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Hydroxyl-Substituted Sulfonylureas as Potent Inhibitors of Specific [^{3}H]Glyburide Binding to Rat Brain Synaptosomes

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Abstract—We are seeking to discover potent CNS-active sulfonylureas with structural features that allow for the formation of several types of prodrugs. We report herein the syntheses of compounds comprising an initial series of hydroxyl-substituted analogues of the potent ATP-sensitive potassium channel blockers glyburide (glibenclamide) and gliquidone. Somewhat unexpectedly, several of the compounds were found to be comparably potent to glyburide as inhibitors of specific [^{3}H]glyburide binding in rat brain preparations.

Introduction

Certain sulfonylureas, such as glyburide (glibenclamide, **1**; Fig. 1) and gliquidone (**2**; Fig. 1), bind to specific sites in the mammalian central nervous system (CNS).[1] At least some of these sites are on protein subunits now referred to as sulfonylurea receptors (SUR), and these can link to potassium channel protein subunits (K_{ir}) to form functional ATP-sensitive potassium channels (K^+_{ATP} channels).[2] A potent blocker of CNS K^+_{ATP} channels might prevent hypoglycemic seizures,[3] and other possible therapeutic applications have been suggested.[4] Of particular interest to us, Roane and co-workers report[5] that icv injections of glyburide, tolbutamide, and diazoxide affect food intake by rats in relatively complex ways (see also refs 6–8). The observed complexities are consonant with what is currently known concerning CNS involvement with the regulation of food intake.[9]

A serious experimental limitation in studies of the CNS effects of sulfonylureas is that most compounds of this class are, at best, modestly soluble in solvent vehicles that are appropriate for injection directly into brain tissue or cerebral ventricles; thus, one of our initial goals was to synthesize compounds incorporating a functional 'handle' that would allow for the synthesis of highly water-soluble prodrugs. Properly designed, these would enable much more drug to be administered per unit volume of strictly aqueous solution, and in turn, greatly improve experimental capabilities to inject drug (via microcannulae) directly into smaller, more carefully restricted regions of the rat brain—including small subregions of the hypothalamus.

A longer-term objective is to address the possibility of CNS-selective compounds. Ample data now documents the existence of molecular diversity among K^+_{ATP} channels in various tissues,[2c–e,10] and the discovery of receptor-subtype-selective compounds is of current interest; even so, little evidence presently suggests the existence of SUR subtypes that are uniquely distributed in the CNS versus pancreatic β-cells or other peripheral tissues[11,12] (see, however, refs 13 and 14). Pending such findings, CNS-selective delivery is one possible key to exploiting the CNS pharmacology of K^+_{ATP} channels, and might be accomplished with a "chemical delivery system" approach such as that which has been extensively explored by Bodor and co-workers.[15] This strategy once again requires a point of attachment for the requisite pro-moieties, and none of the potent hypoglycemic sulfonylureas are structurally suitable for direct conjugation.

As a first step towards the aforementioned aims, we set out to study the effects of introducing polar functional

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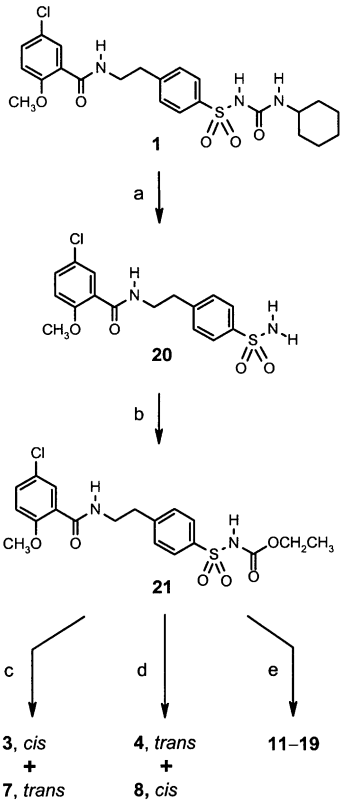
groups, like hydroxyl, into the structure of potent K^+_{ATP} channel blockers. Glyburide (**1**) and gliquidone (**2**) were chosen as the initial structural templates for synthetic modification. Glyburide is a potent blocker of K^+_{ATP} channels in pancreatic β-cells and other tissues, including brain.[1,2,12] Gliquidone was selected because (1) there are many sites on the structure where new functionality might be introduced; (2) gliquidone was reported[3] to be 10^4 times more potent than glyburide in its ability to elicit γ-aminobutyric acid (GABA) release from the rat substantia nigra (possibly due to heterogeneity of brain K^+_{ATP} channels); (3) gliquidone has the exceptional characteristic that greater than 90% of an administered dose is efficiently cleared from the circulation by excretion in bile, at least in large part as sulfate or glucuronide conjugates of hydroxylated metabolites.[16] Reported herein is the synthesis and initial pharmacological evaluation of the first sets of target molecules (Fig. 1). Compounds **3**–**11** are hydroxylated on the cyclohexyl ring, and this series includes the known glyburide metabolites **7**–**8** and the gliquidone metabolite **9**; of these three compounds, only the synthesis of **7** has previously been reported in the literature.[17] The open-chain analogues **12**–**19**, including the diol **17**, were also prepared and tested.

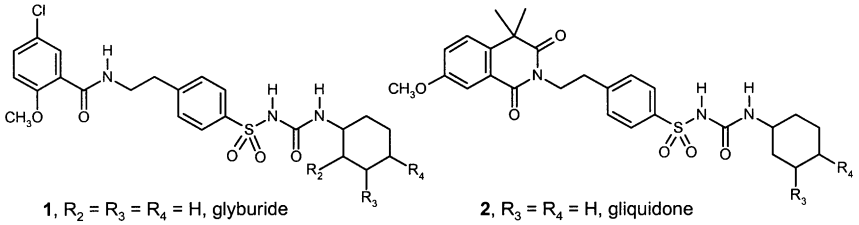
Results

Chemistry

Synthesis of glyburide analogues. Glyburide (**1**) was cleaved with phthalic anhydride in pyridine by the method of Egg et al.[18] to the corresponding sulfonamide, **20** (Scheme 1), which was then converted to the ethyl carbamate **21**.[17,19] The *cis*- and *trans*-4-hydroxycyclohexyl analogues **3** and **7** were obtained by reacting **21** with commercially available *cis*/*trans*-4-aminocyclohexanol under conditions based on those of Kutter et al.,[20] followed by separation of the *cis*/*trans* product mixture by column chromatography. Similarly, the

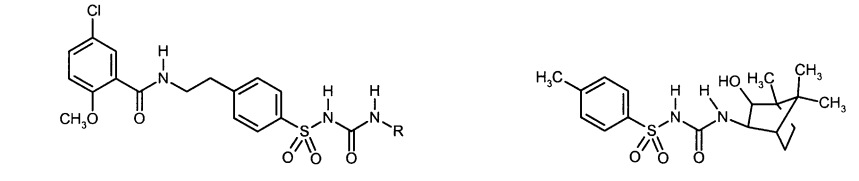


Scheme 1. Reagents: (a) phthalic anhydride, K_2CO_3, DMAP, pyridine; (b) (i) K_2CO_3, acetone; (ii) $ClCO_2Et$, reflux; (c) (i) *cis*/*trans*-4-aminocyclohexanol (2 equiv), dioxane, reflux; (ii) column chromatography; (d) (i) *cis*/*trans*-3-aminocyclohexanol (2 equiv), dioxane, reflux; (ii) column chromatography; (e) amino alcohol (2 equiv), dioxane, reflux.



1, $R_2 = R_3 = R_4 = H$, glyburide
3, $R_2 = R_3 = H$, R_4 = *cis*-OH
4, $R_2 = H$, R_3 = *trans*-OH, $R_4 = H$
7, $R_2 = R_3 = H$, R_4 = *trans*-OH
8, $R_2 = H$, R_3 = *cis*-OH, $R_4 = H$
11, R_2 = *trans*-OH, $R_3 = R_4 = H$

2, $R_3 = R_4 = H$, gliquidone
5, $R_3 = H$, R_4 = *cis*-OH
6, R_3 = *trans*-OH, $R_4 = H$
9, $R_3 = H$, R_4 = *trans*-OH
10, R_3 = *cis*-OH, $R_4 = H$



12, R = CH_2CH_2OH
13, R = $CH_2CH_2CH_2OH$
14, R = $CH_2CH_2CH_2CH_2OH$
15, R = $CH_2CH_2CH_2CH_2CH_2OH$

16, R = $C(CH_3)_2CH_2OH$
17, R = $C(CH_3)(CH_2OH)_2$
18, R = *S*-$CH(CH_3)CH_2OH$
19, R = *R*-$CH(CH_3)CH_2OH$

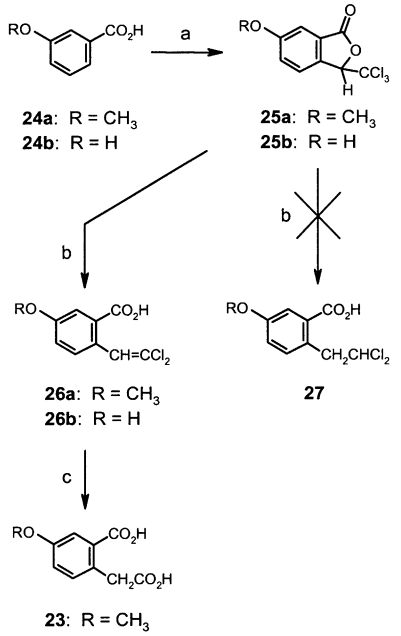
glibornuride

Figure 1. Structures of compounds.

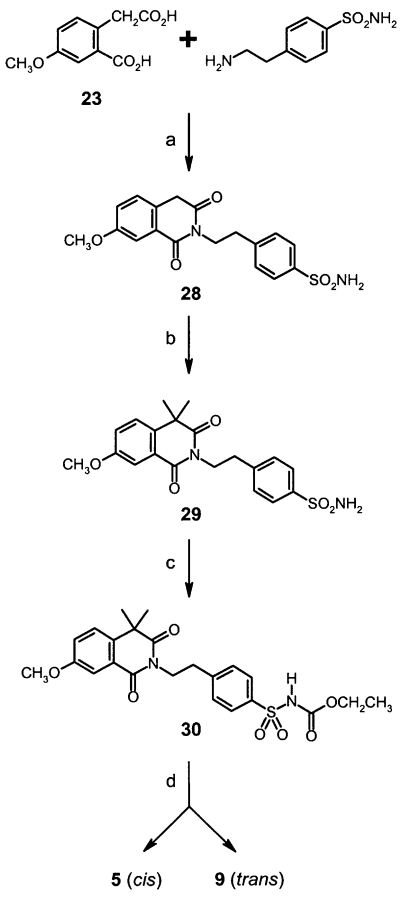
trans- and *cis*-3-hydroxycyclohexyl analogues **4** and **8** were synthesized by reaction with *cis*/*trans*-3-aminocyclohexanol, once again followed by chromatography. This amino alcohol is not commercially available, and was synthesized by the method of Greenhill et al.;[21] of the few known procedures for 3-aminocyclohexanol, this one provides a mixture with the highest known (albeit still minor) proportion of *trans* isomer. The column separation of the 3-hydroxycyclohexyl isomers **4** and **8** was achieved only after investigating *numerous* potential solvent systems and, due to solubility limitations, will not be practical for large-scale preparation of these compounds. The configurations of the isomers (**3** vs **7**, and **4** vs **8**) could be established by comparisons of their NMR spectra; see further discussion below.

