streptozotocin diabetic rats.

Sulfonylureas are known to lower blood glucose in normal animals by releasing insulin from the pancreas and are, therefore, inactive in the streptozotocin-diabetic rat model. Biguanides, on the other hand, produce hypoglycemia in diabetic animals by extrapancreatic mechanisms and are devoid of significant activity in normal animals.[2] We now propose that sulfonyliminoimidazolidines owe their dual activity (i.e., hypoglycemic effect in normal and diabetic rats) to a combination of mechanisms operative in sulfonylureas and biguanides. To test this hypothesis, we studied the effects of selected sulfonyliminoimidazolidines on insulin release in vitro and in vivo (sulfonylurea-type activity) and on glucose oxidation by rat fat cells in vitro (biguanide-type activity). Elucidation of the three-dimensional structure of the highly potent sulfonyliminoimidazolidine analogue 1 by X-ray crystallography then enabled us to analyze the structure–activity relationship at the molecular level.

Chart I



Biological Activity. Three models have been selected to test representative sulfonyliminoimidazolidine compounds for sulfonylurea-type and biguanide-type activities: stimulation of insulin release by pieces of rabbit pancreas in vitro, increase of plasma insulin in normal rats in vivo (sulfonylurea type), and inhibition of glucose oxidation by isolated rat fat cells in vitro (biguanide type). Sulfonylureas show no detectable activity in the latter model, while biguanides are inactive in the first two models. Effects of several sulfonyliminoimidazolidine compounds in these tests are shown in Tables I–III.

Insulin release in vitro was stimulated by the sulfonyliminoimidazolidines 1–4 and by the sulfonylurea tolbutamide (Table I). A two- to threefold increase above basal release was observed with 1–4 in the upper concentration range studied (0.1 mmol/L and above), whereas an approximately fourfold increase was obtained with tolbutamide. In the lower concentration range, 1 was clearly more potent than the other compounds; the minimal effective concentration was 3 times lower than that of **2**, **3**, and tolbutamide and 10 times lower than that of **4**, the analogue with the lowest potency.

Sulfonyliminoimidazolidines also stimulated insulin release in vivo. As shown in Table II, compounds 1–4 increased plasma insulin in normal rats at hypoglycemic doses. A blood glucose decrease in the range of 30–50% correlated with a two- to fourfold increase of plasma insulin. Hypoglycemic and β-cytotropic potency increased in the order **4** < **3** < **2** < **1**. The sulfonylurea tolbutamide showed hypoglycemic and β-cytotropic activity, whereas the biguanide phenformin (1-phenethylbiguanide) was inactive, despite a very high dose.

Glucose oxidation in isolated fat cells was inhibited by the sulfonyliminoimidazolidines 1–4 and by the biguanide phenformin with IC_{50} values between 0.18 and 0.020 mmol/L, while tolbutamide was inactive (Table III). Several quaternary pyridinium salts,[3] shown to lower blood glucose in diabetic animals by a biguanide-type mecha-

† Ciba-Geigy number CGP 11 112.
‡ Research Department, Pharmaceuticals Division.
§ Central Research Services.

(1) E. H. Schweizer, F. Märki, C. Lehmann, and H. Dietrich, *J. Med. Chem.*, preceding paper in this issue.
(2) (a) W. G. Oakley, D. A. Pyke, and K. W. Taylor, in "Diabetes and Its Management", 3rd ed., Blackwell Scientific Publications, Oxford, 1978, p 76. (b) I. Polacek and J. Ouart, *Diabetes*, **23**, 25 (1974). (c) G. Proske, G. Osterloh, R. Beckmann, F. Lagler, G. Michael, and H. Mückter, *Arzneim.-Forsch.*, 12, 314 (1962).
(3) V. J. Bauer, H. P. Dalalian, W. J. Fanshawe, S. R. Safir, E. C. Tocus, and C. R. Boshart, *J. Med. Chem.*, 11, 981 (1968).

