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**Original article**

# Carbonic anhydrase inhibitors. Part 61. Quantum chemical QSAR of a group of benzenedisulfonamides

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**Abstract** – The synthesis of a large group of benzenesulfonamides containing both a primary and secondary sulfonamide moiety is described. These compounds are powerful inhibitors of several isozymes of the enzyme carbonic anhydrase. Separate QSAR's are given for inhibition of three of these isozymes, using descriptors mainly derived from molecular orbital calculations by the semiempirical AM1 method. Activity was found to depend on electrostatic potential-based charges on the atoms of both sulfonamide groups, HOMO and LUMO energies, dipole moments, and lipophilicities. These results are compared with those from other studies. © Elsevier, Paris

**carbonic anhydrase inhibition / QSAR / quantum chemical / synthesis / charge / benzenedisulfonamide**

## 1. Introduction

Aromatic sulfonamides such as sulfanilamide **1**, have been shown to act as inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) in 1940 by Mann and Keilin [1], and this explained some clinical abnormalities related to the diminished $CO_2$ combining power of patients treated with the newly introduced (during the war) therapeutic agents from the class of antibacterial sulfonamides [2]. A very large number of sulfanilamide derivatives had been investigated in that period in the search for more effective antibacterial derivatives, among which were also homosulfanilamide **2** [3, 4] or the N-4-substituted derivatives of type **3** [5, 6]. Although none of these derivatives led to clinical applications, such studies greatly extended our current knowledge regarding the chemistry of this class of compounds [2]. It was thereafter shown by Krebs that the aromatic sulfonamides behave as weaker CA inhibitors as compared to the heterocyclic compounds [7]. This led, on one hand, to the extensive study of compounds from the latter class (with the subsequent introduction of acetazolamide **4** and methazolamide **5** in clinical medicine [8–11]), and on the other hand, to a relative lack of interest in the first class of CA inhibitors, i.e., the aromatic sulfonamides [12]. Only recently it has been shown by several groups that very effective inhibitors can also be designed from this class of compounds, with the Schiff bases of type **6** [13, 14], the oligoethylene glycol containing benzenesulfonamides **7** [15, 16] or the [N-(4-sulfamoylbenzoyl)-leucyl] piperidine-3-carboxylic acid **8** [17], possessing affinities in the nanomolar range for several physiologically relevant isozymes such as CA II and CA IV [13–17].

The above mentioned facts and the lack of CA inhibition studies with sulfonylamido-derivatives of sulfanilamide and related amino-sulfonamides prompted us to prepare a large series of such derivatives (using a compound of type **3** as lead molecule) and to investigate them for the inhibition of the physiologically relevant isozymes CA I, II and IV. Mention should be made that the analogous compounds in the acetazolamide and

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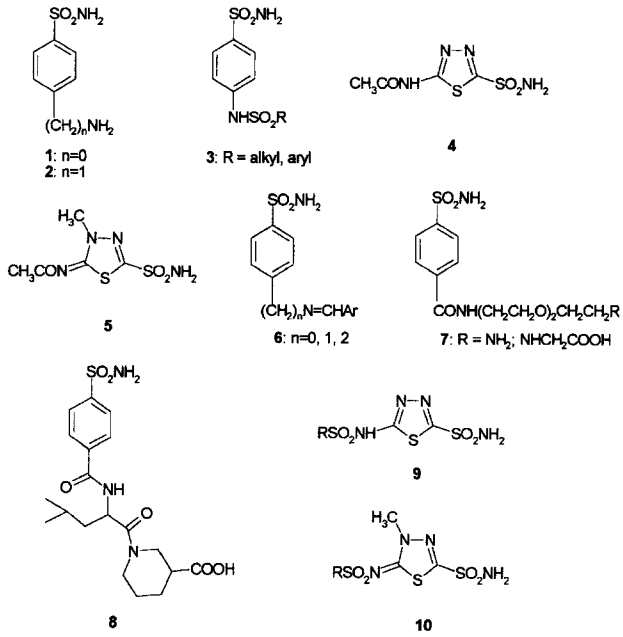
methazolamide series (of type **9** and **10**) recently reported by this group [18] were among the most effective CA inhibitors ever reported, and have been considered as lead molecules for the development of diagnostic tools useful in positron emission tomography (PET) studies [18].