The 2-*trans*-hydroxy analogue **11** and the aliphatic analogues **12**–**19** were synthesized for the most part analogously to compounds **3**/**7** and **4**/**8**. A procedural modification was necessary in the case of the diol **17**; in the initial attempts to react carbamate **21** with 2-amino-2-methyl-1,3-propanediol, the sulfonamide-forming side reaction was quite predominant. The solvent volume in these reactions was usually kept small to increase the reaction rate and facilitate workup; the presence of two hydroxyl groups in this diol amine might raise the dielectric constant sufficiently so as to stabilize the sulfonamide anion more than usual, and thereby enhance its ability to act as a leaving group. Operating with this hypothesis, a larger quantity of solvent (1,4-dioxane) was added, and the formation of sulfonamide byproduct was substantially reduced.

Synthesis of gliquidone analogues. For the synthesis of gliquidone analogues, 4-methoxyhomophthalic acid (**23**) was a key intermediate; this compound was synthesized by modified literature procedures[22–24] (Scheme 2). Although this same basic synthetic sequence for **23**,[22–26] and thence its anhydride,[27] has been reported several times in the literature, we find no prior reports of spectroscopic characterization, other than one IR,[28] for **23** prepared by this or any of several other known routes. Furthermore, no spectral data have been reported for the intermediates in the sequence **25**→**23**, and there has been some confusion concerning the structures of **26a**–**b** (see below). *m*-Methoxybenzoic acid (**24a**) was condensed with chloral hydrate to obtain the lactone **25a**; this lactone was then reduced by zinc in acetic acid. A drying tube was critically needed for the chloral condensation, otherwise the yields were poor. Also, the product of the zinc reduction was the dichlorovinyl derivative **26a**, not the saturated derivative **27** as previously reported by several groups of workers.[22,25,29,30] To corroborate this result, the analogous 5-hydroxy lactone **25b** was also synthesized, and reduced to **26b**. The structures of **26a**–**b** had been correctly reported by Dharwarkar and Alimchandani,[31] who had also obtained the methoxy acid **26a** by a different route, namely methylation of the phenolic group in **26b**. It appears that such *o*-(β,β-dichlorovinyl)benzoic acids are rare in the literature, and we find no prior cases of spectral characterization except for one report[32] of the UV spectrum for the unsubstituted phenyl compound prepared by a different route.



Scheme 2. Reagents: (a) $Cl_3CCH(OH)_2$, concd H_2SO_4, 24 h; (b) Zn°, CH_3CO_2H; (c) warm concd H_2SO_4.



Scheme 3. Reagents: (a) melt, 200 °C; (b) 2 equiv NaOEt, 2 equiv CH_3I, anhyd CH_3OH; (c) (i) K_2CO_3, acetone; (ii) $ClCO_2Et$, reflux; (d) (i) *cis*/*trans*-4-aminocyclohexanol, dioxane, reflux; (ii) column chromatography.

The homophthalic acid **23** was condensed with 4-(2-aminoethyl)benzenesulfonamide by heating an intimate mixture of the two at 200 °C (as mentioned without details by Kutter et al.[20,33]) to obtain the isoquinolinyl derivative **28** in good yield (Scheme 3). The 4-position of the isoquinolinyl ring in **28** was dimethylated to obtain the sulfonamide **29**, also in good yield. Conversion to the ethylcarbamate **30** proceeded as for **20**→**21**. Reaction of **30** with 4-aminocyclohexanol, followed by column chromatography, gave the *cis*-4-hydroxycyclohexyl (**5**) and *trans*-4-hydroxycyclohexyl (**9**) analogues of gliquidone. The configurations were readily assigned by NMR spectral comparisons to the corresponding glyburide analogues. The reaction of **30** with *cis*/*trans*-3-aminocyclohexanol proceeded readily; unfortunately, column separation of these two compounds proved to be quite difficult, and improved methods for the synthesis of the isomeric cyclohexanols are instead being pursued.

Pharmacology: binding studies

The target compounds were tested for their abilities to inhibit specific [^{3}H]glyburide binding in rat whole brain (minus cerebellum) homogenates, using a procedure based on those of Zini et al.[34] and Cherksey and Altszuler.[35] Total specific binding was in excess of 85%, and best fits of the data sets obtained with (unlabelled) glyburide over a concentration range of 10^{-12}–10^{-5} M were consistent with the presence of two specific binding sites, $K_d = 0.25$ nM and $K_d = 730$ nM. Compounds in a given series were evaluated with a single pooled homogenate preparation, resulting in two internally consistent sets of data (Table 1A and B). Although the results for glyburide are a bit different between these two data sets, activity relative to glyburide (and gliquidone in the case of compounds **5** and **6**) was the critical result sought from these studies.

As inhibitors of high-affinity [^{3}H]glyburide binding, the four hydroxyl-substituted glyburide analogues (**3**–**4** and **7**–**8**) were all about 2–3-fold less potent than glyburide, and amongst these four compounds there were no statistically significant contrasts (Table 1A, Fig. 2A). For inhibition of low-affinity [^{3}H]glyburide binding, no significant differences in potency were discerned amongst the four hydroxyl-substituted analogues or between any individual analogue and glyburide. For the 2-*trans*-hydroxycyclohexyl compound (**11**) and the hydroxy-aliphatics **12**–**19** (Fig. 2C), an *F*-test did not support analysis with a two-site equation; the resulting single-site IC_{50} values (Table 1B) presumably pertain to the high-affinity glyburide binding site. The 2-*trans*-hydroxycyclohexyl compound **11**, unlike the 3- and 4-hydroxycyclohexyl compounds, was found to bind with 25-fold lower affinity than glyburide. Most of the acyclic analogues were considerably less active than glyburide and hydroxycyclohexyl analogues **3**–**4** and **7**–**8**,

Table 1. Inhibition of specific [^{3}H]glyburide binding to rat brain synaptosomes[a] by various hydroxy-substituted analogues

Compound	High-affinity site IC_{50} (nM)	Low-affinity site IC_{50} (μM)	High-affinity fraction
A.[b]			
1, glyburide (GLYB)	0.48 (0.38–0.60)[c]	0.68 (0.06–7.5)[c]	0.87
3, *cis*-4-hydroxycyclohexylGLYB	1.3 (1.0–1.6)[c]	0.11 (0.03–0.37)[c]	0.83
7, *trans*-4-hydroxycyclohexylGLYB	0.95 (0.83–1.1)[c]	0.10 (0.05–0.18)[c]	0.82
4, *trans*-3-hydroxycyclohexylGLYB	1.3 (0.9–1.9)[c]	0.13 (0.01–1.6)[c]	0.87
8, *cis*-3-hydroxycyclohexylGLYB	1.5 (1.1–2.0)[c]	0.17 (0.04–0.72)[c]	0.82
2, gliquidone (GLIQ)	2.8 (1.9–4.0)[c]	—	—
5, *cis*-4-hydroxycyclohexylGLIQ	9.0 (6.9–11.7)[c]	—	—
9, *trans*-4-hydroxycyclohexylGLIQ	1.6 (1.4–1.8)[c]	—	—
B.[d]			
1, glyburide	2.5 (1.3–5.0)[e]		
15, 5-hydroxypentyl	11.9 (9.1–15.4)[e]		
14, 4-hydroxybutyl	24 (17–33)[e]		
11, *trans*-2-hydroxycyclohexyl	62 (39–98)[e]		
13, 3-hydroxypropyl	92 (53–161)[e]		
18, *S*-2-hydroxy-*i*-propyl	92 (48–176)[e]		
16, hydroxy-*t*-butyl	130 (81–210)[e]		
19, *R*-2-hydroxy-*i*-propyl	149 (60–371)[e]		
17, 2,2-dihydroxy-*t*-butyl	221 (112–435)[e]		
12, 2-hydroxyethyl	1890 (646—5510)[e]		
1, glyburide[f]	1.6 (0.95–2.7)[f]	1.0 (0.1–8.3)[f]	0.76 (0.65–0.86)[g]
1, glyburide[h]	0.3		
12[h]	30		

[a]See text for full details.
[b]Pharmacologist A (J.K.B.).
[c]50% inhibition of specific [^{3}H]glyburide binding to synaptosomes, 95% confidence interval given in parentheses; data fit to two-site model except compounds **2**, **5**, and **9** (see text).
[d]Pharmacologist B (Y.Z.).
[e]50% inhibition of specific [^{3}H]glyburide binding to synaptosomes, 95% confidence interval given in parentheses; data fit to one-site model except where noted. See text for further information.
[f]Results from two-site fit obtained by Pharmacologist B (Y.Z.); cf. Table 1A.
[g]95% confidence limits.
[h]Data from Ref 1b.

although the 5-hydroxypentyl (**15**) and 4-hydroxybutyl (**14**) compounds were only 5-fold and 10-fold less potent than glyburide, respectively.

For the gliquidone series (Table 1A, Fig. 2B), an *F*-test once again did not support analysis with a two-site equation. The single-site IC_{50} values are in the same concentration range as those of the high-affinity site for the most potent glyburide analogues, and the *trans*-4-hydroxycyclohexyl analogue **9** was about twice as active as gliquidone and about 5-fold more active than the *cis*-4-hydroxy analogue **5**.

Discussion

NMR characterization of *cis* versus *trans* sulfonylureidocyclohexanols

Although hydroxylation of a cyclohexyl ring is a well-established route of mammalian metabolism, and has been reported for several sulfonylureas, we found no prior literature report of NMR spectroscopic examination of *cis*/*trans* isomeric sulfonylureidocyclohexanols; even reports involving the related acylamino- or ureido-cyclohexanols are very few. Metabolism studies of *N*-cyclohexyl compounds[36–39] have most often relied on mass spectral fragmentation patterns in combination with GC or HPLC retention times of synthetic standards (the configurations of which were proven by means other than spectroscopic) although, as early as 1970, Johnson et al.[40] noted bandwidth differences for the carbinol proton signals in their studies of the stereochemistry of microbial oxygenation of *N*-acylcyclohexylamines.