0022-2623/83/1826-0970$01.50/0

Table I. Stimulation of Insulin Release in Pieces of Rabbit Pancreas in Vitro

	insulin release[b]					
compd[a]	0.003 mmol/L	0.01 mmol/L	0.03 mmol/L	0.1 mmol/L	0.3 mmol/L	0.9 mmol/L
1	1.88 ± 0.56 (3)	2.73 ± 0.87 (4)			1.82 ± 0.44 (4)	
2	1.02 ± 0.07 (6)	1.84 ± 0.24 (6)	2.18 ± 0.21 (7)	2.51 ± 0.22 (6)	3.06 ± 0.20 (6)	
3	0.98 ± 0.06 (6)	2.08 ± 0.33 (6)	2.24 ± 0.14 (6)	3.13 ± 0.74 (7)	3.66 ± 0.51 (6)	
4	1.01 ± 0.12 (4)	1.22 ± 0.29 (4)	1.74 ± 0.33 (4)	3.21 ± 0.52 (12)		3.21 ± 0.28 (6)
tolbutamide	0.95 ± 0.19 (3)	1.81 ± 0.23 (5)		4.33 ± 0.68 (10)		4.23 ± 0.29 (7)

[a] Structures are shown in Scheme I. [b] Ratio of release with test compound to without test compound present; mean plus or minus SEM (number of experiments in parentheses).

Table II. Hypoglycemic Effect and Insulin Release in Normal Rats

compd[a]	dose,[b] mmol/kg	N[c]	blood glucose,[d] mmol/L	plasma insulin,[d] pmol/L
control		11	5.87 ± 0.12	95.8 ± 11.0
1	0.0084	6	4.04 ± 0.10 (−31%)**	163.3 ± 35.8 (+71%)
	0.028	6	3.42 ± 0.08 (−42%)**	476.3 ± 28.0 (+397%)**
2	0.008	5	4.86 ± 0.27 (−17%)*	107.5 ± 18.5 (+12%)
	0.024	6	3.62 ± 0.08 (−38%)**	309.5 ± 29.0 (+223%)**
	0.072	6	3.63 ± 0.11 (−38%)**	395.5 ± 66.8 (+313%)**
3	0.18	5	3.08 ± 0.12 (−47%)**	285.0 ± 49.0 (+198%)**
4	0.60	11	4.66 ± 0.10 (−21%)**	136.3 ± 20.5 (+42%)
tolbutamide	0.17	6	3.86 ± 0.05 (−34%)**	277.5 ± 34.3 (+190%)**
phenformin	2.0	7	5.52 ± 0.09 (−6%)	86.1 ± 18.9 (−10%)

[a] Structures are shown in Scheme I. [b] Compounds were administered orally 45 min before assay of blood glucose and plasma insulin. [c] Number of animals used. [d] Mean plus or minus SEM (percent change against control in parentheses). Statistical significance of change against control: * = $p < 0.05$; ** = $p < 0.01$ (Dunnet's test).

Table III. Inhibition of Glucose Oxidation in Isolated Rat Fat Cells in Vitro

compd[a]	IC_{50},[b] mmol/L
1	0.18 ± 0.01 (5)
2	0.071 ± 0.004 (7)
3	0.020 ± 0.002 (4)
4	0.18 ± 0.02 (3)
phenformin	0.18 ± 0.03 (7)
tolbutamide	>0.5[c] (2)

[a] Structures are shown in Scheme I. [b] Concentration causing 50% inhibition; mean plus or minus SEM (number of experiments in parentheses). [c] Highest concentration tested, inactive.

nism,[4] were also found to strongly inhibit glucose oxidation in fat cells with IC_{50} values in the range 0.1–0.04 mmol/L (unpublished results). Although inhibition of glucose oxidation is not known to be directly involved in the hypoglycemic effect in streptozotocin-diabetic rats, it was observed with all hypoglycemic biguanides and quaternary pyridinium compounds studied and is therefore considered an integral part of this type of activity.

The results of these experiments demonstrate that hypoglycemic sulfonyliminoimidozolidines are able to stimulate insulin release like sulfonylureas, as well as to inhibit fat cell glucose oxidation like biguanides.

