was removed by filtration and washed with EtOH (2 × 25 mL). The combined filtrates were evaporated to furnish 0.44 g (91%) of **27a** as a white foam. In a similar way compounds **18**, **20**, and **26** were hydrogenated to furnish **19**, **21**, and **26a**, respectively (Table I).

Method F (E.g., 23 → 28, 24 → 29, 25 → 30, 26a → 31) (27a → 32: A Representative Example). To a suspension of **27a** (0.24 g, 0.5 mmol) in dry MeOH (25 mL) was added freshly prepared NaOMe in MeOH (1 N) until a pH of 9 was reached. After 2 h, TLC of the reaction mixture indicated no further reaction. To the clear solution, Dowex 50 (H^+) resin was added to adjust the pH to pH ~6. The resin was removed by filtration, washed with MeOH (25 mL) and the filtrate evaporated to dryness. Crystallization of the residue from EtOH furnished 0.10 g (87%) of **32**. Detoluoylation of **23–25** and **26a** by this method furnished **28–31**, respectively (Table I).

Method G (35 → 40). To a solution of **35** (1.66 g, 3 mmol) in dry dichloromethane (50 mL) at −78 °C was added BCl_3 (25 mL, 1 M in dichloromethane). The reaction mixture was stirred at this temperature for 2 h and then at −40 °C for an additional 2 h. To the reaction mixture was added MeOH (50 mL) at −40 °C and stirring was continued at room temperature for 30 min. The mixture was then neutralized with NH_4OH and filtered to remove inorganic salts. The filtrate was evaporated and the residue was purified by flash silica gel column chromatography using $CHCl_3$/MeOH (6:1, v/v) to yield **40** (0.8 g, 93%) after crystallization from acetone.

Method H (E.g., 34 → 39, 36 → 41, 37 → 42) (38 → 43: A Representative Example). To a solution of **38** (0.56 g, 1 mmol) in absolute EtOH (30 mL) were added cyclohexene (30 mL) and $Pd(OH)_2$ (0.2 g of 20%), and the mixture was refluxed for 48 h. After filtration of the reaction mixture through a Celite pad, the filtrate was evaporated to dryness and the residue was purified by flash silica gel column chromatography using $CHCl_3$/MeOH (6:1, v/v) to give 0.18 g (70%) of **43**. Following this method nucleosides **34**, **36**, and **37** were converted to **39**, **41**, and **42**, respectively (Table I).

Acknowledgment. We wish to thank T. S. Breen for expert technical assistance in the biochemical assays and R. H. Springer for large-scale synthesis of certain chemical intermediates. Our thanks are also due to Sandy Young for her very competent and meticulous typing of this manuscript.

Registry No. 4, 271-73-8; **5**, 29274-28-0; **6**, 119368-03-5; **7**, 119368-04-6; **8**, 119368-05-7; **9**, 102690-94-8; **10**, 119391-46-7; **11**, 119368-06-8; **12**, 119368-07-9; **13**, 119391-47-8; **14**, 119368-08-0; **15**, 49834-63-1; **16**, 35010-60-7; **17**, 119368-09-1; **18**, 119368-10-4; **19**, 119368-11-5; **20**, 119368-12-6; **21**, 49834-61-9; **22**, 4330-21-6; **23**, 119368-13-7; **24**, 119368-14-8; **25**, 119368-15-9; **26**, 119368-16-0; **26a**, 119368-31-9; **27**, 119368-17-1; **27a**, 119368-32-0; **28**, 119368-18-2; **29**, 119368-19-3; **30**, 119368-20-6; **31**, 119368-21-7; **32**, 119391-48-9; **33**, 4060-34-8; **34**, 119368-22-8; **35**, 119368-23-9; **36**, 119368-24-0; **37**, 119391-49-0; **38**, 119368-25-1; **39**, 119368-26-2; **40**, 119368-27-3; 41, 119368-28-4; **42**, 119368-29-5; **43**, 119368-30-8; 5-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-isopropylidene-D-ribose, 68703-51-5.

The Binding of Benzenesulfonamides to Carbonic Anhydrase Enzyme. A Molecular Mechanics Study and Quantitative Structure-Activity Relationships

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Molecular mechanics methods have been applied to study the interaction between a series of 20 deprotonated benzenesulfonamides and the enzyme carbonic anhydrase. The different contributions to the binding energy have been evaluated and correlated with experimental inhibition data and molecular orbital indices of the sulfonamides in their bound conformation. The results suggest that the discrimination shown by the enzyme toward these inhibitors is dominated by the short-range van der Waals forces.