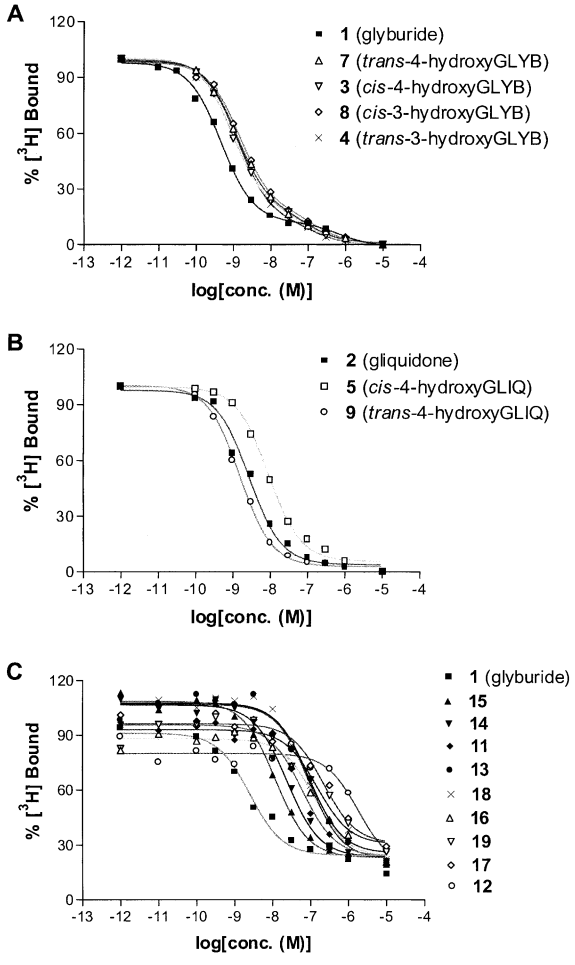


Figure 2. Inhibition of specific [^{3}H]glyburide binding to rat brain homogenates by various compounds: effect versus concentration. See Experimental for details.

Definitive assignment of the identities of the chromatographically separated 1,4-hydroxycyclohexyl isomers (**3** vs **7**) could be made based on selected literature comparisons, notably with reports from Zell et al.[36] and May et al.[37] For the assignment of the 1,3-hydroxycyclohexyl isomers (**4** vs **8**), however, the literature contained surprisingly little directly pertinent NMR spectral data. This is consistent with the synthetic challenges involved in obtaining the *cis* and *trans* isomers of cyclohexanols having a nitrogen-linked substituent at the 3-position. Reports from May et al.,[37] Bartoli et al.,[41] Nishi et al.,[39] and Johnson et al.[42] were helpful but not definitive; in the latter two reports, the carbinol and NHC*H* proton signals are the only discerning spectroscopic information relative to isomer assignment. With our *trans*-1,3 compound (**4**), these signals are partially occluded by others, making it impossible to observe the *J*-splittings needed for comparison. On the other hand, in both of those reports the shifts for the carbinol and NHC*H* proton signals were reported to be higher for the *trans* isomer than with the *cis*, and our results are consistent (δ 3.68 and 3.75 in the spectrum of **4** [*trans*-1,3] versus 3.25 and 3.36 for **8** [*cis*-1,3]). Rader[43] reported that, for isomers of various 3-alkyl-substituted cyclohexanols in DMSO solution, the *J*-splitting for the hydroxyl proton in diequatorially situated compounds was consistently larger than for the hydroxyl proton in molecules with equatorial-axial arrangement. Consistently, J_{CH-OH} in the spectrum of **4** (*trans*-1,3) is 2.8 Hz, versus 4.0 Hz for **8** (*cis*-1,3). As one further piece of supporting evidence, Kortynyk et al.,[44] in assigning the configurations of *cis*- and *trans*-1,4-acetamidocyclohexanol, noted that the ^{13}C NMR signals for the C-1 (hydroxyl-substituted) and C-3/C-5 ring carbons were shifted upfield for the *cis*-1,4 isomer versus the *trans*. We observed concordant shift differences for both the 4-hydroxy (**3**/**7**) and 3-hydroxy (**4**/**8**) pairs (in the 3-hydroxy pair, the relative upfield shift occurs in the *trans* isomer, as expected). This phenomenon is thought to be due to steric crowding and ring flattening when a non-hydrogen substituent is forced axial.[45] The recent assignments of *cis* and *trans* configurations for some isomeric 4-[(2-aminobenzyl)amino]cyclohexanols[46] also apparently rely on this effect, as do the spectral assignments for *cis*/*trans* mixtures of 3-aminocyclohexanol[41] and 4-dimethylaminocyclohexanol;[47] none of these last three reports provides a rationale or references for the given assignments, however.

Cyclohexyl ring conformation

Ring conformational equilibria do not appear to have been thoroughly investigated for cyclohexanol isomers

having an *NH*-linked substituent at the 3- or 4-position and no additional ring substitutuents. The opportunity for intramolecular ('flagpole–flagpole') hydrogen bonds in the *cis* isomers **3**, **5**, and **8** might significantly affect the conformational populations and dynamics. In the ^{1}H NMR spectrum of the *trans*-1,4 compound **7** dissolved in DMSO, the observed distinct signals for the axial (δ 1.1–1.2) and equatorial (δ 1.6–1.8) protons (Fig. 3A) are consistent with the expected stable conformation in which both non-hydrogen substituents are equatorial. In contrast, the merger of the corresponding methylene signals for the *cis*-1,4 analogue **3** into a single narrow band (δ 1.4–1.5, Fig. 3B) seems indicative of rapid equilibrium, possibly between the two chair conformers (if so, then presumably with the ring inverting while the large sulfonylureido substituent remains effectively stationary). This conclusion is consonant with the conformational mobility reported by Johnston et al.[48] for an N^1-(*cis*-4-*carboxy*cyclohexyl)urea. One or more twist-boat conformers might, however, be more energetically accessible than normal due to intramolecular *H*-bonding; although such an *H*-bond is not expected to be important in aqueous solution, it could become significant in DMSO-d_6, and also—biologically—in a receptor binding pocket. The 'freezing out' of conformational mobility upon binding to a receptor is entropically unfavorable, but simultaneous formation of an intramolecular *H*-bond could compensate for lost binding enthalpy if the resultant conformation is readily accommodated in the receptor pocket.

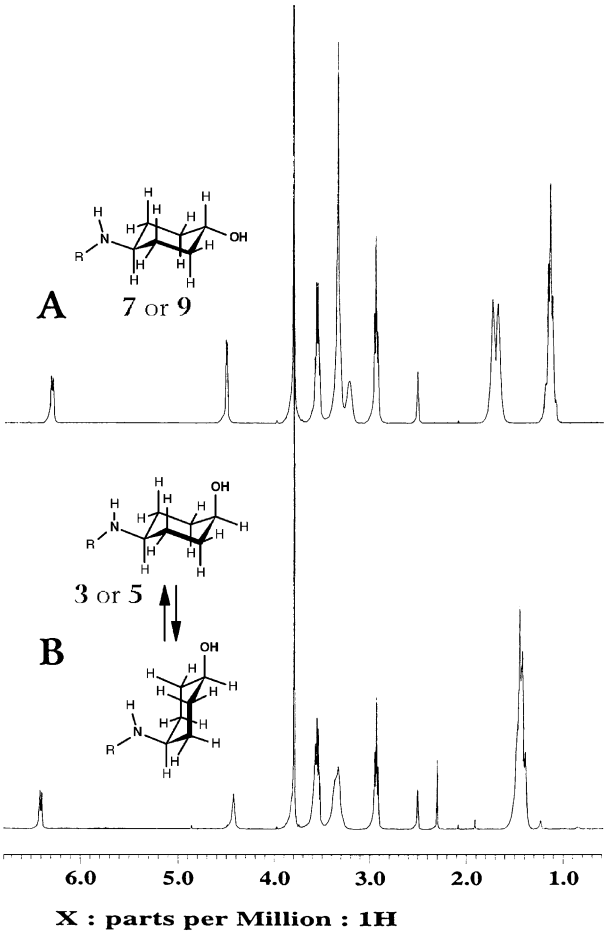


Figure 3. ^{1}H NMR of compounds **3** (A) and **7** (B), aliphatic regions.

In our pharmacological studies of the hydroxycyclohexyl-substituted sulfonylureas, statistically significant differences in IC_{50} values were observed for gliquidone (**2**) and its 4-hydroxycyclohexyl analogues **5** and **9**. The rank ordering (*trans*-4-hydroxy > gliquidone > *cis*-4-hydroxy) is in accord with the expected effects of conformational dynamics—that is, from predicted binding entropy differences. Of course, steric interference by the *cis*-hydroxyl group in **5** cannot be ruled out as a cause for its reduced binding affinity. For the corresponding glyburide series (**1**, **3**, and **7**), the same rank ordering was not observed, however. Neither was it seen for the 3-hydroxycyclohexyl glyburide analogues (i.e., **1**, **4**, and **8**). These discrepancies remain to be explained, and the planned synthesis of additional substituted-cyclohexyl analogues coupled with studies of the temperature-dependence of equilibrium binding is expected to provide further clarification.

Pharmacological significance

No receptor binding data, in pancreatic β-cells or any other tissue, has previously been reported for the two known hydroxylated metabolites of glyburide, **7** and **8**, and earlier reports indicated that these two compounds were poorly active as hypoglycemic agents (see Table 2). When we began this project, we had thus expected that the hydroxyl-substituted analogues would lose activity considerably versus the parent compounds, and were somewhat surprised by the high relative potencies of **3**–**5** and **7**–**9** in our binding assay. It is now rather clear that, with oral administration, a high first-pass metabolism greatly reduces the observed hypoglycemic activities of **7** and **8**. In a recent human study[49] (published about the

Table 2. Summary of literature data on the activities of the two hydroxycyclohexyl metabolites of glyburide

Compound	Hypoglycemia po in rabbit[a]	Hypoglycemia ip in rat[b]	Insulin release, isolated islets of Langerhans[c]	Hypoglycemia iv in human[d]
1, glyburide	1	1	1	1
7, *trans*-4-hydroxycyclohexyl	0.0025	0.15	~0.025	0.72
8, *cis*-3-hydroxycyclohexyl	0.025			0.5

[a]Ref 56.
[b]Ref 57. See also refs 58 and 59.
[c]Ref 60.
[d]Ref 49.

same time as our preliminary presentation of a portion of this work[50]), both the *trans*-4-hydroxy (**7**) and *cis*-3-hydroxy (**8**) metabolites were found to be potent hypoglycemics when administered intravenously, nearly as active as glyburide. Our observation of the potent inhibition of specific [^{3}H]glyburide binding in rat brain by **7** and **8** is in good agreement with that result because, in general, when a series of sulfonylureas is tested in binding or functional studies, it has been repeatedly observed that the same rank-ordering of potencies generally obtains in β-cells and brain;[1a,1b,1e,51] furthermore, excellent correlations have typically been obtained for plots of activity indices (i.e., IC_{50} or EC_{50} values) from one tissue versus those in the other. The receptor-level pharmacological basis for this correspondence has recently become more evident.[12]

Aside from glibornuride (Fig. 1), wherein the hydroxyl group is sterically occluded by alkyl groups, only one other *N*′-hydroxyalkyl sulfonylurea appears to have been evaluated in binding studies: Geisen et al.[1b] previously reported that the hydroxyethyl analogue **12** bound with 100-fold lower affinity in rat brain neuronal (cortical) membranes than glyburide (Table 1B). We found the relative activity of **12** to be even poorer, only 1/760 that of glyburide, though this still represents a modest level of potency. Our results suggest that, at least for high-affinity SUR binding in rat brain, the alkylurea substituent does not need to be strictly lipophilic. On the other hand, our observation that the stereochemical orientation of the hydroxyl substituent has only a modest effect (gliquidone series) or no effect (glyburide series) on the binding affinity of the 3- and 4-hydroxycyclohexyl analogues argues against any specific hydroxyl group interaction (e.g., hydrogen bonding) with the receptor. Given, however, that the 3-hydroxy compounds **4** and **8** were each tested as racemic mixtures, the possibility of enantiomeric activity differences has not yet been addressed.