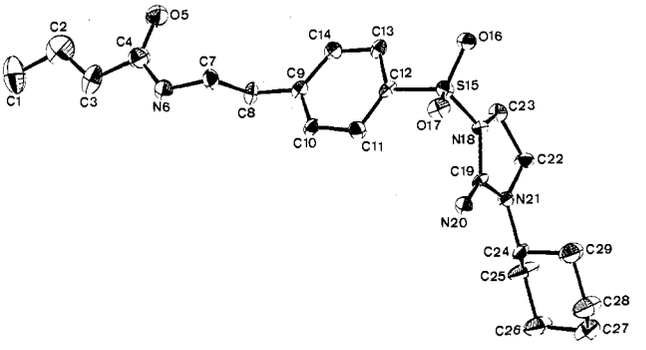


Figure 1. ORTEP drawing of 1 with numbering of the atoms.

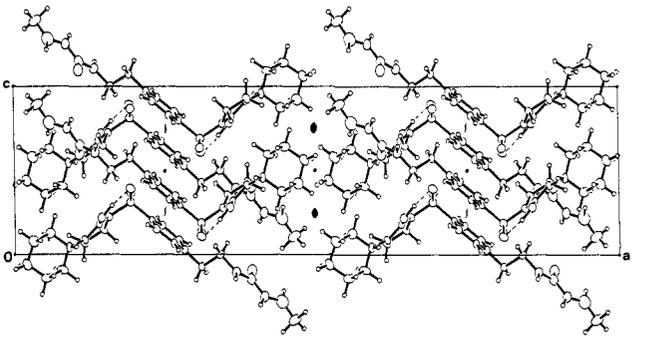


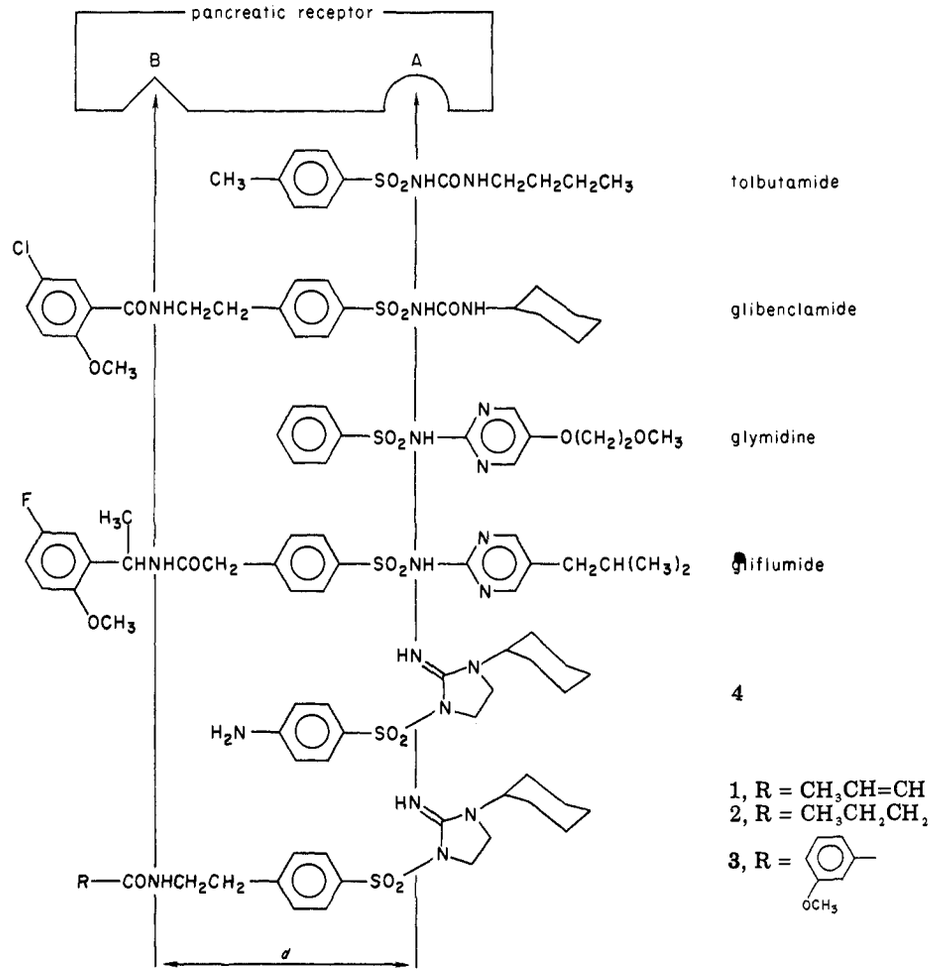
Figure 2. Packing of 1.