Calculating binding energies between inhibitors and macromolecular targets is not enough. If the results are to be usable in molecular design, we need to be able to partition the energies into the various contributing aspects. Here this is done for sulfonamides binding to carbonic anhydrase.

The zinc metalloenzyme carbonic anhydrase (EC 4.2.1.1) catalyzes the reversible hydration of carbon dioxide. Aromatic and heterocyclic sulfonamides with an unsubstituted sulfonamido group constitute a class of highly active inhibitors possessing unusual selectivity toward different carbonic anhydrase isozymes.[1] The structure-activity relationships of sulfonamides have been analyzed both qualitatively[2,3] and quantitatively.[4-14] In earlier studies[12,14] we showed that certain calculated properties of these molecules, such as charge distribution and frontier molecular orbital indices, are good predictors of the inhibitory activities of sulfonamides on carbonic anhydrase.

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Table I. Energies (kcal/mol) for Interactions between Benzenesulfonamides and Carbonic Anhydrase

no.	substit	BE	E_{CA-S}	E_S^D	E_{CA}^D	E_{EL}	E_{vdW}	E_{HB}
1	4-NO_2	-44.10	-53.47	0.112	9.24	-14.88	-29.12	-1.53
2	4-$NHCOCH_3$	-42.87	-53.24	0.716	9.64	-13.90	-30.84	-0.94
3	3-Cl	-42.16	-51.69	0.181	9.36	-14.13	-28.34	-1.31
4	3-Cl, 4-NO_2	-41.81	-53.40	0.380	11.21	-12.97	-29.82	-1.06
5	3-NO_2	-41.25	-49.56	0.230	8.08	-12.49	-29.51	-0.94
6	3,4-Cl	-40.61	-51.92	0.258	11.06	-9.41	-29.48	-0.50
7	3-NO_2, 4-Cl	-40.37	-51.52	0.154	10.98	-10.73	-28.81	-0.91
8	2-Cl	-40.31	-48.72	0.230	8.18	-13.41	-27.47	-1.06
9	4-$COCH_3$	-40.31	-52.92	0.600	12.01	-14.17	-28.26	-1.19
10	4-Cl	-40.03	-49.72	0.260	9.41	-13.22	-28.96	-1.09
11	4-OCH_3	-38.39	-49.64	0.370	10.87	-12.65	-25.76	-0.94
12	4-CH_3	-38.35	-48.48	0.292	9.84	-12.63	-27.12	-0.84
13	3-CH_3	-38.21	-51.19	0.430	12.54	-13.78	-26.26	-1.12
14	2-NH_2	-37.93	-49.00	0.329	10.73	-13.00	-23.63	-0.81
15	3-NH_2	-37.02	-48.30	0.220	11.05	-12.97	-22.84	-0.84
16	4-H	-36.97	-47.57	0.218	10.38	-13.15	-24.94	-1.00
17	2-CH_3	-36.76	-47.69	0.380	10.55	-14.05	-23.69	-1.09
18	4-NH_2	-35.23	-47.72	0.291	12.19	-12.54	-20.48	-1.09
19	4-$NHCH_3$	-34.94	-48.93	0.300	13.30	-12.23	-21.75	-0.87
20	3,4-CH_3	-34.24	-49.31	0.250	14.83	-11.81	-22.67	-0.87

Furthermore, some speculations about the mechanism of action were inferred from the reactivity criteria.

To the best of our knowledge, extensive interaction energy studies have not been reported for this class of molecules. Two important exceptions come from the studies of Vedani et al.[15-18] and Höltje.[19,20] Vedani used the molecular mechanics program YETI to refine details of complexes of carbonic anhydrase mainly with heterocyclic compounds. Höltje studied the binding of five benzenesulfonamides and three heterocyclic sulfonamides to carbonic anhydrase by means of semiempirical quantum chemical methods and found that graduation in inhibitory potency within the two groups of compounds was satisfactorily reproduced by the calculated interaction energies. However, although NMR experiments[21,22] have shown that sulfonamides bind via a deprotonated N atom to the zinc ion in the active site, these studies dealt with the neutral form of the inhibitor.

In the present study we have computed, using molecular mechanics, the binding energies of an extended series of deprotonated benzenesulfonamides to carbonic anhydrase with the aims of further clarifying the principles that govern this interaction and of testing the validity of reactivity criteria in representing a particular mechanism of drug action.