Four series of compounds have been prepared in the present work, by reaction of sulfonyl halides (or sulfonic acid cyclic anhydrides) with sulfanilamide (compounds of type **A1–A18**), metanilamide (compounds **B19–B36**), *p*-aminomethyl-benzenesulfonamide (derivatives **C37–C54**) and *p*-(2-aminoethyl)-benzenesulfonamide (derivatives **D55–D72**), respectively. The 72 inhibitors were characterized by standard procedures and assayed for inhibition of human red cell isozymes CA I and II, and the bovine membrane-bound isozyme CA IV. A quantum chemical QSAR study has also been performed in order to better explain the biological activity of the new inhibitors.

We have previously developed QSAR equations based on molecular orbital calculations for several series of sulfonamide CA inhibitors. In a group of sulfanilamide Schiff's bases it was shown that CA inhibitory activity could be related to lipophilicity, pKa, dipole moment and electrostatic potential-based atomic charges and other reactivity indices from a COSMO AM1 calculation [19]. Similar results were obtained in a group of thiadiazole and thiadiazoline disulfonamides closely related to the present series. In this case, solvation energy was also implicated [20]. In both these series, and also in a noncongeneric series of sulfonamides, the frontier orbital energies $E_H$ and $E_L$ were found to have a significant influence on activity [21].

## 2. Experimental protocols

### *2.1. Chemistry*

Melting points were recorded with a heating plate microscope and are not corrected. IR spectra were recorded in KBr pellets with a Carl Zeiss IR-80 instrument. NMR spectra were recorded in DMSO-$d_6$ as solvent with a Bruker CPX200 instrument working at 200 MHz. Chemical shifts are reported as δ values, relative to $Me_4Si$ as internal standard. Thin layer chromatography (TLC) was done on precoated silicagel 60 plates, from Merck, and spots were visualized in UV light. Elemental analysis was done by combustion (for C, H, N) with a Carlo Erba automated analyser (Milan, Italy). The values obtained were within ± 0.4% of the theoretical values, calculated for the proposed formulae.

Sulfonyl chlorides, pyridine, triethylamine, solvents as well as inorganic reagents were from Aldrich, E. Merck, Sigma and Carlo Erba. 2-Sulfobenzoic acid cyclic anhydride was from Acros. Aminosulfonamides used in the synthesis (sulfanilamide, metanilamide, *p*-aminomethyl-benzenesulfonamide and *p*-(2-aminoethyl)-benzenesulfonamide) were from E. Merck or Sigma.

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/HCA I and pACA/HCA II described by Forsman et al. [22] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group [23], and enzymes were purified by affinity chromatography according to the method of Khalifah et al. [24]. Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1}.cm^{-1}$ for CA I and 54 $mM^{-1}.cm^{-1}$ for CA II, respectively, based on $M_r$ = 28.85 kDa for CA I, and 29.3 kDa for CA II, respectively [25, 26]. CA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with ethoxzolamide [27].

### *2.2. General procedure for the preparation of the benzene-disulfonamides* (**A–D**)**1–72**

An amount of 5 mmol of amino-benzene-sulfonamide derivative (sulfanilamide, metanilamide, *p*-aminomethyl-

benzenesulfonamide or *p*-(2-aminoethyl)-benzenesulfonamide) were suspended in 15 mL anhydrous acetonitrile and cooled to 2–5 °C in a salt-ice bath. Finely powdered sulfonyl chloride (5 mmol) was added in small portions, concomitantly with 5 mmol of pyridine or triethylamine, maintaining the temperature under 10 °C. The reaction mixture was then stirred at room temperature for 3–5 h (t.l.c. control of the progress of the reaction), the solvent evaporated in vacuo, and a small volume of water added. The precipitated sulfonamides were filtered and recrystallized from aqueous ethanol. Derivatives **A11, B29, C47** and **D65** were prepared by reaction of the corresponding amino-benzenesulfonamide derivatives with 2-sulfobenzoic acid cyclic anhydride in equimolar amount, in anhydrous acetonitrile at reflux for 2 h. All compounds have been extensively characterized and analysed as mentioned above. Some typical spectral data for a compound of each series are shown below.