X-ray Crystal Structure. Crystal data of compound 1 are as follows: crystal system, monoclinic; space group, $C2/c$, centrosymmetric; a = 38.517, b = 10.970, c = 10.568 Å; β = 90.98°; number of reflections, 2556; final R value, 0.069. A perspective view of the molecule, drawn with the aid of the ORTEP program,[5] is shown in Figure 1. Bond lengths and angles between non-hydrogen atoms are within the range of normally expected values. The double-bond between C_2 and C_3 forms an angle of 14° with the amide group. The atoms N_6, C_7, C_8, and C_9 are lying within a plane that forms an angle of 88° with the amide group and an angle of 77° with the aromatic part of the molecule. The five-membered ring is not fully planar: C_{23} is lying 0.184 Å below the plane and C_{22} is lying 0.097 Å above the plane defined by the atoms N_{18}, C_{19}, N_{20}, and N_{21}. The cyclohexane ring is in the chair conformation. N_{20} and O_{17} are connected by an intramolecular hydrogen bond (N_{20}–H_{44}, 1.03 Å; H_{44}···O_{17}, 2.17 Å; N_{20}···O_{17}, 2.891 Å; N_{20}–H_{44}···O_{17}, 125°). The distance between N_{20} and N_6 is 9.34 Å. Packing is effected by an intermolecular hydrogen bond (N_6–H_{35}, 1.01 Å; H_{35}···N_{20}, 1.94 Å; N_6···N_{20}, 2.949 Å; N_6–H_{35}···N_{20}, 173°) and by van der Waals contacts. A projection of the unit cell, which consists of eight molecules, onto the (a, c) plane is shown in Figure 2. It should be noted that N_{20} is involved in two hydrogen bonds: It

(4) D. A. Blickens and S. J. Riggi, *J. Pharmacol. Exp. Ther.*, 177, 536 (1971).

(5) ORTEP program: C. K. Johnson, ORTEP Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN, 1965.

Scheme I



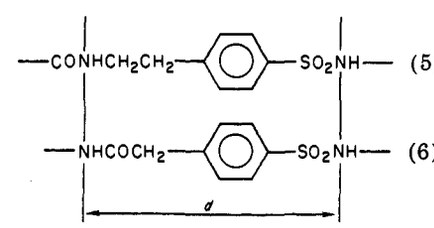
acts as a proton donor in an intramolecular bond (indicated as dotted lines in Figure 2) and as a proton acceptor in an intermolecular bond (nearly parallel to the *b* axis).

Discussion

It has been described previously that sulfonyliminoimidazolidines lower blood glucose in normal and in streptozotocin-diabetic rats.[1] In this study we show that representative analogues from this class stimulate release of insulin from the pancreas comparable to tolbutamide and inhibit glucose oxidation in fat cells similar to phenformin. Therefore, it seemed reasonable to speculate that different, specific regions of the sulfonyliminoimidazolidine molecule may be responsible for the sulfonylurea-type and biguanide-type activities.

As first stated in 1961, the presence of a hydrogen on the sulfonamide nitrogen of sulfonylureas is an obligatory requirement for hypoglycemic activity in this class.[6] Sulfonyliminoimidazolidines, which also lower blood glucose by stimulating insulin release, appear to violate this rule, since no hydrogen is covalently attached to the sulfonamide nitrogen (Chart I). However, the X-ray structural analysis of 1 reveals that—at least in the crystal—the molecule does not assume an extended linear conformation but forms an angle with its vertex at the sulfamide moiety. This conformation is stabilized by an intramolecular hydrogen bond between N_{20} and O_{17}. Thus, bending of the molecule brings the NH group (N_{20}) into close vicinity of the SO_2 group. It is tempting to speculate that this steric arrangement may bear sufficient similarity to that of the sulfonamide group of a sulfonylurea to enable stimulation of insulin release in the pancreas. Unfortunately, a direct test of this assumption is presently not possible because crystallographic data of sulfonylureas have not been published so far.