Methods

Energy calculations of the free molecules and the bound complexes for carbonic anhydrase and 20 benzenesulfonamides were performed with use of the AMBER suite of programs.[23] The geometries and the conformational preferences of the anionic sulfonamides considered were optimized by means of the semiempirical molecular orbital AM1 method within the AMPAC program[24,25] and the atomic partial charges obtained were included in the force field. Any system by which atomic point charges are produced is open to question, but here we concentrate on differences between similar compounds and there is no reason to suspect that the values for charged species are any less valuable than those for neutral molecules. In this context, any change in the charges employed (molecular electrostatic potential-derived for the macromolecules and AM1 for the ligands) would only produce a parallel shift in binding energies and would not influence the variation between compounds. The various methods of computing atomic charges, while giving different results, do correlate strongly with each other.[26]

The drug atoms were assigned the van der Waals and hydrogen bonding parameters of corresponding AMBER atom types; the additional parameters necessary were obtained in accordance with the interpolation method presented by Weiner et al.[27] Additionally, we have supplemented the force field with appropriate parameters for the Zn atom[28] and the atom types defined in NO_2,[29] OCH_3,[30] and Cl[31] derivatives. An *all atom* force field representation was used for the small molecules while, in the case of the enzyme, the hydrogens bonded to C atoms were not explicitly included (*united atom* approximation) for computational efficiency.[32] A distance-dependent dielectric constant ($\epsilon = 4r$) was employed in order to reduce the long-range electrostatic term and to incorporate some effects of the solvent.

The crystal structure[33] of the native human erythrocyte

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Table II. MO Indices and Biological Data of Aromatic Benzenesulfonamides

no.	$q(NH^-)$	$q(O^*)$	$q(O)$	$q(SO_2NH^-)$	E_{HOMO}	log II_{50} obsd[a]	log II_{50} calcd[b]
1	−0.6766	−0.7843	−0.8326	−0.5245	−5.1869	1.41	1.19
2	−0.6931	−0.7940	−0.8479	−0.5606	−4.6937	1.48	1.43
3	−0.6881	−0.7925	−0.8437	−0.5476	−4.8413	1.00	1.08
4	−0.6934	−0.7948	−0.8387	−0.5499	−4.8009	1.74	1.29
5	−0.6700	−0.7820	−0.8375	−0.5197	−5.1425	1.25	1.24
6	−0.6771	−0.7904	−0.8385	−0.5365	−4.9495	1.76	1.24
7	−0.6660	−0.7786	−0.8339	−0.5099	−5.2698	2.13	1.14
8	−0.6854	−0.7852	−0.8347	−0.5359	−4.7729	0.88	0.95
9	−0.6867	−0.7910	−0.8355	−0.5429	−4.9010	1.32	1.06
10	−0.6885	−0.7930	−0.8436	−0.5526	−4.8004	1.08	1.16
11	−0.6863	−0.7984	−0.8389	−0.5702	−4.7504	0.71	0.71
12	−0.6945	−0.7976	−0.8479	−0.5653	−4.6056	0.78	0.90
13	−0.7017	−0.7989	−0.8414	−0.5631	−4.6198	0.66	0.78
14	−0.6955	−0.8018	−0.8662	−0.5846	−4.5689	0.66	0.41
15	−0.7011	−0.7966	−0.8475	−0.5712	−4.5305	0.40	0.30
16	−0.6953	−0.7973	−0.8476	−0.5659	−4.6203	0.58	0.59
17	−0.6896	−0.7971	−0.8484	−0.5638	−4.6555	0.16	0.42
18	−0.7090	−0.8026	−0.8526	−0.5808	−4.4226	0.00	−0.04
19	−0.7061	−0.7996	−0.8511	−0.5752	−4.5023	0.18	0.14
20	−0.6967	−0.7953	−0.8466	−0.5674	−4.6081	0.48	0.27
Δ	0.0430	0.0240	0.0336	0.0747	0.8472		

[a] Reference 12 and references therein. [b] Calculated by eq 4.

Carbonic Anhydrase C enzyme (HCAC)[34,35] at 2.0-Å resolution was retrieved from the Protein Data Bank[36,37] at Brookhaven National Laboratory. This was used as the starting model together with the coordinates for *p*-aminobenzenesulfonamide and its associate Zn atom available from the crystallographic analysis of the sulfonamide–carbonic anhydrase complexes published by Kannan et al.[38] With the aid of the computer graphics program HYDRA[39] implemented on a Silicon Graphics Iris 3120 workstation, several orientations for the inhibitors with respect to the experimental structure of the enzyme were generated for energy refinement. Although this does not solve the "local minimum problem", it allows one to make a reasonably extensive search of conformational space.

All of the amino acids in the first and second layers pointing into the cleft of the active site were included in the energy minimization, even though not all of them may directly participate in the enzymatic mechanism. These are Thr 7, Asn 61, His 64, Ala 65, Asn 67, Ile 91, Gln 92, Phe 93, His 94, His 96, Gln 106, Glu 117, His 119, Val 121, Phe 131, Leu 141, Leu 143, Gly 145, Leu 198, Thr 199, Thr 200, Pro 201, Pro 202, Leu 204, Cys 206, Val 207, and Val 211.