*2.2.1. 4-(4-Fluorobenzenesulfonylamido)-benzenesulfonamide* **A7**

White crystals, m.p. 157–159 °C (from ethanol-water 3:1, v/v); IR (KBr), $cm^{-1}$: 629, 710, 883, 1 165, 1 170, 1 290, 1 367, 1 425, 1 575, 3 075; $^1$H-NMR (DMSO-$d_6$), δ, ppm: 7.10–7.58 (m, AA'BB',8H, ArH); 8.13 (br s, 2H, $SO_2NH_2$); 8.46 (s, 1H, $SO_2NH$); Anal $C_{12}H_{11}FN_2O_4S_2$ (C, H, N).

*2.2.2. 3-(2-Nitrobenzenesulfonylamido)-benzenesulfonamide* **B30**

Pale yellow crystals, m.p. 201–204 °C, IR (KBr), $cm^{-1}$: 685, 717, 810, 943, 1 080 and 1 250 ($NO_2$), 1 144 and 1 181 ($SO_2^{sym}$), 1 314 ($SO_2^{as}$), 3 275 and 3 400 (NH and $NH_2$); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 6.50 (br s, 3H, $NH_2$ + NH); 7.15–7.50 (m, 4H, ArH from 1,3-phenylene); 7.33–7.78 (m, 4H, Ar H from *ortho*-substituted phenyl). Anal $C_{12}H_{11}N_3O_6S_2$ (C, H, N).

*2.2.3. 4-(4-Nitrobenzenesulfonylamidomethyl)-benzenesulfonamide* **C50**

Pale yellow crystals, m.p. 249–251 °C, IR (KBr), $cm^{-1}$: 661, 715, 762, 820, 884, 1 075 and 1 249 ($NO_2$), 1 151 and 1 173 ($SO_2^{sym}$), 1 325 ($SO_2^{as}$), 3 270 and 3 390 (NH and $NH_2$); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 4.90 (s, 2H, $SO_2NHCH_2$); 6.86 (br s, 3H, $NH_2$ + NH); 7.05 (m, AA'BB', 4H, ArH from phenylene-sulfonamido); 7.23 (m, AA'BB', 4H, ArH from nitro-phenylene). Anal $C_{13}H_{13}N_3O_6S_2$ (C, H, N).

*2.2.4. 4-(4-Nitrobenzenesulfonylamidoethyl)-benzenesulfonamide* **D68**

Pale yellow crystals, m.p. 237–240 °C, IR (KBr), $cm^{-1}$: 690, 728, 779, 860, 898, 970, 1 045, 1 080 and 1 255 ($NO_2$), 1 150 and 1 171 ($SO_2^{sym}$), 1 328 ($SO_2^{as}$), 3 270 and 3 390 (NH and $NH_2$); $^1$H-NMR (DMSO-$d_6$), δ, ppm: 3.15 (t, 2H, ($CH_2$)); 3.71 (t, 2H, ($CH_2$)); 6.86 (br s, 3H, $NH_2$ + NH); 7.06 (m, AA'BB', 4H, ArH from phenylene-sulfonamido); 7.21 (m, AA'BB', 4H, ArH from nitro-phenylene). Anal $C_{14}H_{15}N_3O_6S_2$ (C, H, N).

*2.3 Pharmacology*

*2.3.1. Carbonic anhydrase inhibition*

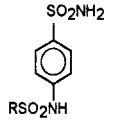
Initial rates of 4-nitrophenyl acetate (*p*-NPA) hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC [28]. Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2 \times 10^{-2}$ and $1 \times 10^{-6}$ M, working at 25 °C. A molar absorption coefficient ε of 18 400 $M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40), as reported in the literature [28]. Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations [18]) and dilutions up to 0.01 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The $IC_{50}$, representing the molarity of inhibitor producing a 50% decrease of enzyme catalysed *p*-NPA hydrolysis, has been determined as described by Pocker and Stone [25, 28]. Enzyme concentrations were 3.5 nM for CA II, 10.5 nM for CA I and 35 nM for CA IV (this isozyme has a decreased esterase activity [29] and higher concentrations had to be used for the measurements).

*2.4. Calculations*

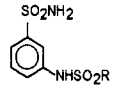
The atomic coordinates of the compounds listed in *table I* were calculated using the program PC-MODEL [30], using the MMX molecular mechanics algorithm and the dihedral driver in an attempt to find a global minimum. The geometry was further optimized using the AM1 Hamiltonian and the program MOPAC version 6 [31]. The first order polarizability was also calculated at this stage.

The optimized geometry was defined with the numbering of the common atoms from 1–17 as in *figure 1* (**D58,**

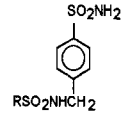
**Table I.** Structures and inhibitory concentrations against carbonic anhydrase isozymes of all compounds considered in this study.