Conclusions

All of the hydroxycyclohexyl analogues except the 2-*cis* compound, **11**, were comparably potent to the unsubstituted derivatives as inhibitors of specific [^{3}H]glyburide binding in rat brain preparations. This finding was somewhat unexpected at the outset, but is in accord with more recent reports of the high hypoglycemic potencies of **7** and **8**, and several of our hydroxyl-substituted analogues are easily potent enough to allow for the synthesis of various types of prodrugs. The findings of Rydberg and co-workers[52] suggest, however, that installation of a hydroxyl group in the alkylurea portion of the molecule may bring about certain unexpected, fundamental changes in pharmacodynamic character; this alone is of sufficient scientific interest to warrant further research. We plan further studies of pharmacological activity, biodistribution, and metabolism, directed towards enhanced understanding—and eventual therapeutic exploitation—of the pharmacology of K^+_{ATP} channels in the CNS.

Experimental

Instrumentation and materials

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. Infrared spectra of samples (as KBr pellets) were obtained on a Nicolet Analytical 5MX FT spectrometer, and NMR spectra on a JEOL FX-90Q spectrometer (90 MHz) or a JEOL JNM GSX-270 (270 MHz) or a Brüker AM-400 spectrometer (400 MHz). Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Electron impact (EI) mass spectra were recorded on Finnigan MAT TSQ 4510 or Finnigan MAT 900 double-focusing magnetic sector spectrometers. Fast atom bombardment (FAB) mass spectra were acquired in a glycerol or 3-nitrobenzyl alcohol matrix with a Finnigan MAT TSQ-70 equipped with an ANTEK cesium ion source and an acceleration voltage of 5 kV; or a Finnigan MAT 900 double-focusing instrument with a Finnigan cesium ion source and an acceleration voltage of 6 kV. Gas chromatography/electron impact (GC/EI) mass spectra were recorded on a Hewlett-Packard 5971 GC/MS with mass-selective detector, using a 30 mL × 0.25 cm i.d., 0.25-μm DB-5 capillary column with 1 mL/min helium gas flow rate and a 40–280 °C temperature ramp. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Elemental analyses were obtained from Galbraith Laboratories, Inc., Knoxville, TN, USA, or from Atlantic Microlab, Inc., Norcross, GA, USA. All commercially available reagents and solvents were used without further purification, unless otherwise noted. TLC plates (silica gel HLF, 250 μ layer) were obtained from Analtech, Inc.

Synthetic methods

4-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulfonamide (20). The procedure of Egg et al.[18] was followed, starting with 12.5 g of glyburide, except that after refluxing for 4 h with a drying tube (Drierite®) in place, four drops of water were added to the reaction mixture, and upon refrigerating overnight the product **20** crystallized (yield 5.66 g, 61%, lit.[18] 29%); recrystallization from 95% EtOH gave an analytical sample, mp 214–215 °C (lit.[18] mp 210–211 °C).

Ethyl 4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzene sulfonamide carbamate (21). The procedure was based on the general method of Marshall and Sigal[19] (who did not, however, synthesize glyburide); thus, a mixture of the sulfonamide **20** (3.0 g, 8.1 mmol) and K_2CO_3 (2.92 g, 21.1 mmol, dried overnight at 100 °C) in acetone (90 mL, dried over molecular sieves) was brought to reflux with a drying tube (Drierite®) in place. Ethyl chloroformate (0.86 mL, 10.7 mmol) was added dropwise during a period of 2 h. The reaction mixture was further refluxed for 24 h, while the progress was monitored by TLC on silica (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$). The solvent was then evaporated in vacuo. Water (100 mL) was added to the residue, and the mixture was acidified with 1 N HCl to pH 2–3. The precipitated product was collected by filtering, washed

with water, and air-dried. Recrystallization from 95% EtOH gave white crystals of compound **21** (2.5 g, 70%), mp 154–156 °C (lit.[17] mp 154–155 °C). The ^{1}H NMR and IR were also basically consistent with the literature,[17] but are given here due to additions and minor variances, whereas ^{13}C NMR data appears absent from the literature: ^{1}H NMR (90 MHz, DMSO-d_6) δ 11.95 (s, 1H, SO_2NHCO), 8.26 (t, 1H, CONH), 7.85 (d, 2H, J = 8.2 Hz, Ar–H), 7.70–7.35 (m, 4H, Ar–H), 7.15 (d, 1H, J = 8.9 Hz, Ar–H), 4.00 (q, 2H, J = 7.0 Hz, CH_2CH_3), 3.80 (s, 3H, OCH_3), 3.56 (dt, 2H, $NHCH_2CH_2$), 2.94 (t, 2H, J = 6.5 Hz, $NHCH_2CH_2$), 1.09 (t, 3H, J = 7.0 Hz, CH_2CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 163.2 (ArCONH), 155.7 (SO_2NHCO), 151.1 (C-OCH_3), {146.2, 137.8, 134.4, 132.9, 132.5, 129.6, 128.0, 127.3, 114.2} (Ar–C), 63.1 (CH_2CH_3), 56.2 (OCH_3), 40.6 ($NHCH_2$), 35.6 (CH_2–Ar), 14.1 (CH_2CH_3); IR (cm^{-1}) 3350 (N–H), {1736, 1634} (C=O), 1531 (NH bend), 1451 (C=C), 1352 (SO_2 asym), 1159 (SO_2 sym).

1-[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl]-3-(*cis*-4-hydroxycyclohexyl)urea (3) and 1-[4-[2-(5-chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl]-3-(*trans*-4-hydroxycyclohexyl)urea (7). Toluene (5 mL) was added to 4-aminocyclohexanol (0.5 mL of a 50% w/w solution in water, 2.3 mmol) and the water/toluene azeotropic mixture was distilled off in a rotary evaporator. This procedure was repeated twice. To the resulting 4-aminocyclohexanol, carbamate **21** (1.0 g, 2.3 mmol) and dioxane (9 mL) were added, and the reaction mixture was refluxed for 2 h with a drying tube (Drierite®) over the condenser. The reaction was monitored by TLC (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$), and upon completion (approx. 2 h) the solvent was evaporated from the reaction mixture in vacuo. Water (30 mL) was added to the residue, and the mixture was acidified with 1 N HCl to pH 2–3. The resulting somewhat-gummy solid, containing a mixture of compounds **3** and **7**, was filtered off and air-dried to give an amorphous solid (1.0 g, ca. 85%). The product was dissolved in a sufficient quantity of methanol and adsorbed on silica gel (10 g) by evaporating to dryness (rotary evaporator). This silica adsorbate was packed in a short precolumn (Michel-Miller®, Ace Glass, 130 mm L × 22 mm i.d.), and eluted onto the main chromatographic silica gel column (450 mm L × 51 mm i.d., presolvated with benzene) with 10% MeOH/1% glacial AcOH in benzene. The two product fractions, the first containing compound **3** and the second containing compound **7**, each eluted in approximately 200 mL of eluent. For each fraction, the eluent was distilled off on a rotary evaporator, and the residue was subsequently mixed with toluene (300 mL) and again evaporated to remove acetic acid by azeotropic codistillation. This procedure was repeated twice to provide compound **3** (0.27 g) and compound **7** (0.2 g; some material was accidentally lost during the separation process). Compound **7** corresponded by TLC to the standard sample of the *trans*-4 compound graciously provided by The Upjohn Co., Kalamazoo, MI, USA (eluent: 10% MeOH/0.5% glacial AcOH in benzene). Analytical data for compound **3:** mp 143–147 °C (95% EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H, $SO_2NHCONH$), 8.26 (t, 1H, J = 5.2 Hz, $NHCH_2CH_2$), 7.82 (d, 2H, J = 8.0 Hz, Ar–H), 7.63 (d, 1H, J = 2.5 Hz, Ar–H), 7.54–7.38 (m, 3H, Ar–H), 7.13 (d, 1H, J = 8.9 Hz, Ar–H), 6.38 (d, 1H, J = 7.3 Hz, $SO_2NHCONH$), 4.42 (s, 1H, OH), 3.78 (s, 3H, OCH_3), 3.63–3.42 (m, 3H, $NHCH_2CH_2$, NHCH or CHOH), 3.33 (br s, CHOH or NHCH overlapped with H_2O in DMSO-d_6), 2.92 (t, 2H, J = 6.8 Hz, $NHCH_2CH_2$), 1.58–1.24 (m, 8H, cyclohexyl CH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ 163.61 (ArCONH), 155.65 (NHCONH), 150.71 ($COCH_3$), 145.06 [(Ar)CSO_2NH], {138.37, 131.47, 129.47, 129.23, 127.24, 124.82, 124.30, 114.12} (Ar–C), 64.71 (CHOH), 56.18 (OCH_3), 46.31 (CHNH), 40.14 ($ArCONHCH_2$), 34.63 (CH_2–Ar), {30.76, 27.23} (cyclohexyl CH_2); IR (cm^{-1}) 3406 (O–H), 3350 (N–H), {1686, 1649} (C=O), 1535 (NH bend), 1483 (C=C), 1273 (SO_2 asym), 1165 (SO_2 sym); MS (FAB) *m/e* 512 (MH^+, ^{37}Cl), 510 (MH^+, ^{35}Cl). Anal. calcd for $C_{23}H_{28}ClN_3O_6S{\cdot}H_2O$: C, 52.32; H, 5.73; N, 7.96; found: C, 52.17; H, 5.61; N, 7.89. Analytical data for compound **7**: mp 184–186 °C (95% EtOH) (lit.[17] mp 174–175 °C; standard sample provided by Upjohn: mp 181–183 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H, $SO_2NHCONH$), 8.26 (t, 1H, J = 5.1 Hz, $NHCH_2CH_2$), 7.82 (d, 2H, J = 8.2 Hz, Ar–H), 7.62 (d, 1H, J = 2.1 Hz, Ar–H), 7.54–7.41 (m, 3H, Ar–H), 7.13 (d, 1H, J = 8.9 Hz, Ar–H), 6.28 (d, 1H, J = 7.1 Hz, $SO_2NHCONH$), 4.49 (d, 1H, J = 3.7 Hz, OH), 3.78 (s, 3H, OCH_3), 3.53 (dt, 2H, $NHCH_2CH_2$), 3.40–3.25 (s, NHCH or CHOH overlapped with H_2O in DMSO-d_6), 3.19 (br s, 1H, NHCH or CHOH), 2.92 (t, 2H, J = 6.8 Hz, $NHCH_2CH_2$), 1.83–1.55 (m, 4H, cyclohexyl CH_2), 1.12 (m, 4H, cyclohexyl CH_2); ^{1}H NMR (400 MHz, $CDCl_3$) gave δ 3.40 (tt, J = 10.3. 3.8 Hz, CHOH) and 3.30 (br m, CHNH); ^{13}C NMR (400 MHz, DMSO-d_6) δ 163.62 (ArCONH), 155.65 (NHCONH), 150.56 ($COCH_3$), 145.19 [(Ar)CSO_2NH], {138.15, 131.47, 129.47, 129.25, 127.29, 124.83, 124.31, 114.12} (Ar–C), 67.71 (CHOH), 56.18 (OCH_3), 47.94 (NHCH), 40.13 ($ArCONHCH_2$), 34.64 (CH_2–Ar), {33.54, 30.09} (cyclohexyl CH_2); IR (cm^{-1}) 3405 (O–H), 3352 (N–H), {1668, 1636} (C=O), 1539 (NH bend), 1462 (C=C), 1275 (SO_2 asym), 1165 (SO_2 sym); MS (FAB) *m/e* 512 (MH^+, ^{37}Cl), 510 (MH^+, ^{35}Cl). Anal. calcd for $C_{23}H_{28}ClN_3O_6S$: C, 54.17; H, 5.53; N, 8.24; found: C, 54.20; H, 5.65; N, 8.04.