Minimization was carried out, by using the conjugate gradient method, until the root mean square (rms) of the gradient was less than 0.1 kcal/(mol Å).

Results and Discussion

(a) Binding Energies. The lowest minimized energies for the carbonic anhydrase–benzenesulfonamide complexes are reported in Table I. The binding energy (BE) is obtained according to the following equation:

$$BE = E_{CA-S} + E_S^D + E_{CA}^D$$

where E_{CA-S} is the total interaction energy between the ligand and the enzyme, E_S^D is the distortion energy of the ligand calculated with respect to the optimized energy of the free molecule, and E_{CA}^D is a measure of the conformational energy change in carbonic anhydrase induced by drug binding. The component analysis of the binding energies of these complexes is also reported in this table: E_{EL} is the electrostatic contribution, E_{vdW} corresponds to the 6–12 nonbonded dispersion and repulsion terms, and E_{HB} is the 10–12 term for hydrogen bonding interactions.[32]

Inspection of the data reported in Table I highlights a number of important points. Firstly, it suggests that the complexes are stabilized by ligands carrying electron-acceptor substituents. Secondly, the spread in computed binding energies is mostly due to the changes in the van der Waals part of the potential, and not to the electrostatic term. Thirdly, the values of the distortion energy for carbonic anhydrase (average 10.8 kcal/mol, corresponding to 4% of the total energy of the enzyme) indicate that the enzyme does not undergo profound changes upon binding of the drugs, as expected from the fact that in this series of compounds there are no bulky substituents on the benzene ring. The sulfonamide derivatives, on the other hand, experience an induced fit within the active site of the enzyme (average distortion energy of 0.4 kcal/mol, corresponding to more than 10% of the mean energy of the inhibitor), in agreement with experimental evidence.[40] It is noticed in these simulations that, in order to remove the unfavorable steric contacts with the protein on binding, the aromatic ring rotates by 40°–90° (depending on the compound considered) from the orientation observed in the crystal structure of α-sulfanilamide, where both the nitrogen lone pair and the aromatic p orbital lie on the bisector of the O–S–O internuclear angle.

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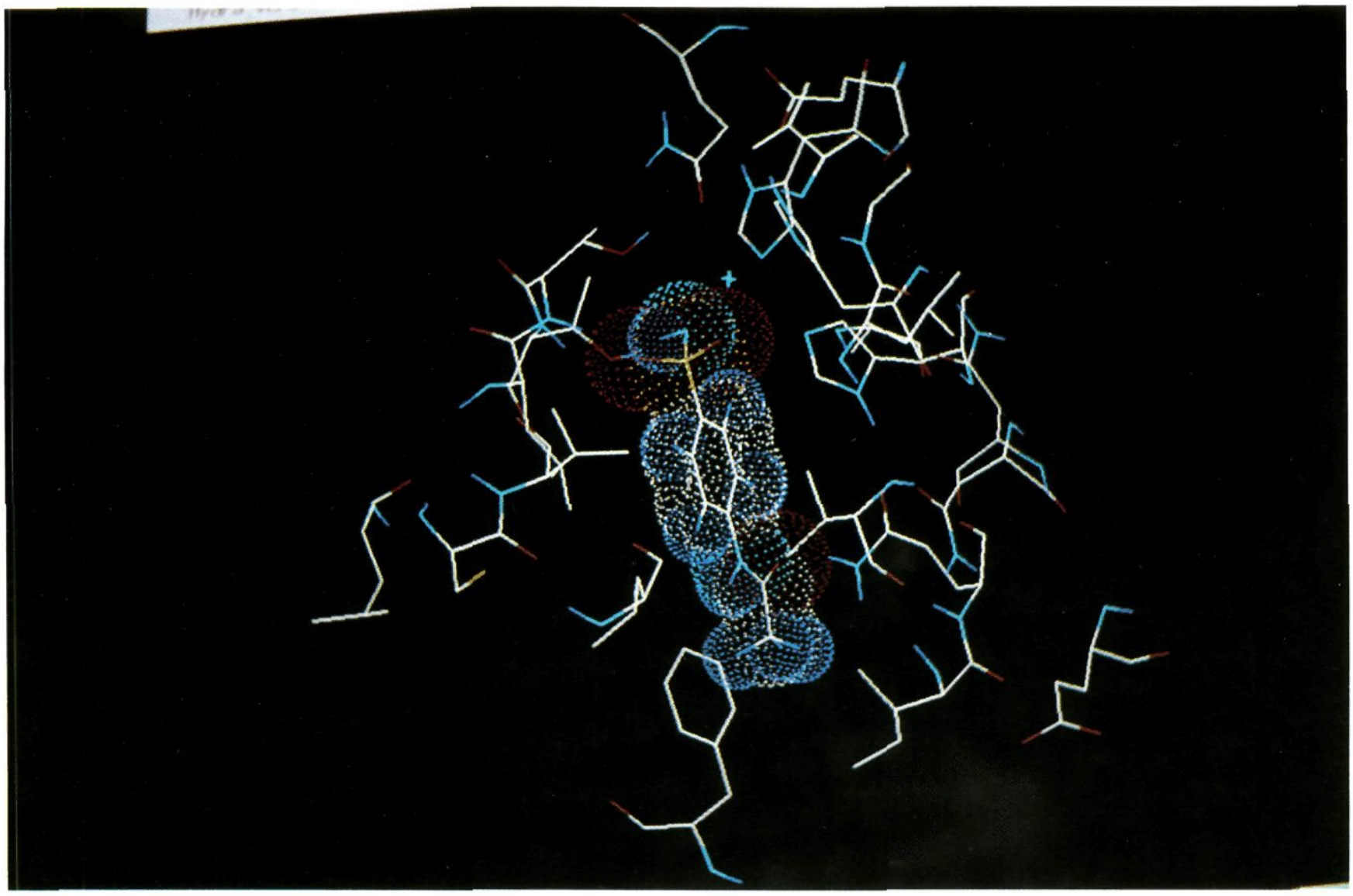