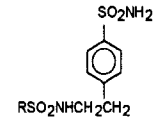

**A**




**B**




**C**




**D**

| Drug | Type | R | $IC_{50}$ (nM) CA I | CA II | CA IV |
|---|---|---|---|---|---|
| **1** | **A** | Me | 125 | 64 | 98 |
| **2** | **A** | $PhCH_2$ | 109 | 17 | 51 |
| **3** | **A** | $Me_2N$ | 114 | 12 | 30 |
| **4** | **A** | Ph | 103 | 49 | 85 |
| **5** | **A** | 4-Me-$C_6H_4$ | 96 | 42 | 83 |
| **6** | **A** | 2,4,6-$Me_3$-$C_6H_2$ | 69 | 55 | 60 |
| **7** | **A** | 4-F-$C_6H_4$ | 40 | 9 | 15 |
| **8** | **A** | 4-Cl-$C_6H_4$ | 38 | 9 | 13 |
| **9** | **A** | 4-Br-$C_6H_4$ | 35 | 8 | 10 |
| **10** | **A** | 4-MeO-$C_6H_4$ | 44 | 30 | 14 |
| **11** | **A** | 2-HOOC-$C_6H_4$ | 26 | 7 | 10 |
| **12** | **A** | 2-$O_2N$-$C_6H_4$ | 29 | 11 | 18 |
| **13** | **A** | 3-$O_2N$-$C_6H_4$ | 33 | 10 | 21 |
| **14** | **A** | 4-$O_2N$-$C_6H_4$ | 31 | 10 | 22 |
| **15** | **A** | 4-AcNH-$C_6H_4$ | 245 | 82 | 164 |
| **16** | **A** | 3-$H_2N$-$C_6H_4$ | 168 | 43 | 114 |
| **17** | **A** | 4-$H_2N$-$C_6H_4$ | 164 | 46 | 129 |
| **18** | **A** | 4-Cl-3-$O_2N$-$C_6H_3$ | 60 | 12 | 31 |
| **19** | **B** | Me | 176 | 79 | 125 |
| **20** | **B** | $PhCH_2$ | 150 | 28 | 77 |
| **21** | **B** | $Me_2N$ | 210 | 31 | 65 |
| **22** | **B** | Ph | 295 | 77 | 126 |
| **23** | **B** | 4-Me-$C_6H_4$ | 301 | 76 | 110 |
| **24** | **B** | 2,4,6-$Me_3$-$C_6H_2$ | 98 | 67 | 81 |
| **25** | **B** | 4-F-$C_6H_4$ | 65 | 10 | 22 |
| **26** | **B** | 4-Cl-$C_6H_4$ | 44 | 9 | 14 |
| **27** | **B** | 4-Br-$C_6H_4$ | 40 | 9 | 12 |
| **28** | **B** | 4-MeO-$C_6H_4$ | 59 | 40 | 48 |
| **29** | **B** | 2-HOOC-$C_6H_4$ | 32 | 7 | 20 |
| **30** | **B** | 2-$O_2N$-$C_6H_4$ | 33 | 10 | 26 |
| **31** | **B** | 3-$O_2N$-$C_6H_4$ | 35 | 9 | 22 |
| **32** | **B** | 4-$O_2N$-$C_6H_4$ | 36 | 10 | 24 |
| **33** | **B** | 4-AcNH-$C_6H_4$ | 298 | 101 | 188 |
| **34** | **B** | 3-$H_2N$-$C_6H_4$ | 176 | 47 | 129 |
| **35** | **B** | 4-$H_2N$-$C_6H_4$ | 185 | 50 | 144 |
| **36** | **B** | 4-Cl-3-$O_2N$-$C_6H_3$ | 74 | 15 | 46 |
| **37** | **C** | Me | 104 | 51 | 87 |
| **38** | **C** | $PhCH_2$ | 96 | 10 | 45 |
| **39** | **C** | $Me_2N$ | 90 | 8 | 21 |
| **40** | **C** | Ph | 81 | 40 | 64 |
| **41** | **C** | 4-Me-$C_6H_4$ | 85 | 31 | 60 |
| **42** | **C** | 2,4,6-$Me_3$-$C_6H_2$ | 60 | 52 | 57 |
| **43** | **C** | 4-F-$C_6H_4$ | 33 | 7 | 12 |
| **44** | **C** | 4-Cl-$C_6H_4$ | 29 | 7 | 10 |
| **45** | **C** | 4-Br-$C_6H_4$ | 24 | 5 | 8 |
| **46** | **C** | 4-MeO-$C_6H_4$ | 43 | 18 | 29 |
| **47** | **C** | 2-HOOC-$C_6H_4$ | 21 | 6 | 10 |