1-[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl]-3-(*trans*-3-hydroxycyclohexyl)urea (4) and 1-[4-[2-(5-chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl]-3-(*cis*-3-hydroxycyclohexyl)urea (8). A procedure similar to that used for the synthesis of compounds **3** and **7** was followed. The carbamate **21** (1.20 g, 2.7 mmol) and 3-aminocyclohexanol[21] (0.32 g, 2.8 mmol) were reacted in dioxane (6.4 mL) to yield a gummy off-white product (1.1 g, ca. 80%) containing a mixture of compounds **4** and **8**. These were separated by column chromatography in a way similar to that for compounds **3** and **7** (eluent: 30% CH_3CN/1% glacial AcOH in benzene). Compound **8** eluted first, then compound **4**, each in approximately 1 L of eluent; TLC (30% CH_3CN/0.5% glacial AcOH in benzene) showed that compound **8** corresponded to an analytical standard sample of the

cis-3 compound provided by Upjohn. The eluent was evaporated in vacuo from each of the collected fractions; further codistillation with toluene, as for compounds **3** and **7**, followed by crystallization from EtOAc and 95% EtOH, respectively, yielded compound **4** (0.37 g) and compound **8** (0.12 g; some material was lost during the separation process). Analytical data for compound **4**: mp $\geq$140 °C (abs EtOH/benzene); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H, $SO_2NHCONH$), 8.26 (t, 1H, J = 5.3 Hz, $NHCH_2CH_2$), 7.82 (d, 2H, J = 8.0 Hz, Ar–H), 7.63 (d, 1H, J = 2.6 Hz, Ar–H), 7.56–7.39 (m, 3H, Ar–H), 7.14 (d, 1H, J = 8.9 Hz, Ar–H), 6.29 (d, 1H, J = 7.6 Hz, $SO_2NHCONH$), 4.43 (d, 1H, J = 2.8 Hz, OH), 3.78 (s, 3H, OCH_3, overlapped with multiplet at δ 3.80–3.72), 3.80–3.72 (m, 1H, CHOH or NHCH), 3.71–3.51 (m, 1H, CHOH or NHCH), 3.53 (dt, 2H, $NHCH_2CH_2$), 2.91 (t, 2H, J = 6.8 Hz, $NHCH_2CH_2$), 1.62–1.02 (m, 8H, cyclohexyl CH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ 163.63 (ArCONH), 155.67 (NHCONH), 150.66 ($COCH_3$), 145.09 [(Ar)CSO_2NH], {138.36, 131.49, 129.48, 129.25, 127.26, 124.83, 124.31, 114.13} (Ar–C), 64.54 (CHOH), 56.20 (OCH_3), 44.40 (NHCH), 40.15 ($ArCONHCH_2$), 34.65 (CH_2–Ar), {(1C imbedded under solvent peaks), 32.62, 31.52, 19.01} (cyclohexyl CH_2); IR (cm^{-1}) 3500 (O–H), 3350 (N–H), {1699, 1636} (C=O), 1543 (NH bend), 1483 (C=C), 1275 (SO_2 asym), 1163 (SO_2 sym); MS (FAB) *m*/*e* 512 (MH^+, ^{37}Cl), 510 (MH^+, ^{35}Cl). Anal. calcd for $C_{23}H_{28}ClN_3O_6S$: C, 54.17; H, 5.53; N, 8.24; found: C, 54.52; H, 5.81; N, 7.50. Analytical data for compound **8**: mp 191–193 °C (95% EtOH), standard sample from Upjohn: mp 184–187 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H, $SO_2NHCONH$), 8.27 (t, 1H, J = 5.6 Hz, $NHCH_2CH_2$), 7.83 (d, 2H, J = 8.2 Hz, Ar–H), 7.62 (d, 1H, J = 2.7 Hz, Ar–H), 7.55–7.42 (m, 3H, Ar–H), 7.13 (d, 1H, J = 8.9 Hz, Ar–H), 6.53 (d, 1H, J = 7.2 Hz, $SO_2NHCONH$), 4.67 (d, 1H, J = 4.0 Hz, OH), 3.78 (s, 3H, OCH_3), 3.53 (dt, 2H, $NHCH_2CH_2$), 3.46–3.25 (m, CHOH and NHCH overlapped with H_2O in DMSO-d_6), 2.92 (t, 2H, J = 7.0 Hz, $NHCH_2CH_2$), 1.81 (multiplet appearing as a gross doublet, 1H, J = 11.8 Hz, $NHCHCH_{equat}H$CHOH), 1.74–1.46 (m, 3H, other equatorial CHs in cyclohexyl CH_2), 1.11–0.90 (m, 4H, axial CH in cyclohexyl CH_2). In a separate experiment, D_2O was added to reveal the signals for CHOH (δ 3.36, m, 1H) and CHNH (δ 3.25, m, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 163.64 (ArCONH), 155.66 (NHCONH), 150.44 ($COCH_3$), 145.18 [(Ar)CSO_2NH], {138.21, 131.49, 129.47, 129.27, 127.28, 124.84, 124.31, 114.13} (Ar–C), 67.00 (CHOH), 56.19 (OCH_3), 46.78 (NHCH), 40.15, 34.65, {40.95, 34.12, 31.39, 20.47} (cyclohexyl CH_2); IR (cm^{-1}) 3555 (O–H), 3350 (N–H), 1661 (C=O), 1531 (NH bend), 1456 (C=C), 1275 (SO_2 asym), 1171 (SO_2 sym); MS (FAB) *m*/*e* 512 (MH^+, ^{37}Cl), 510 (MH^+, ^{35}Cl). Anal. calcd for $C_{23}H_{28}ClN_3O_6S{\cdot}0.5H_2O$: C, 53.23; H, 5.63; N, 8.10; found: C, 53.57; H, 5.48; N, 7.83.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(*trans*-2-hydroxycyclohexyl)urea (11). *trans*-2-Aminocyclohexanol hydrochloride (2.0 g) was dissolved in a saturated solution of Na_2CO_3 (15 mL). The free base was extracted with EtOAc (100 mL), which was then distilled off in a rotary evaporator to give 1.06 g of the free base (70%). A mixture of the resulting *trans*-2-aminocyclohexanol (0.62 g, 5.4 mmol) and the carbamate **21** (1.2 g, 2.7 mmol) was refluxed in dry 1,4-dioxane (10 mL) for 10 h, with a drying tube (Drierite®) over the condenser. The progress was monitored by TLC on silica (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$). The 1,4-dioxane was distilled off in vacuo, and water (150 mL) was added to the residue. The insoluble material was filtered out, and the filtrate was acidified with 1 N HCl (30 mL). Recrystallization of the resulting product in 95% EtOH yielded 0.35 g (25%) of **11**: mp 165–166 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 11.07 (s, 1H, SO_2NH), 8.25 (t, 1H, ArCONH), 7.88–7.13 (m, 7H, Ar–H), 6.38 (d, 1H, NHCONH), 4.71 (br s, 1H, OH), 3.80 (s, 3H, OCH_3), 3.67–3.37 (br m, Ar–$CONHCH_2$, NHCH, and CHOH overlapped with H_2O in DMSO-d_6), 2.98 (br t, CH_2–Ar), 1.80–1.21 [br m, 8H, –CH_2–(cyclohexyl)]; ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.6 (ArCONH), 155.7 (NHCONH), 151.1 (C-OCH_3), {145.2, 138.3, 131.5, 129.5, 129.3, 127.3, 124.7, 124.3, 114.1} (Ar–C), 70.9 (CHOH), 56.2 (OCH_3), 55.1 (NHCH), 40.2 ($ArCONHCH_2$), 34.7 (CH_2–Ar), {34.1, 30.9, 23.8, 23.6} (cyclohexyl–CH_2); IR (cm^{-1}) {3550, 3315} (O–H, N–H), {1716, 1622} (C=O), 1522 (NH bend), 1461 (C=C), 1277 (SO_2 asym), 1166 (SO_2 sym). Anal. calcd for $C_{23}H_{28}ClN_3O_6S$: C, 54.28; H, 5.53; N, 8.24; found: C, 54.28; H, 5.52; N, 8.30.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(2-hydroxyethyl)urea (12). A mixture of the carbamate **21** (1.53 g, 3.50 mmol) and 2-aminoethanol (0.5 mL, 7 mmol) in 1,4-dioxane (10 mL, dried over 3-Å molecular sieves) was refluxed for 4 h with a drying tube (Drierite®) over the condenser. The progress was monitored by TLC on silica (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$). Two layers formed after the reaction mixture was cooled to room temperature and allowed to stand overnight. The 1,4-dioxane layer was decanted, and 1 N HCl (30 mL) and EtOAc (10 mL) were added to the gummy bottom layer. After stirring for about 20 min, the resulting solid was collected by filtration, and recrystallized from 95% EtOH to give 0.52 g (33%) of **12** as the monoethanolate: mp 125–127 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.41 (s, 1H, SO_2NH), 8.38 (br t, 1H, Ar–CONH), 7.83 (d, 2H, J = 8.1 Hz, Ar–H), 7.54 (d, 1H, J = 3.1 Hz, Ar–H), 7.53–7.44 (m, 3H, Ar–H), 7.10 (d, 1H, J = 9.0 Hz, Ar–H), 6.48 (t, 1H, CONHCH_2CH_2OH), 5.54–4.34 (br m, 2H, OH in **12** and OH in solvate EtOH), 3.81 (s, 3H, OCH_3), 3.54–3.32 (br m, Ar–$NHCH_2$, CH_2CH_2OH, CH_2OH, ethanol CH_3CH_2OH overlapped with H_2O in DMSO-d_6), 2.95 (t, 2H, J = 6.5 Hz, CH_2–Ar), 1.07 (t, 3H, J = 7.1 Hz, $HOCH_2CH_3$, solvate ethanol); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.7 (ArCONH), 155.8 (NHCONH), 151.4 (C–OCH_3), {145.3, 138.2, 131.6, 129.6, 129.4, 127.4, 124.8, 124.4, 114.2} (Ar–C), 59.7 ($NHCH_2CH_2OH$), 56.2 ($HOCH_2CH_3$, ethanol), 55.1 (OCH_3), 41.8 (Ar–$CONHCH_2$), 34.7 (CH_2–Ar), 18.6 ($HOCH_2CH_3$, ethanol); IR (cm^{-1}) {3458, 3363} (O–H, N–H), {1772, 1693} (C=O), 1537 (NH bend), 1477 (C=C), 1273 (SO_2 asym), 1160 (SO_2 sym). Anal. calcd for $C_{19}H_{22}ClN_3O_6S{\cdot}CH_3CH_2OH$: C, 50.25; H, 5.62; N, 8.37; found: C, 50.50; H, 5.41; N, 8.53.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(3-hydroxypropyl)urea (13). Following the method described for the synthesis of **12**, carbamate **21** (1.5 g, 3.4 mmol) and 3-amino-1-propanol (0.60 mL, 6.8 mmol) in dry 1,4-dioxane (15 mL) yielded 0.58 g (36%) of compound **13**: mp 91–93 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.31 (s, 1H, SO_2NH), 8.27 (t, 1H, J = 4.4 Hz, ArCONH), 7.90–7.15 (m, 7H, Ar–H), 6.52 (br t, 1H, NHCONH), 4.48 (br s, 1H, OH), 3.81 (s, 3H, OCH_3), 3.63–3.42 (br m, 4H, Ar–CONHCH_2, NHCONHCH_2 or CH_2OH), 3.59–2.94 (m, CH_2–Ar and CH_2OH or NHCONHCH_2 overlapped with H_2O in DMSO-d_6), 1.55–1.41 (m, 2H, CH_2C$H_2$$CH_2$); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.6 (ArCONH), 155.6 (NHCONH), 151.3 (C–OCH_3), {145.1, 138.2, 131.5, 129.5, 129.3, 127.2, 124.8, 124.3, 114.1} (Ar–C), 58.4 (CH_2OH), 56.2 (OCH_3), 40.8 (NHCONHCH_2), 40.1 (ArCONHCH_2), 34.6 (CH_2–Ar), 32.1 (CH_2CH_2OH); IR (cm^{-1}) {3381, 3375} (O–H, N–H), {1662, 1641} (C=O), 1562 (NH bend), 1468 (C=C), 1273 (SO_2 asym), 1165 (SO_2 sym). Anal. calcd for $C_{20}H_{24}ClN_3O_6S{\cdot}H_2O$: C, 49.22; H, 5.37; N, 8.61; found: C, 49.55; H, 5.21; N, 8.82.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(4-hydroxybutyl)urea (14). Following the method described for the synthesis of **12**, carbamate **21** (2.0 g, 4.5 mmol) and 4-amino-1-butanol (0.80 mL, 6.8 mmol) in dry 1,4-dioxane (20 mL) yielded 0.92 g (42%) of compound **14**: mp 135–136 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.31 (s, 1H, SO_2NH), 8.24 (t, 1H, J = 4.4 Hz, ArCONH), 7.81–7.14 (m, 7H, Ar–H), 6.52 (br t, 1H, NHCONH), 4.41 (br s, 1H, OH), 3.81 (s, 3H, OCH_3), 3.62–3.29 (br m, C$H_2$$CH_2$Ar, NHCONHC$H_2$, C$H_2$OH overlapped with H_2O in DMSO-d_6), 2.98 (t, 2H, CH_2–Ar), 1.41–1.25 (br m, 4H, CH_2CH_2C$H_2$$CH_2$); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.6 (ArCONH), 155.6 (NHCONH), 151.3 (C–OCH_3), {145.1, 138.2, 131.5, 129.5, 129.3, 127.3, 124.8, 124.3, 114.1} (Ar–C), 60.3 (CH_2OH), 56.2 (OCH_3), 40.2 (NHCONHCH_2), 38.8 (Ar–CONHCH_2), 34.6 (CH_2–Ar), 29.6 (C$H_2$$CH_2OH$), 25.9 (NHCONHC$H_2CH_2$); IR (cm^{-1}) {3389, 3349} (O–H, N–H), {1663, 1639} (C=O), 1542 (NH bend), 1462 (C=C), 1275 (SO_2 asym), 1179 (SO_2 sym). Anal. calcd for $C_{21}H_{26}ClN_3O_6S{\cdot}0.5H_2O$: C, 51.16; H, 5.52; N, 8.52; found: C, 51.22; H, 5.52; N, 8.52.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(5-hydroxypentyl)urea (15). Following the method described for the synthesis of **12**, carbamate **21** (1.5 g, 3.4 mmol) and 5-amino-1-pentanol (0.80 mL, 6.8 mmol) in dry 1,4-dioxane (15 mL) yielded 0.82 g (48%) of compound **15**: mp 122–124 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.42 (s, 1H, SO_2NH), 8.28 (t, 1H, ArCONH), 7.85–7.13 (m, 7H, Ar–H), 6.47 (br t, 1H, NHCONH), 4.34 (br s, 1H, OH), 3.80 (s, 3H, OCH_3), 3.46–3.25 (br m, Ar–CONHCH_2, NHCONHCH_2, and CH_2OH overlapped with H_2O in DMSO-d_6), 2.94 (t, 2H, CH_2–Ar), 1.49–1.21 (br m, 6H, CH_2CH_2CH_2CH_2CH_2OH); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.6 (ArCONH), 155.6 (NHCONH), 151.2 (C–OCH_3), {145.1, 138.2, 131.5, 129.5, 129.3, 127.2, 124.8, 124.3, 114.1} (Ar–C), 60.5 (CH_2OH), 56.2 (OCH_3), 40.1 (NHCONHCH_2), 39.2 (Ar–CONHCH_2), 34.6 (CH_2–Ar), 32.0 (C$H_2$$CH_2OH$), 29.0 (NHCONHC$H_2CH_2$), 22.7 ($CH_2CH_2CH_2$$CH_2$$CH_2$); IR (cm^{-1}) {3464, 3349} (O–H, N–H), {1669, 1609} (C=O), 1535 (NH bend), 1455 (C=C), 1275 (SO_2 asym), 1166 (SO_2 sym). Anal. calcd for $C_{22}H_{28}ClN_3O_6S{\cdot}0.5H_2O$: C, 52.12; H, 5.77; N, 8.29; found: C, 51.26; H, 5.68; N, 8.30.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(1,1-dimethyl-2-hydroxyethyl)urea (16). A mixture of the carbamate **21** (1.0 g, 2.3 mmol) and 2-amino-2-methyl-1-propanol (0.85 mL, 9.2 mmol) in dry 1,4-dioxane (10 mL) was refluxed for 10 h with a drying tube (Drierite®) over the condenser. The progress was monitored by TLC on silica (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$). Upon cooling at room temperature overnight, a white solid had crystallized. This solid was collected by filtration and washed with EtOAc, and then added to 150 mL of water. The insoluble material was filtered out, and the solution was then acidified with 1 N HCl (30 mL), causing the product to crystallize as an amorphous solid. Recrystallization from 95% EtOH yielded 0.50 g (45%) of **16**: mp 162–164 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.39 (s, 1H, SO_2NH), 8.29 (t, 1H, ArCONH), 7.86–7.14 (m, 7H, Ar–H), 6.30 (s, 1H, NHCONH), 4.95 (br s, 1H, OH), 3.81 (s, 3H, OCH_3), 3.63–3.24 (br m, Ar–CONHCH_2 and CH_2OH overlapped with H_2O in DMSO-d_6), 2.94 (t, 2H, CH_2–Ar), 1.20 (s, 6H, $C(CH_3)_2$); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.7 (ArCONH), 155.8 (NHCONH), 150.4 (C–OCH_3), {145.2, 138.3, 131.6, 129.6, 129.4, 127.3, 124.8, 124.4, 114.2} (Ar–C), 67.8 (CH_2OH), 56.3 (OCH_3), 53.9 (C$(CH_3)_2$), 40.3 (Ar–CONHCH_2), 34.7 (CH_2–Ar), 23.3 (C$(CH_3)_2$); IR (cm^{-1}) {3540, 3381} (O–H, N–H), {1716, 1622} (C=O), 1545 (NH bend), 1462 (C=C), 1275 (SO_2 asym), 1159 (SO_2 sym). Anal. calcd for $C_{21}H_{26}ClN_3O_6S$: C, 52.12; H, 5.42; N, 8.68; found: C, 52.24; H, 5.39; N, 8.61.