Figure 1. Model fit of 4-$NHCOCH_3$-C_6H_4-SO_2NH^- in carbonic anhydrase active site.

Table III. Correlation Matrix between the Data Given in Tables I and II

	BE	E_{CA-S}	E_S^D	E_{CA}^D	E_{EL}	E_{vdW}	E_{HB}	$q(NH^-)$	$q(O^*)$	$q(O)$	$q(SO_2NH^-)$	E_{HOMO}	log II_{50}
BE	1												
E_{CA-S}	0.776	1											
E_S^D	−0.075	−0.313	1										
E_{CA}^D	0.683	0.074	0.174	1									
E_{EL}	0.259	0.123	−0.278	0.294	1								
E_{vdW}	0.917	0.746	−0.156	0.590	0.056	1							
E_{HB}	0.378	0.299	−0.063	−0.244	0.813	0.162	1						
$q(NH^-)$	−0.652	−0.408	−0.281	−0.530	0.225	−0.699	0.005	1					
$q(O^*)$	−0.639	−0.429	−0.327	−0.488	0.132	−0.663	0.182	0.889	1				
$q(O)$	−0.573	−0.527	−0.149	−0.273	0.025	−0.637	0.320	0.668	0.766	1			
$q(SO_2NH^-)$	−0.699	−0.524	−0.297	−0.471	0.139	−0.735	0.219	0.897	0.972	0.823	1		
E_{HOMO}	0.733	0.588	0.275	0.448	−0.119	0.740	0.236	−0.948	−0.914	−0.754	−0.949	1	
log II_{50}	−0.803	−0.790	0.037	−0.342	0.241	−0.885	0.001	0.756	0.725	0.620	0.786	−0.817	1

(b) Molecular Orbital Indices and Inhibitory Activities. With the aim of evaluating the substituent effects on the charge distribution of the anionic sulfonamide derivatives in the bound conformation, we have computed their molecular orbital (MO) indices in the AM1 parameterization and approximation. The molecular descriptors chosen to represent the main features of the electronic structure of sulfonamides with respect to the mechanism of enzymic inhibition appear in Table II. They are (1) the total net charge ($\sigma + \pi$) on the amidic group ($q(NH^-)$), (2) the total net charge on each of the two oxygen atoms of the SO_2 group ($q(O^*)$ and $q(O)$, where O* is the oxygen atom closer to the Zn ion in the complex), and (3) the total net charge on the SO_2NH^- group ($q(SO_2NH^-)$). In addition, the frontier orbital energy of the highest occupied MO (E_{HOMO}) is also reported.

The range of variation of the MO indices (Δ values in the table) is very close to that previously observed in a CNDO/2 study[12] in which the X-ray conformation of α-sulfanilamide was considered for the same series of compounds. This result suggests that there is little charge distribution rearrangement on changing molecular conformation.