An essential role of the sulfonamide moiety of sulfonylureas and related sulfonylaminopyrimidines regarding insulin release has been postulated. According to the hypothesis of Rufer and Losert,[7] insulin release is triggered by binding of the sulfonylurea molecule via its sulfonamide group to a pancreatic receptor site (A, Scheme I). Hypoglycemic agents of the "second generation" (e.g., glibenclamide and gliflumide) have drastically increased potencies in vivo. They differ from the earlier compounds (e.g., tolbutamide and glymidine) by carrying a carboxamidoalkyl substitution on the benzene ring para to the sulfonyl group. The fact that optimal activity is restricted to analogues with the structural elements **5** or **6** suggested

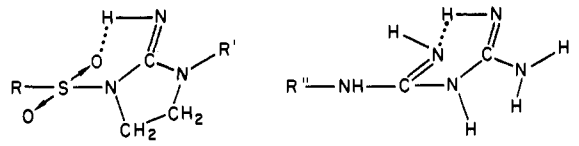


that the distance "*d*" between the nitrogen atoms may be

(6) A. Bänder, in "Proceedings, 4e Congrès de la Fédération Internationale du Diabète, Genève", M. Demole, Ed., Editions Médecine et Hygiène, Genève, 1961, p 694.

(7) C. Rufer and W. Losert, *J. Med. Chem.*, **22**, 750 (1979).

Chart II



sulfonyliminoimidazolidines biguanides

crucial for increased activity[8] and led to the speculation that the carboxamide nitrogen could bind to a second pancreatic receptor site (B, Scheme I), located at the distance *d* from receptor site A.[7] According to this hypothesis, increased potency of "second generation" hypoglycemic agents would result from occupation of the sulfonamide receptor (A) and the carboxamide receptor (B) by the same molecule. It is an intriguing possibility that sulfonyliminoimidazolidines conform to the same—so far hypothetical—mechanism. As shown in the lower part of Scheme I, "first-generation" analogues (e.g., 4) with relatively low potency in vivo (about one-third that of tolbutamide in the normal rat) can only bind to receptor A. "Second-generation" analogues with increased potency (e.g., 1; 20 times that of tolbutamide) containing a carboxamide group at the appropriate distance (*d*) may, in addition, also bind to receptor B. Thus, the sulfonylurea-type activity of sulfonyliminoimidazolidines appears to rely on the same molecular mechanism as postulated for sulfonylureas. The structural elements directly involved in this hypothetical mechanism may be identified as those corresponding to the region S_{15}–N_{20} (fitting receptor site A) and C_4–N_6 (fitting receptor site B) of compound 1.

Recently, direct experimental evidence for the existence of highly specific receptors for sulfonylureas has been obtained in brain and in a β-cell tumor of the rat.[9] It is interesting to note that the binding affinity of these receptors for second-generation sulfonylureas is about two orders of magnitude higher than that for first-generation analogues. This study lends strong support to the hypotheses discussed above.

The biguanide-type activity of sulfonyliminoimidazolidines may be related to chelation of a biologically important metal ion. Several authors have noted that hypoglycemic biguanides form stable complexes with certain bivalent metal ions,[10] and others have suggested that this property may be involved in the mechanism of the hypoglycemic activity of biguanides.[11] Although stable complexes of sulfonyliminoimidazolidines with metal ions have so far not been isolated, the six-membered ring of 1, formed by the atoms O_{17}, S_{15}, N_{18}, C_{19}, and N_{20} with its attached hydrogen forming an intramolecular hydrogen bond with O_{17} (Figure 1, Chart II), is likely to be a good chelating ligand. Thus, the possibility exists that this structural part of the sulfonyliminoimidazolidine molecule may be essential for the biguanide-like activity. Further studies are needed, however, to substantiate the role of metal chelates in the mode of action of sulfonyliminoimidazolidines—and of biguanides as well.

In conclusion, the proposed hypothesis that sulfonyliminoimidazolidines owe their dual hypoglycemic action in normal and in diabetic rats to a combination of mechanisms operative in sulfonylureas and biguanides appears to be valid. It is supported by the demonstration of typical sulfonylurea-like and biguanide-like effects (stimulation of insulin release, inhibition of glucose oxidation). Furthermore, the X-ray structure analysis of 1 enabled us to identify specific regions presumed to be involved in the molecular mode of action of sulfonyliminoimidazolidines.