1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl}-3-(2-hydroxy-1-hydroxymethyl-1-methylethyl)urea (17). A mixture of the carbamate **21** (1.0 g, 2.3 mmol) and 2-amino-2-methyl-1,3-propanediol (0.48 g, 4.6 mmol) was reacted in dry 1,4-dioxane (20 mL), proceeding as for compound **11**. Recrystallization of the resulting solid in 95% EtOH yielded 0.22 g (19%) of **17**: mp 80–82 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.91 (s, 1H, SO_2NH), 8.26 (t, 1H, ArCONH), 7.85–7.15 (m, 7H, Ar–H), 6.34 (s, 1H, NHCONH), 4.90 (br s, 2H, OH), 3.80 (s, 3H, OCH_3), 3.81–3.39 (br m, Ar–CONHCH_2 and both CH_2OH overlapped with H_2O in DMSO-d_6), 2.95 (t, 2H, CH_2–Ar), 1.07 (s, 3H, CCH_3); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.7 (ArCONH), 155.8 (NHCONH), 150.7 (C–OCH_3), {145.2, 138.3, 131.6, 129.6, 129.4, 127.2, 124.7, 124.4, 114.2} (Ar–C), 63.5 (CH_2OH), 57.4 (NHCONHC), 56.2 (OCH_3), 40.2 (Ar–CONHCH_2), 34.7 (CH_2–Ar), 18.3 (CCH_3); IR (cm^{-1}) {3562, 3369} (O–H, N–H), {1703, 1639} (C=O), 1541 (NH bend), 1475 (C=C), 1242 (SO_2 asym), 1157 (SO_2 sym). Anal. calcd for $C_{21}H_{26}ClN_3O_7S$: C, 50.45; H, 5.24; N, 8.40; found: C, 50.36; H, 5.22; N, 8.38.

(+)-1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]-sulfonyl}-3-(2-hydroxy-1*S*-methylethyl)urea (18). A mixture of the carbamate **21** (1.0 g, 2.3 mmol) and (*S*)-(+)-

2-amino-1-propanol (0.72 mL, 9.2 mmol) in dry 1,4-dioxane (10 mL) was refluxed for 10 h, and the product was isolated in the same way as compound **17** above. Recrystallization from 95% EtOH yielded 0.48 g (44%) of **18**: mp 169–171 °C; $[\alpha]_D^{23} = +18°$ (1,4-dioxane, *c* 5); ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.39 (s, 1H, SO_2NH), 8.27 (t, 1H, ArCONH), 7.85–7.13 (m, 7H, Ar–H), 6.36 (d, 1H, NHCONH), 4.83 (br s, 1H, OH), 3.80 (s, 3H, OCH_3), 3.63–3.42 (br m, $NHCH_2CH_2Ar$, NHCH, and CH_2OH overlapped with H_2O in DMSO-d_6), 2.94 (br t, 2H, CH_2–Ar), 0.96 (d, 3H, $J = 6.3$ Hz, $CHCH_3$); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.7 (ArCONH), 155.7 (NHCONH), 150.8 (C–OCH_3), {145.2, 138.2, 131.5, 129.5, 129.3, 127.3, 124.8, 124.4, 114.1} (Ar–C), 64.0 (CH_2OH), 56.2 (OCH_3), 47.1 (NHCONHCH), 40.2 ($ArCONHCH_2$), 34.7 (CH_2–Ar), 17.3 ($CHCH_3$); IR (cm^{-1}) {3564, 3338} (O–H, N–H), {1709, 1618} (C=O), 1531 (NH bend), 1477 (C=C), 1278 (SO_2 asym), 1159 (SO_2 sym). Anal. calcd for $C_{20}H_{24}ClN_3O_6S$: C, 51.11; H, 5.15; N, 8.94; found: C, 51.19; H, 5.14; N, 8.90.