The values of the enzymic inhibition indices (log II_{50}) are also given in Table II. The inhibition constants, defined according to Bar[2] and normalized with respect to the p-NH_2 derivative,[12] represent, on a logarithmic scale, how many times a given sulfonamide is more active than the reference compound p-aminobenzenesulfonamide. Unfortunately, a large set of literature inhibition data for sulfonamides and human carbonic anhydrase isozyme C is not available. Therefore, we used the inhibition constants measured on the bovine erythrocyte B enzyme (BCAB) for more extensive correlation. These two isozymes can be considered practically identical, the main differences being due to the replacement of Ile 91 with Val 91 and of Cys 206 with Ser 206 in the active site.[41,42] These substitutions are not expected to modify significantly ei-

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Table IV. Interaction Energies (kcal/mol) between Benzenesulfonamides and Individual Residues of Carbonic Anhydrase

	Zn										
no.	TOT	EL	Thr 199	Thr 200	His 94	His 119	Leu 198	Val 121	Phe 131	Leu 141	Gln 92
1	-27.59	-20.47	-5.01	-0.46	-2.26	-1.68	-4.50	-2.66	-1.68	-0.75	-2.17
2	-29.31	-22.23	-2.64	-0.49	-2.09	-1.16	-4.40	-2.65	-2.56	-0.73	-2.69
3	-29.13	-22.25	-2.74	-0.51	-1.58	-1.48	-5.33	-2.79	-0.83	-1.31	-0.61
4	-29.00	-22.05	-5.25	-1.33	-1.62	-1.83	-5.33	-1.69	-1.66	-0.66	-0.52
5	-28.10	-20.87	-2.57	-0.54	-3.08	-1.05	-4.03	-2.41	-0.96	-0.51	-2.82
6	-28.96	-22.05	-3.47	-2.41	-0.75	-1.33	-5.86	-1.58	-1.33	-0.90	-0.26
7	-27.54	-20.36	-2.42	-0.56	-3.22	-0.99	-4.12	-2.96	-1.74	-0.70	-3.10
8	-28.96	-21.68	-2.50	-0.84	-2.09	-0.68	-4.69	-2.51	-1.02	-0.94	-1.54
9	-28.79	-21.77	-5.31	-1.56	-1.84	-1.59	-5.07	-1.41	-1.40	-0.64	-1.21
10	-29.54	-22.54	-3.25	-1.49	-0.69	-1.24	-5.36	-1.50	-1.27	-1.02	-0.23
11	-29.23	-21.96	-2.51	-0.67	-2.11	-1.11	-4.43	-2.25	-1.54	-0.69	-1.72
12	-30.19	-22.96	-2.84	-2.03	-0.53	-0.93	-5.19	-1.46	-1.19	-0.90	-0.25
13	-29.20	-22.09	-5.30	-1.05	-2.28	-1.80	-4.47	-1.57	-0.81	-0.53	-0.62
14	-29.82	-22.76	-2.45	-0.42	-1.86	-0.96	-4.73	-2.45	-1.02	-0.83	-1.30
15	-29.53	-22.44	-2.41	-0.42	-2.03	-0.91	-4.19	-2.36	-1.01	-0.51	-1.91
16	-30.08	-23.14	-3.20	-1.22	-0.84	-1.39	-4.68	-1.48	-0.65	-0.67	-0.21
17	-30.40	-23.15	-2.68	-1.86	-0.61	-0.79	-5.35	-1.59	-0.83	-0.85	-0.28
18	-29.64	-22.64	-2.65	-1.32	-1.08	-0.91	-5.22	-1.56	-1.18	-0.72	-0.50
19	-29.39	-22.25	-2.42	-0.78	-1.69	-0.88	-4.62	-1.83	-1.63	-0.74	-1.44
20	-28.83	-21.88	-3.23	-1.31	-1.40	-1.18	-5.28	-1.60	-1.32	-0.51	-0.69

ther the geometry or the peculiar chemicophysical properties of the enzyme.

(c) Relationships between Theoretical Indices and Inhibition Data. The general trend between the theoretical data presented in Tables I and II and the inhibitory activity is provided by the correlation matrix reported in Table III where the correlation coefficients (r) can be found. Linear regression analysis allows a quantitative expression of the qualitative conclusions reported above: the lack of correlation between inhibitory activity and the distortion energy of both carbonic anhydrase and sulfonamides supports the idea that conformational factors do not play a major role in determining the activity of these compounds;[26] in addition, 86% of the variation in the binding energy is explained by the variation in the nonbonded dispersion and repulsion term (r = 0.92). The electrostatic term shows a spread of about 5 kcal/mol with no apparent trend over the class of molecules studied.