**Table I.** Continued.

| Drug | Type | R | $IC_{50}$ (nM) CA I | CA II | CA IV |
|---|---|---|---|---|---|
| **48** | **C** | 2-$O_2N$-$C_6H_4$ | 24 | 10 | 16 |
| **49** | **C** | 3-$O_2N$-$C_6H_4$ | 25 | 9 | 17 |
| **50** | **C** | 4-$O_2N$-$C_6H_4$ | 20 | 6 | 11 |
| **51** | **C** | 4-AcNH-$C_6H_4$ | 205 | 77 | 149 |
| **52** | **C** | 3-$H_2N$-$C_6H_4$ | 123 | 35 | 86 |
| **53** | **C** | 4-$H_2N$-$C_6H_4$ | 109 | 33 | 72 |
| **54** | **C** | 4-Cl-3-$O_2N$-$C_6H_3$ | 58 | 11 | 27 |
| **55** | **D** | Me | 93 | 40 | 71 |
| **56** | **D** | $PhCH_2$ | 81 | 8 | 38 |
| **57** | **D** | $Me_2N$ | 75 | 6 | 13 |
| **58** | **D** | Ph | 69 | 28 | 39 |
| **59** | **D** | 4-Me-$C_6H_4$ | 70 | 27 | 54 |
| **60** | **D** | 2,4,6-$Me_3$-$C_6H_2$ | 46 | 40 | 45 |
| **61** | **D** | 4-F-$C_6H_4$ | 28 | 5 | 9 |
| **62** | **D** | 4-Cl-$C_6H_4$ | 24 | 5 | 8 |
| **63** | **D** | 4-Br-$C_6H_4$ | 19 | 3 | 7 |
| **64** | **D** | 4-MeO-$C_6H_4$ | 38 | 15 | 25 |
| **65** | **D** | 2-HOOC-$C_6H_4$ | 15 | 5 | 9 |
| **66** | **D** | 2-$O_2N$-$C_6H_4$ | 22 | 6 | 9 |
| **67** | **D** | 3-$O_2N$-$C_6H_4$ | 22 | 8 | 13 |
| **68** | **D** | 4-$O_2N$-$C_6H_4$ | 20 | 4 | 10 |
| **69** | **D** | 4-AcNH-$C_6H_4$ | 187 | 75 | 119 |
| **70** | **D** | 3-$H_2N$-$C_6H_4$ | 101 | 30 | 84 |
| **71** | **D** | 4-$H_2N$-$C_6H_4$ | 95 | 30 | 72 |
| **72** | **D** | 4-Cl-3-$O_2N$-$C_6H_3$ | 50 | 9 | 21 |

drawn with the program Schakal [32]), with the origin at the midpoint of atoms 1 and 4, atom 1 on the positive X axis, the midpoint of atoms 2 and 3 in the X–Y plane, and with the Z coordinate of nitrogen 8 positive. A single point AM1 calculation was run on this geometry by the COSMO method [33], calculating ESP-based atomic charges by the Merz-Besler method [34] with the program MOPAC93 [35]. The charges will be denoted by, for example, $Q_{S7}$ for the charge of sulfur atom 7 in *figure 1*, except that $Q_H$ will be the sum of the charges on H11 and H12, $Q_{O1}$ will be the sum of the charges on O9 and O10, and $Q_{O2}$ will be the sum of the charges on O15 and O16.

In addition to the ESP-based charges and the orbital energies, the mean absolute Mulliken charge, the local dipole index [36], and the sum of electrophilic superdelocalizabilities over all atoms were calculated from the MOPAC93 output. The area and volume of the molecule were calculated using the GEPOL [37] tessellation option of the ARVOMOL program [38].