Experimental Section

Biological Activity. Insulin Release. Stimulation of insulin release from pieces of rabbit pancreas incubated in bicarbonate-buffered medium containing 0.6 g/L of glucose was evaluated as described in detail by Coore and Randle.[12] Stimulation by the test compound was expressed as the ratio of insulin release (nanograms of insulin per flask) in the presence of test compound vs. basal insulin release (mean of two preceding incubation periods, 20 min each, in medium without test compound). Eight pieces of pancreas pooled from four rabbits were used in each experiment.

Insulin release in vivo and hypoglycemic effect were determined by assaying plasma insulin and blood glucose in female rats (body weight 175–195 g, fasted for 6–9 h) 45 min after oral administration of the test compounds as suspensions in 0.5% (w/v) methylcellulose (5 mL/kg). Plasma insulin was determined by radioimmunoassay, and blood glucose was determined by an enzymatic method described previously.[13]

Glucose Oxidation. Fat cells were isolated from epididymal adipose tissue of five young rats (body weight 140–160 g) by the collagenase technique of Rodbell[14] and incubated with D-[1-^{14}C]glucose (40 nCi/flask, 0.1 g of glucose/L) in the presence of various concentrations of the test compound. The 50% inhibitory concentration (IC_{50}, in millimoles per liter) was determined graphically from a plot of percent inhibition vs. log concentration.

X-ray Structure Analysis. Intensity measurements were made on a Philips PW 1100 diffractometer with monochromated Mo $K\alpha$ radiation. The structure was solved by direct methods by using the computer program MULTAN 77.[15] Block diagonal least-squares refinements of the 29 non-hydrogen atoms converged at $R = 0.098$. Eighteen out of the thirty hydrogen atoms could be located in different Fourier maps; positions of the remaining ones were calculated.

Acknowledgment. We thank J. Motz, L. Hartmann, R. Schwaller, H. R. Walter, and P. Häner for technical assistance and E. Koncz and E. Scholer for typing the manuscript.

Registry No. 1, 85337-29-7; 2, 25864-94-2; 3, 25919-85-1; 4, 21541-18-4.

Supplementary Material Available: Fractional atomic coordinates with estimated standard deviation of 1-[[*p*-[2-(crotonylamino)ethyl]phenyl]sulfonyl]-3-cyclohexyl-2-iminoimidazolidine (1) (1 page). Ordering information is given on any current masthead page.

(8) C. Rufer, H. Biere, H. Ahrens, O. Loge, and E. Schröder, *J. Med. Chem.*, 17, 708 (1974).
(9) N. Kaubisch, R. Hammer, C. Wollheim, A. E. Renold, and R. E. Offord, *Biochem. Pharmacol.*, 31, 1171 (1982).
(10) (a) W. O. Foye, R. L. O'Laughlin, and R. N. Duvall, *J. Pharmacol. Sci.*, 50, 641 (1961). (b) P. Rây, *Chem. Rev.*, 61, 313 (1961). (c) G. Schwarzenbach and G. Anderegg, *Pharm. Acta Helv.*, 38, 547 (1963).
(11) (a) F. Davidoff, *N. Engl. J. Med.*, 289, 141 (1973). (b) G. Schäfer, *Pharmacol. Ther.*, 8, 275 (1980).
(12) H. G. Coore and P. J. Randle, *Biochem. J.*, 93, 66 (1964).
(13) F. Märki, B. Kamber, H. Rink, and P. Sieber, *J. Endocrinol.*, 81, 315 (1979).
(14) M. Rodbell, *J. Biol. Chem.*, 239, 375 (1964).
(15) MULTAN 77: A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data (1977): (a) P. Main, L. Lessinger, and M. M. Woolfson, Department of Physics, University of York, York, England; (b) G. Germain and J. P. Declercq, Laboratoire de Chimie Physique et de Crystallographie, Bâtiment Lavoisier, 1348 Louvain-La Neuve, Belgique.