A more accurate analysis of the relationships between theoretical descriptors and biological activity furnishes the following equations:

$$\log II_{50} = -0.171\ (\pm 0.052)BE - 5.77\ (\pm 2.03) \quad (1)$$

$$n = 20,\ r = 0.80,\ s = 0.36,\ F = 32.8$$

$$\log II_{50} = -0.159\ (\pm 0.029)BE - 5.43\ (\pm 1.14) \quad (2)$$

$$n = 16,\ r = 0.93,\ s = 0.17,\ F = 92.5$$

$$\log II_{50} = -0.167\ (\pm 0.036)E_{vdW} - 3.50\ (\pm 0.96) \quad (3)$$

$$n = 20,\ r = 0.88,\ s = 0.28,\ F = 64.8$$

$$\log II_{50} = -0.142\ (\pm 0.022)E_{vdW} - 2.95\ (\pm 0.58) \quad (4)$$

$$n = 16,\ r = 0.95,\ s = 0.15,\ F = 128.8$$

where n represents the number of sulfonamides considered, r is the correlation coefficient, s is the standard deviation from the regression, F is the significance Fisher test value, and the numbers in parentheses give the 95% confidence intervals.

Equations 2 and 4 refer to the somewhat more homogeneous series of monosubstituted benzenesulfonamides. Compounds **4**, **6**, **7**, and **20** are disubstituted derivatives and they show, in both regressions, slightly higher activities than those predicted by the theoretical indices. The explanation for this may be connected with the choice of parameters for these compounds. The force field used is probably unable to represent entirely the strength and directionality of the intermolecular interactions when two substituents are present at the 3- and 4-positions, particularly in the case of compound **7**. However, the agreement between the calculated interaction energies and the experimental data is gratifying. This result, besides making us optimistic about the use of this sort of approach to aid in the future design of pharmacologically active agents in this and other systems, allows us to identify the particular molecular forces involved in the interaction and verify the role of reactivity characteristics in the inhibitory mechanism.

(d) Residue Contribution. A qualitative inspection of the interactions between sulfonamides and some individual residues of carbonic anhydrase, reported in Table IV, suggests that all of the sulfonamide inhibitors studied bind to the active site in a rather similar way (see Figure 1). The ionized amino group occupies the fourth coordination site of the zinc ion (N–Zn average distance = 2.4 Å) and is hydrogen bonded to the hydroxyl O atom of Thr 199 (N–O = 2.9 Å); one oxygen atom of the sulfonamido group approaches the zinc atom (O*–Zn = 2.6 Å) while the other one is involved in a hydrogen bond with the main chain –NH– group of Thr 199 (O–N = 3.0 Å). A weaker interaction between the sulfonamido group and Thr 200 also occurs. The aromatic ring and the aliphatic substituents are involved in hydrophobic interactions with His 94, His 119, Val 121, Phe 131, Leu 141, and Leu 198. As observed in the case of heterocyclic compounds,[15] the contacts of the aromatic ring with the side chain of Val 121 seem to be less pronounced than those proposed by X-ray crystallographic studies.[38,43] Hydrophilic substituents interact with the side chain of Gln 92 but, with the exception of the p-NO_2 derivative, they usually form very weak hydrogen bonds, probably due to poor directionality.

It is interesting to note (Table IV) that about 60% of the E_{CA-S} value is contributed for by the interaction energy between sulfonamides and zinc. Moreover, the expected trend for a nucleophilic attack of sulfonamides toward the zinc ion is shown in the following equations:

(42) Hewett-Emmett, D.; Hopkins, J. P.; Tashian, R. E.; Czelusniak, J. Biology and Chemistry of the Carbonic Anhydrases. *Ann. N.Y. Acad. Sci.* **1984**, *429*, 338.

(43) Eriksson, E. A.; Jones, T. A.; Liljas, A. *Zinc Enzymes*; Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., Eds.; Birkhauser: Boston, 1986; p 317.

$$E_{EL}(Zn) = -2.800\ (\pm 0.756)E_{HOMO} - 35.41\ (\pm 3.60) \quad (5)$$

$$n = 20,\ r = 0.83,\ s = 0.44,\ F = 41.0$$

$$E_{EL}(Zn) = 98.881\ (\pm 26.512)q(O^*) + 56.39\ (\pm 21.04) \quad (6)$$

$$n = 20,\ r = 0.84,\ s = 0.44\ F = 41.6$$

This interaction, however, does not seem to be responsible for the modulation of the inhibitory activity in the series of compounds considered, as already suggested by eq 3 and 4. The values obtained for this term (Table IV) do not vary among the different complexes to a significant extent (the maximum and minimum values are only 2.8 kcal/mol apart), and when they are plotted against the experimental inhibition data, a negative slope and a rather poor regression coefficient ($r = 0.67$) are found. The biofunctional role of the zinc ion seems, therefore, to be exhausted in the generation of an electrostatic potential pattern that can guide the incoming ligand, in the early stages of the interaction, toward the cationic site.