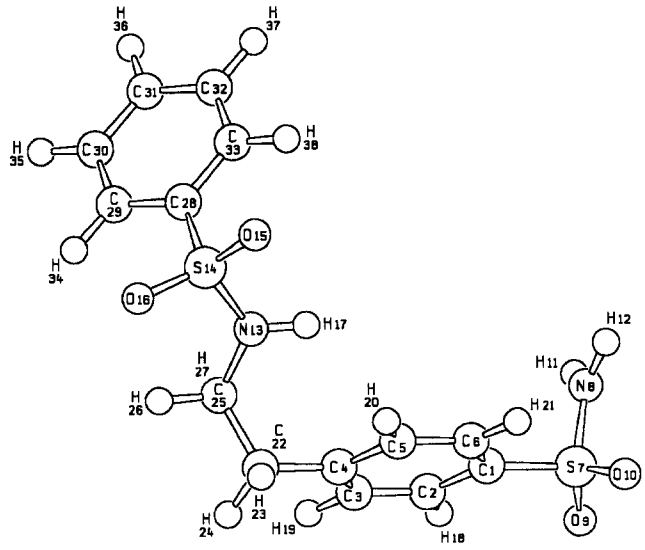



**Figure 1.** Compound **D58** showing atom numbering. Atoms 1–17 are common to all compounds.

**Table II.** Calculated descriptors used in this study.

| Symbol | Descriptor | Units |
|---|---|---|
| $\Pi_{xx}$ | Polarizability along axis of lowest inertia | a.u. |
| $\Pi_{yy}$ | Polarizability along medium inertial axis | a.u. |
| $\Pi_{zz}$ | Polarizability along axis of highest inertia | a.u. |
| $\mu$ | Magnitude of dipole moment | Debye |
| $\mu_x$ | Dipole moment component in X direction | Debye |
| $\mu_y$ | Dipole moment component in Y direction | Debye |
| $\mu_z$ | Dipole moment component in Z direction | Debye |
| $Q_{C1}$ | ESP based charge on carbon atom 1 | e |
| $Q_o$ | Sum of ESP based charges on carbon atoms 2 and 6 | e |
| $Q_m$ | Sum of ESP based charges on carbon atoms 3 and 5 | e |
| $Q_p$ | ESP based charge on carbon atom 4 | e |
| $Q_{S7}$ | ESP based charge on sulfur atom 7 | e |
| $Q_{N8}$ | ESP based charge on nitrogen atom 8 | e |
| $Q_{N13}$ | ESP based charge on nitrogen atom 13 | e |
| $Q_{S14}$ | ESP based charge on sulfur atom 14 | e |
| $Q_{H17}$ | ESP based charge on hydrogen atom 17 | e |
| $Q_{O1}$ | Sum of ESP based charges on oxygen atoms 9 and 10 | e |
| $Q_{O2}$ | Sum of ESP based charges on oxygen atoms 15 and 16 | e |
| $Q_H$ | Sum of ESP based charges on hydrogen atoms 11 and 12 | e |
| $Q_M$ | Mean of absolute Mulliken charges on all atoms | e |
| $D_I$ | Local dipole index based on Mulliken charges | e |
| $E_H$ | Energy of highest occupied molecular orbital | eV |
| $E_L$ | Energy of lowest unoccupied molecular orbital | eV |
| $A_w$ | Van der Waals area of molecule | Å$^2$ |
| $V_w$ | Van der Waals volume of molecule | Å$^3$ |
| Log P | Log of calculated octanol-water partition coefficient | – |
| $\Sigma H_S$ | Difference between heats of formation, COSMO and vacuum | kcal |
| $\Sigma S^E$ | Sum of electrophilic superdelocalizabilities, all atoms | eV$^{-1}$ |
| $I_1$ | Indicator variable: 1 for metanilamides, 0 otherwise | – |
| $I_2$ | Indicator variable: 1 for p-aminomethylbenzenesulfonamides | – |
| $I_3$ | Indicator variable: 1 for p-aminoethylbenzenesulfonamides | – |

Lipophilicity was calculated using the program ClogP [39]. Solvation energy was calculated as the difference between the standard SCF heat of formation and the COSMO value.

Three indicator variables, $I_1$, $I_2$ and $I_3$ were defined. All three were set to zero for the series of sulfanilamides. For the metanilamides, $I_1$ was set to unity and $I_2$ and $I_3$ to zero. For the *p*-aminomethyl-benzenesulfonamides, $I_2$ was set to unity and $I_1$ and $I_3$ to zero. For the *p*-aminoethyl-benzenesulfonamides $I_3$ was set to unity and $I_1$ and $I_2$ to zero. All of the descriptors tested are listed in *table II*.