(−)-1-{[4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenyl]-sulfonyl}-3-(2-hydroxy-1*R*-methylethyl)urea (19). Following the method described for the synthesis of **18**, the carbamate **21** (1.0 g, 2.3 mmol) and *R*-(−)-2-amino-1-propanol (0.72 mL, 9.2 mmol) yielded 0.50 g (46%) of compound **19**: mp 168–170 °C; $[\alpha]_D^{23} = -18°$ (1,4-dioxane, *c* 5). The ^{1}H NMR, ^{13}C NMR, and IR spectra were identical to those obtained for the *S* isomer (compound **18**). Anal. calcd for $C_{20}H_{24}ClN_3O_6S$: C, 51.11; H, 5.15; N, 8.94; found: C, 51.22; H, 5.18; N, 8.87.

3-Trichloromethyl-6-methoxy-1(3*H*)-isobenzofuranone (25a). The procedure followed was essentially that of Desai and Usgaonkar,[22] which was in turn based on those of Fritsch[23] and of Chakravarti and Perkin;[24] however, a drying tube over the flask was essential to obtain the literature yield of **25a**, mp 132–134 °C (lit.[22] mp 130–132 °C as obtained, 136–137 °C from AcOH); ^{1}H NMR (90 MHz, DMSO-d_6) δ 7.88 (dd, 1H, $J_{ortho} = 7.6$ Hz, $J_{meta} = 3.2$ Hz, Ar–H), 7.58–7.25 (m, 2H, Ar–H), 6.53 (s, 1H, $CHCCl_3$), 3.90 (s, 3H, OCH_3); IR (cm^{-1}) 1780 (C=O), 1499 (C=C), 1289, 1069, 1005, 866, 826, 814.

3-Trichloromethyl-6-hydroxy-1(3*H*)-isobenzofuranone (25b). A round-bottomed flask fitted with a drying tube (Drierite®) was charged with *m*-hydroxybenzoic acid (**24b**; 2.0 g, 15 mmol) and chloral hydrate (2.4 g, 15 mmol) in concd sulfuric acid (6 mL). This mixture was stirred at room temperature for 48 h. A workup similar to that for compound **25a** gave compound **25b** (3.2 g, 82%), mp 195–197 °C (lit.[22] mp 195–197 °C as obtained, 199–200 °C from AcOH; lit.[53] mp 199–200 °C from alcohol); ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.56 (s, 1H, OH), 7.79 (d, 1H, $J_{ortho} = 8.1$ Hz, Ar–H), 7.42–7.10 (m, 2H, Ar–H), 6.46 (s, 1H, Ar–H); with two drops of D_2O added there was no peak at δ 10.56; IR (cm^{-1}) 3290 (v br, O–H), 1746 (C=O), 1508, 1370, 1312, 1225, 1069, 1005, 882, 821, 787, 770.

2-(β,β-Dichloroethenyl)-5-methoxybenzoic acid (26a). The procedure of Desai and Usgaonkar[22] (essentially also that of Hurry and Meldrum[30]) was followed. Reduction of **25a** (4.0 g, 14 mmol) with zinc dust (3.16 g) in glacial acetic acid (30 mL) yielded **26a** (3.0 g, 84%), mp 166–168 °C (MeOH). Desai and Usgaonkar[22] reported the product to be 2-(β,β-dichloroethyl)-5-methoxybenzoic acid (mp 167–168 °C, MeOH), as did Hurry and Meldrum[30] (mp 165 °C, AcOH); Dharwarkar and Alimchandani,[31] who correctly reported the structure, gave mp 167–168 °C (EtOH). Analytical data for **26a**: ^{1}H NMR (90 MHz, DMSO-d_6) δ 13.23 (br s, 1H, COOH), 7.70–7.36 (m, 3H, Ar–H, $CH{=}CCl_2$), 7.20 (dd, 1H, $J_{ortho} = 8.6$ Hz, $J_{meta} = 3.1$ Hz, Ar–H), 3.82 (s, 3H, OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ 167.19 (COOH), 158.96 ($COCH_3$), 131.46, 131.18, 129.48, 126.11, 118.41, 117.72, 115.30, 55.44 (OCH_3); IR (cm^{-1}) 1690 (C=O), 1607, 1566, 1495, 1429, 1310, 1240, 1078, 1044, 843; MS (EI) *m*/*e* 250 (M^+, $^{37}Cl_2$), 248 (M^+, $^{35}Cl^{37}Cl$), 246 (M^+, $^{35}Cl_2$). Anal. calcd for $C_{10}H_8Cl_2O_3$: C, 48.61; H, 3.26; Cl, 28.70; found: C, 48.77; H, 3.31; Cl, 28.56.

2-(β,β-Dichloroethenyl)-5-hydroxybenzoic acid (26b). In a way similar to that for **25a**, reducing **25b** (3.0 g, 11 mmol) with zinc dust (2.25 g) in glacial acetic acid (21 mL) yielded **26b** (2.2 g, 84%), mp 198–199 °C (MeOH). Desai and Usgaonkar[22] reported the product to be 2-(β,β-dichloroethyl)-5-hydroxybenzoic acid (80%, mp 196–197 °C), as did Hurry and Meldrum[30] (mp 194 °C, AcOH); Dharwarkar and Alimchandani,[31] who correctly reported the structure, gave mp 196–197 °C (MeOH). Analytical data for **26b**: ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.04 (br s, 1H, OH), 7.58–7.29 (m, 3H, Ar–H, $CH{=}CCl_2$), 7.04 (dd, 1H, $J_{ortho} = 8.2$ Hz, $J_{meta} = 2.7$ Hz, Ar–H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 167.40 (COOH), 157.65 ($COCH_3$), 131.48, 131.10, 129.69, 124.55, 119.02, 117.83, 117.02; IR (cm^{-1}) 3262 (v br, O–H), 3051 (v br, O–H), 1684 (C=O), 1453, 1288, 1246, 840, 826; MS (EI) *m*/*e* 232 (M^+, $^{35}Cl_2$), 233 (MH^+, $^{35}Cl_2$), 234 (M^+, $^{35}Cl^{37}Cl$), 235 (MH^+, $^{35}Cl^{37}Cl$), 236 (M^+, $^{37}Cl_2$), 237 (MH^+, $^{37}Cl_2$). Anal. calcd for $C_9H_6Cl_2O_3$: C, 46.38; H, 2.59; Cl, 30.42; found: C, 46.22; H, 2.71; Cl, 30.49.

(2-Carboxy-5-methoxyphenyl)acetic acid (23). A procedure similar to that of Desai and Usgaonkar[22] was followed, but profitable modifications and spectral data absent from the literature are noted here. To stirred concd sulfuric acid (14 mL), the dichloro compound **26a** (7.0 g, 28 mmol) was added in small portions. Each additional portion of the compound was added only after the previous one had dissolved, and the reaction mixture was occasionally and carefully warmed on a hot plate (~60 °C). After the addition was complete, concd sulfuric acid (7 mL) was added and the reaction was monitored by TLC (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$). After about 2 h, the reaction mixture was poured onto ice (60 g), and the precipitated product was collected by filtration, washed with cold water, and air-dried to give compound **23** (5.8 g, 98%), mp 180–182 °C (water), lit.[22] mp 186–187 °C, lit.[54] 185–186 °C, lit.[55] 191 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 12.41 (br, 2H, 2COOH), 7.41 (d, 1H, $J_{meta} = 2.7$ Hz, Ar–H), 7.25 (d, 1H, $J_{ortho} = 8.3$ Hz, Ar–H), 7.07 (dd, 1H, $J_{ortho} = 8.5$ Hz, $J_{meta} = 2.7$ Hz, Ar–H), 3.86 (s, 2H, CH_2), 3.79

(s, 3H, OCH_3); IR (cm^{-1}) 2960 (v br, O–H), 1690 (C=O), 1675 (C=O), 1574, 1302, 1233 (cf. ref 28).

4-[2-(3,4-Dihydro-7-methoxy-1,3-dioxo-2(1*H*)-isoquinolinyl)ethyl]benzenesulfonamide (28). An intimate mixture of **23** (2.0 g, 9.5 mmol) and 4-(2-aminoethyl)benzenesulfonamide (2.1 g, 10.5 mmol) was heated with an oil bath maintained at 200 °C for 20 min. Upon cooling, the brown-colored melt solidified, and this solid was dissolved in EtOAc (~1.2 L); the resulting solution was transferred to a separatory funnel and washed with 1 N HCl (3 × 600 mL). The EtOAc layer was dried over anhyd $MgSO_4$ and evaporated under reduced pressure to a small volume, whereupon the product **28** crystallized (3.2 g, 90%). This compound was quite pure, adequately so for further synthetic transformation. An analytical sample was prepared by column chromatography on silica (eluent: 8% MeOH in $CHCl_3$, followed successively by 8% MeOH/1% glacial AcOH in $CHCl_3$, and then 15% MeOH/1% glacial AcOH in $CHCl_3$), and recrystallization from EtOAc, mp 198–200 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.75 (d, 2H, J_{ortho} = 8.3 Hz, Ar–H), 7.49 (d, 1H, J_{meta} = 2.6 Hz, Ar–H), 7.42 (d, 2H, J_{ortho} = 8.3 Hz, Ar–H), 7.29 (m, 3H, Ar–H, SO_2NH_2), 7.27 (d, 1H, J_{meta} = 2.6 Hz, Ar–H), 4.11–3.99 (m, 4H, $C\underline{H}_2CH_2Ar$, CH_2), 3.82 (s, 3H, OCH_3), 2.88 (t, 2H, J = 7.7 Hz, $CH_2C\underline{H}_2Ar$); ^{13}C NMR (400 MHz, DMSO-d_6) δ {170.04, 164.25} (C=O), 158.29 ($\underline{C}OCH_3$), 142.89, 142.28, 129.10, 128.94, 127.37, 125.85, 125.69, 121.12, 110.79, 55.41 (OCH_3), 40.30, 35.31, 33.19; IR (cm^{-1}) {3350, 3250} (N–H), {1717, 1641} (C=O), 1341 (SO_2 asym), 1163 (SO_2 sym); MS (EI) m/e 375 (MH^+). Anal. calcd for $C_{18}H_{18}N_2O_5S$: C, 57.74; H, 4.85; N, 7.48; found: C, 58.18; H, 5.06; N, 7.16.