Further information can be obtained by considering in full detail the most significant correlations between interaction energies, inhibition data and MO indices:

$$E_{vdW} = -113.312\ (\pm 35.085)q(SO_2NH^-) - 89.04\ (\pm 19.45) \quad (7)$$

$$n = 19,\ r = 0.81,\ s = 1.81,\ F = 31.6$$

$$E_{vdW} = 10.160\ (\pm 3.088)E_{HOMO} + 22.16\ (\pm 14.73) \quad (8)$$

$$n = 19,\ r = 0.81,\ s = 1.79,\ F = 32.8$$

$$\log II_{50} = -2.100\ (\pm 0.536)E_{HOMO} - 9.09\ (\pm 2.56) \quad (9)$$

$$n = 19,\ r = 0.86,\ s = 0.31,\ F = 46.4$$

Equations 7 and 8 show the correlations between reactivity characteristics computed on the isolated inhibitors and the most significant enzyme–inhibitors interaction energy terms. Equation 9 indicates that poor nucleophilic reactivity characteristics increase the inhibitory potency of the compounds studied; i.e. the most active compound possesses the least electron-rich biofunctional group SO_2NH^- and the deepest frontier energy level HOMO. Compound **2** has been omitted from the regressions because of its large deviation from the equations reported. The explanation for this exception may be connected with the nature of the substituent.[12] Additional strong interactions by the p-$NHCOCH_3$ group can be invoked in order to explain the higher activity observed for this compound with respect to the MO indices, as quantitatively shown in Table IV.

The results are conducive to the same working hypothesis previously proposed,[12,14] in agreement with the King and Burgen two-step reaction,[44,45] in which the drug approaches and becomes attached to the enzyme by lipophilic and ionic forces, the deprotonation of the drug being a critical factor at the active site. In fact, the electronic features of the SO_2NH^- group have a determining and concordant role in both steps: the less nucleophilic the group is, the more favored the hydrophobic interactions are (first step) and the more difficult it is to regain the proton that would lead to the dissociation of the complex.

This full agreement with the previous results supports the use of reactivity characteristics as a powerful tool for the elucidation of the molecular basis of drug action, once care is taken in their interpretation.[46]

Conclusions

The good correlations found between calculated binding energies and experimental inhibition indices suggest that the relaxation of the whole protein is not necessary for those enzymes whose inhibition mechanism does not involve large-scale conformational changes, since we did not include the entire enzyme in the minimization. It also seems that the differential solvation effects are not a key factor in carbonic anhydrase inhibition by these sulfonamides. Nevertheless, a more realistic representation of the solvent will be required if the goal is to obtain quantitatively correct estimates of energy changes.

This work also provides confirmation of our previous conclusion[12,14] that the reactivity characteristics of the biofunctional group SO_2NH^- are mainly responsible for the modulation of the inhibitory activity of this class of compounds. This group, in fact, reflects the variation in the electronic structure of the whole molecule as a function of the variable substitution. In this context, it would be of use and interest to employ the YETI method of Vedani,[18] which includes directional potential functions for H bonds, salt linkages, and metal ligand interactions, to determine how consistent the binding results are with respect to the different force fields employed.

The most important conclusion is drawn, however, from the breakdown of the separate contributions to the intermolecular binding energy. We have found that the discrimination shown by the enzyme toward these inhibitors is dominated by the short-range van der Waals forces, rather than by the electrostatic interactions.

Registry No. 1, 6325-93-5; **2**, 121-61-9; **3**, 17260-71-8; **4**, 93745-74-5; **5**, 121-52-8; **6**, 23815-28-3; **7**, 97-09-6; **8**, 6961-82-6; **9**, 1565-17-9; **10**, 98-64-6; **11**, 1129-26-6; **12**, 70-55-3; **13**, 1899-94-1; **14**, 3306-62-5; **15**, 98-18-0; **16**, 98-10-2; **17**, 88-19-7; **18**, 63-74-1; **19**, 16891-79-5; **20**, 6326-18-7; carbonic anhydrase, 9001-03-0.

(44) Taylor, P. W.; King, R. W.; Burgen, A. S. V. *Biochemistry* **1970**, *9*, 2638.
(45) King, R. W.; Burgen, A. S. V. *Proc. R. Soc. London, Ser. B* **1976**, *193*, 107.
(46) Richards, W. G. *Quantum Pharmacology*, 2nd ed.; Butterworth: London, 1983.