The logarithm of the $IC_{50}$ of the drugs as CA inhibitors against CA I, CA II and CAIV was regressed on the calculated descriptors using the statistical package BMDP [40] and a principal components program written by the authors, in order to find regression equations free from colinearity, as described previously [21]. A test was also made for the possible occurrence of chance correlation, using the randomization technique described previously.

## 3. Results

### *3.1. Chemistry*

The 72 compounds prepared by reaction of alkyl-/arylsulfonyl halides with sulfanilamide, metanilamide, *p*-aminomethyl-benzenesulfonamide or *p*-(2-aminoethyl)-benzenesulfonamide of type **A–D**, and their CA inhibitory properties against isozymes I, II and IV are shown in *table I*.

### 3.2. Calculations

#### 3.2.1. CA I

The best equation obtained on the Mallow's $C_p$ criterion was:

$$\text{Log } IC_{50} = C_1\, Q_{S7} + C_2\, Q_{H17} + C_3\, \mu_x + C_4\, E_H + C_5\, E_L + C_6\, \Pi_{yy} + C_7\, \Delta H_S + C_8\, Q_{C1} + C_9 \quad (1).$$

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* |
|---|---|---|---|---|---|---|---|---|---|
| C | 5.79 | 4.26 | –0.0326 | 0.299 | 0.600 | $-2.12 \times 10^{-3}$ | –0.0209 | 3.13 | –10.30 |
| σ | 2.12 | 1.01 | 0.011 | 0.108 | 0.145 | $1.14 \times 10^{-3}$ | 0.0088 | 0.96 | 5.58 |
| α | 0.00834 | 0.00008 | 0.00448 | 0.00008 | 0.00011 | 0.06647 | 0.02102 | 0.00182 | 0.06976 |

N = 72, $R^2$ = 0.619, $Q^2$ = 0.512, s = 0.22, F = 12.8, $P = 1 \times 10^{-12}$, Λ = 3.10.

$$\text{Log } IC_{50} = C_1\, Q_{C1} + C_2\, Q_{H17} + C_3\, E_H + C_4\, E_L + C_5\, Q_{O1} + C_6\, \Delta H_S + C_7\, I_3 + C_8 \quad (2).$$

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
|---|---|---|---|---|---|---|---|---|
| C | 3.73 | 4.74 | 0.310 | 0.821 | –13.84 | –0.0201 | –0.328 | –20.73 |
| σ | 1.30 | 1.12 | 0.103 | 0.170 | 5.25 | 0.0089 | 0.084 | 9.56 |
| α | 0.00548 | 0.00007 | 0.00368 | 0.00001 | 0.01049 | 0.02677 | 0.00024 | 0.03362 |

N = 72, $R^2$ = 0.610, $Q^2$ = 0.517, s = 0.22, F = 14.3, $P = 5 \times 10^{-11}$, Λ = 4.85

$$\text{Log } IC_{50} = C_1\, \Pi_{zz} + C_2\, E_H + C_3\, E_L + C_4 \quad (3).$$

| | *1* | *2* | *3* | *4* |
|---|---|---|---|---|
| C | $-3.33 \times 10^{-3}$ | 0.533 | 0.378 | 7.91 |
| σ | $9.2 \times 10^{-4}$ | 0.087 | 0.117 | 0.83 |
| α | 0.00054 | 0.00000 | 0.00194 | 0.00000 |

N = 72, $R^2$ = 0.540, $Q^2$ = 0.500, s = 0.23, F = 26.6, $P = 2 \times 10^{-11}$, Λ = 1.04

#### 3.2.2. CA II

The best equation on the $C_p$ criterion was:

$$\text{Log } IC_{50} = C_1\, \Pi_{yy} + C_2\, \Pi_{zz} + C_3\, Q_{N13} + C_4\, \mu + C_5\, E_L + C_6\, \log P + C_7\, Q_{O2} + C_8 I_3 + C_9 \quad (4).$$

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* |
|---|---|---|---|---|---|---|---|---|---|
| C | $2.63 \times 10^{-3}$ | $3.67 \times 10^{-3}$ | –1.12 | 0.0239 | 0.644 | –0.115 | –4.16 | –0.592 | –7.50 |
| σ | $1.34 \times 10^{-3}$ | $1.23 \times 10^{-3}$ | 0.244 | 0.0117 | 0.173 | 0.046 | 1.14 | 0.095 | 2.26 |
| α | 0.05441 | 0.00416 | 0.00002 | 0.04607 | 0.00045 | 0.01587 | 0.00055 | 0.00000 | 0.00152 |