4-[2-(3,4-Dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1*H*)-isoquinolinyl)ethyl]benzenesulfonamide (29). A suspension of the sulfonamide **28** (4.9 g, 13 mmol) and CH_3I (1.63 mL, 26.2 mmol) in MeOH (anhyd, 35 mL) was refluxed under dry argon atmosphere for 20 min. To this refluxing mixture, sodium ethoxide (26.2 mmol) in EtOH (abs, 12 mL) was added dropwise with a glass syringe during a period of 3 h. The reaction mixture was further refluxed for 2 h while monitoring by TLC (eluent: 5% MeOH/0.5% glacial AcOH in $CHCl_3$). After cooling, the solvent was removed from the reaction mixture in vacuo. Water (150 mL) was added to the residue, and the resulting mixture was acidified (pH 2–3) with 1 N HCl. The product precipitated from solution, and was filtered off and dried to yield **29** (4.7 g, 90%) with some minor impurities. This material was used without further purification in subsequent synthetic procedures. An analytical sample was prepared by column chromatography on silica (eluent: 2% MeOH/1% glacial AcOH in $CHCl_3$) and crystallized from EtOAc: hexane, mp 200–202 °C [lit.[20] mp 203–205 °C, lit.[18] mp 200–202 °C (MeOH)]; ^1H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, 2H, J_{ortho} = 7.4 Hz, Ar–H), 7.59 (d, 1H, J_{ortho} = 8.6 Hz, Ar–H), 7.51 (d, 1H, J_{meta} = 2.5 Hz, Ar–H), 7.39 (d, 2H, J_{ortho} = 7.3 Hz, Ar–H), 7.34–7.24 (m, 3H, Ar–H, SO_2NH_2), 4.12 (t, 2H, J = 7.2 Hz, $C\underline{H}_2CH_2Ar$), 3.82 (s, 3H, OCH_3), 2.92 (t, 2H, J = 7.2 Hz, $CH_2C\underline{H}_2Ar$), 1.44 (s, 6H, 2×CH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ {176.46, 163.24} (C=O), 158.11 ($\underline{C}OCH_3$), {142.67, 142.31, 137.24, 129.22, 127.48, 125.73, 124.20, 121.53, 110.64} (Ar–C), 55.42 (OCH_3), 42.44 [$\underline{C}(CH_3)_2$], 40.47 (NCH_2), 33.05 (CH_2–Ar), 28.92 (2×CH_3); IR (cm^{-1}) 3200 (N–H), {1705, 1655} (C=O), 1335 (SO_2 asym), 1165 (SO_2 sym); MS (GC/EI) m/e 402 (M^+). Anal. calcd for $C_{20}H_{22}N_2O_5S{\cdot}0.5H_2O$: C, 58.38; H, 5.63; N, 6.81; found: C, 58.26; H, 5.66; N, 6.57.

Ethyl 4-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1*H*)-isoquinolinyl)ethyl]benzenesulfonamide carbamate (30). A procedure similar to that described for the synthesis of **21** was followed. The sulfonamide **29** (3.4 g, 8.5 mmol) and K_2CO_3 (3.04 g, 22.0 mmol, dried overnight at 100 °C) was refluxed in acetone (200 mL, dried over molecular sieves) under a dry argon atmosphere for 30 min. Ethyl chloroformate (0.88 mL, 11.0 mmol) was then added dropwise during a period of 3 h. Reflux was continued, and the progress was monitored by TLC (4% MeOH/0.5% glacial AcOH in $CHCl_3$). After 24 h, the solvent was evaporated in vacuo and the residue taken up in EtOAc/water (ca. 300 mL/100 mL). The resulting mixture was extracted with one-10th-saturated $NaHCO_3$ solution (4 × ~400 mL). The aqueous phase was then acidified to pH 2–3 with 1 N HCl, and the resulting cloudy mixture was extracted with EtOAc (3 × ~300 mL). The combined EtOAc extracts were then dried over $MgSO_4$ and evaporated under reduced pressure to an oily residue of compound **30** (2.8 g, 70%, somewhat impure as indicated by TLC), which was suitable for use in the subsequent step without further purification. ^1H NMR (90 MHz, DMSO-d_6) δ 7.70 (d, 2H, J = 8.3 Hz, Ar–H), 7.60–6.99 (m, 5H, Ar–H), 4.14 (t, 2H, $C\underline{H}_2CH_2Ar$), 4.00–3.64 (m, 5H, OCH_3, $C\underline{H}_2CH_3$), 2.94 (t, 2H, $CH_2C\underline{H}_2Ar$), 1.42 (s, 6H, 2×CH_3), 1.05 (t, 3H, J = 7.2 Hz, $CH_2C\underline{H}_3$); IR (cm^{-1}) 3230 (N–H), {1752, 1711, 1663} (C=O), 1613 (NH bend), 1352 (SO_2 asym), 1163 (SO_2 sym).

1-[4-[2-(3,4-Dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1*H*)-isoquinolinyl)ethyl]phenyl]sulfonyl]-3-(*cis*-4-hydroxycyclohexyl)urea (5) and 1-[4-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1*H*)-isoquinolinyl)ethyl]phenyl]-sulfonyl]-3-(*trans*-4-hydroxycyclohexyl)urea (9). A procedure similar to that described for the synthesis of **3** and **7** was followed. The carbamate **30** (0.7 g, 1.5 mmol) in dioxane (3.1 mL) was reacted with 4-aminocyclohexanol (0.34 mL of a 50% w/w solution in water, the water previously removed by azeotropic distillation with toluene) to yield an off-white amorphous solid (0.80 g, 98%) containing a mixture of compounds **5** and **9**. The compounds were separated by column chromatography in a way similar to that for compounds **3** and **7**, except that the product mixture was introduced onto the column as a dry silica adsorbate (in vacuo evaporation of a $CHCl_3$ solution with 8 g of silica gel). Upon eluting with 30% CH_3CN/1% glacial AcOH in benzene, compound **5** eluted first, and then compound **9**, each in approximately 600 mL of eluent. For each fraction, the eluent was evaporated to dryness and the residue coevaporated with toluene, followed by crystallization from EtOH/water to yield compounds **5** (0.22 g) and **9** (0.24 g).

Analytical data for compound **5**: mp 110–116 °C (EtOH/water); ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.23 (s, 1H, $SO_2NHCONH$), 7.79 (d, 2H, J = 8.1 Hz, Ar–H), 7.69–7.41 (m, 3H, Ar–H), 7.41–7.16 (m, 2H, Ar–H), 6.36 (d, 1H, J = 7.7 Hz, $SO_2NHCONH$), 4.44 (d, 1H, J = 2.9 Hz, OH), 4.16 (t, 2H, J = 6.7 Hz, CH_2CH_2Ar), 3.84 (s, 3H, OCH_3), 3.71–3.48 (m, 1H, CHOH or NHCH), 3.33 (s, CHOH or CHNH overlapped with H_2O in DMSO-d_6), 2.96 (t, 2H, CH_2CH_2Ar), 1.41 (br s, 14H, 2×CH_3 + cyclohexyl CH_2); ^{13}C NMR (90 MHz, DMSO-d_6) δ {176.45, 163.29} (C=O), 158.14 ($COCH_3$), 150.34 (NHCONH), 144.27 [(Ar)CSO_2NH], {138.31, 137.28, 129.43, 127.48, 127.37, 124.23, 121.50, 110.68} (Ar–C), 65.00 (CHOH), 55.48 (OCH_3), 46.38 (NHCH), 42.38 ($C(CH_3)_2$), (NCH_2 under solvent), 33.20 (CH_2–Ar), {30.83, 27.26} (cyclohexyl CH_2), 28.94 (2×CH_3); IR (cm^{-1}) 3389 (v br, O–H and N–H), {1701, 1678, 1661} (C=O), 1352 (SO_2 asym), 1161 (SO_2 sym); MS (FAB) m/e 544 (MH^+). Anal. calcd for $C_{27}H_{33}N_3O_7S{\cdot}2H_2O$: C, 55.95; H, 6.43; N, 7.25; found: C, 55.99; H, 6.58; N, 6.72. Analytical data for compound **9**: mp 159–161 °C (EtOAc); ^{1}H NMR (90 MHz, DMSO-d_6) δ 10.32 (s, 1H, $SO_2NHCONH$), 7.79 (d, 2H, J = 8.4 Hz, Ar–H), 7.69–7.40 (m, 3H, Ar–H), 7.40–7.14 (m, 2H, Ar–H), 6.27 (d, 1H, J = 7.8 Hz, $SO_2NHCONH$), 4.51 (d, 1H, J = 4.3 Hz, OH), 4.17 (t, 2H, J = 7.5 Hz, CH_2CH_2Ar), 3.84 (s, 3H, OCH_3), 3.58–3.10 (m, CHOH and CHNH overlapped with H_2O in DMSO-d_6), 2.97 (t, 2H, CH_2CH_2Ar), 1.96–1.53 (m, 4H, cyclohexyl CH_2), 1.41 (s, 6H, 2×CH_3), 1.29–0.89 (m, 4H, cyclohexyl CH_2); IR (cm^{-1}) 3331 (br, O–H and N–H), {1713, 1665, 1620} (C=O), 1348 (SO_2 asym), 1161 (SO_2 sym); MS (FAB) m/e 544 (MH^+). Anal. calcd for $C_{27}H_{33}N_3O_7S$: C, 59.65; H, 6.12; N, 7.73; found: C, 59.63; H, 6.46; N, 7.30.

Glyburide binding studies

Male Sprague–Dawley rats were decapitated, and whole brains (without cerebella) were removed and placed on ice. Membranes were prepared for assay as described by Zini et al.[34] and Cherksey and Altszuler.[35] Briefly, each brain was homogenized with 20 volumes (vol/wt) of 5 mM Tris–HCl/0.32 M sucrose buffer (pH 7.4) using a Polytron® homogenizer (Brinkman, Inc.). The homogenate was centrifuged at 1000g for 10 min at 4 °C, and the pellet was discarded. The supernatant was centrifuged at 40,000g for 20 min (4 °C); the resulting supernatant was discarded, the pellet was resuspended in 20 volumes of 50 mM Tris buffer (pH 7.4), and this suspension was centrifuged at 40,000g for 20 min. The supernatant was again discarded, and a resuspension in 20 volumes of 50 mM Tris buffer (pH 7.4) was again prepared. Brain membranes from 12 rats were pooled, so that all compounds in a given set were tested in a single pooled membrane preparation.

The assays were carried out in a final prepared volume of 1.0 mL, consisting of 900 μL of tissue resuspension (containing about 1.0 mg of protein as determined by Bradford protein assay), 50 μL of 1% DMSO/25 mM $NaHCO_3$ buffer containing 0.2 nM [^{3}H]glyburide (50.9 Ci/mmol, NEN-DuPont), and 50 μL of 1% DMSO/25 mM $NaHCO_3$ buffer with or without varying drug concentrations. Duplicate tubes were prepared at each of 12 drug concentrations spanning a concentration range from 10^{-12}–10^{-5} M (see Fig. 2). Membranes were incubated for 1.5 h at 15 °C, then filtered on glass fiber filters (Whatman GFB) with a Brandel tissue harvester, washing with three 5-mL portions of ice-cold 50 mM Tris buffer. The filters were placed in 7-mL vials, and scintillation fluid (4 mL, NBCS104, Amersham) was added. Nonspecific binding was determined as tritium binding in the presence of 10^{-5} M glyburide. The data were analyzed by nonlinear fitting with GraphPad Prism®, and an F-test of significance was used as the criterion for selecting a one-site or two-site standard sigmoidal binding model.

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