N = 72, $R^2$ = 0.682, $Q^2$ = 0.594, s = 0.24, F = 16.9, $P = 4 \times 10^{-13}$, Λ = 1.75

$$\text{Log } IC_{50} = C_1\, Q_{C1} + C_2\, \mu_x + C_3\, E_L + C_4\, V_w + C_5 \log P + C_6\, Q_{O2} + C_7\, Q_{m+p} + C_8 \quad (5).$$

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
|---|---|---|---|---|---|---|---|---|
| C | 4.53 | –0.0251 | 0.551 | $3.49 \times 10^{-3}$ | –0.183 | –2.91 | 3.06 | –6.98 |
| σ | 0.97 | 0.0103 | 0.176 | $1.06 \times 10^{-3}$ | 0.049 | 1.09 | 0.58 | 2.17 |
| α | 0.00002 | 0.01774 | 0.00262 | 0.00154 | 0.00043 | 0.00964 | 0.00000 | 0.00207 |

N = 72, $R^2 = 0.663$, $Q^2 = 0.591$, s = 0.25, F = 18.0, $P = 3 \times 10^{-13}$, $\Lambda = 2.81$

$$\text{Log } IC_{50} = C_1\, Q_{N13} + C_2\, E_L + C_3\, D_l + C_4 \log P + C_5\, Q_{O2} + C_6\, I_3 + C_7 \quad (6).$$

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* |
|---|---|---|---|---|---|---|---|
| C | –1.45 | 0.649 | –1.25 | –0.189 | –3.18 | –0.532 | –3.30 |
| σ | 0.26 | 0.153 | 0.330 | 0.0486 | 1.08 | 0.086 | 2.04 |
| α | 0.00000 | 0.00007 | 0.00035 | 0.00025 | 0.00465 | 0.00000 | 0.11068 |

N = 72, $R^2 = 0.652$, $Q^2 = 0.581$, s = 0.25, F = 20.3, $P = 3 \times 10^{-13}$, $\Lambda = 1.69$

### *3.2.3. CA IV*

$$\text{Log } IC_{50} = C_1\, Q_{N13} + C_2\, E_H + C_3\, E_L + C_4\, D_l + C_5 \log P + C_6\, Q_{O2} + C_7\, I_3 + C_8 \quad (7).$$

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
|---|---|---|---|---|---|---|---|---|
| C | –0.891 | 0.322 | 0.377 | –0.921 | –0.184 | –2.885 | –0.350 | 0.45 |
| σ | 0.317 | 0.134 | 0.166 | 0.367 | 0.060 | 1.108 | 0.101 | 2.16 |
| α | 0.00651 | 0.01937 | 0.02676 | 0.01468 | 0.00311 | 0.01144 | 0.00102 | 0.83731 |

N = 72, $R^2 = 0.661$, $Q^2 = 0.591$, s = 0.25, F = 17.9, $P = 6 \times 10^{-13}$, $\Lambda = 2.21$

$$\text{Log } IC_{50} = C_1\, Q_{S14} + C_2\, \mu_x + C_3\, E_H + C_4\, E_L + C_5 \log P + C_6 \quad (8).$$

| | *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|---|
| C | 1.409 | –0.0261 | 0.405 | 0.327 | –0.122 | 2.296 |
| σ | 0.405 | 0.0104 | 0.117 | 0.134 | 0.045 | 1.68 |
| α | 0.00091 | 0.01450 | 0.00091 | 0.01741 | 0.00903 | 0.17772 |

N = 72, $R^2 = 0.630$, $Q^2 = 0.581$, s = 0.26, F = 22.5, $P = 4 \times 10^{-13}$ $\Lambda = 1.36$

$$\text{Log } IC_{50} = C_1\, \mu_x + C_2\, E_H + C_3\, Q_{O2} + C_4 \quad (9).$$

| | *1* | *2* | *3* | *4* |
|---|---|---|---|---|
| C | –0.0210 | 0.604 | –4.44 | –0.73 |
| σ | 0.0101 | 0.109 | 0.85 | 1.99 |
| α | 0.04072 | 0.00000 | 0.00000 | 0.71544 |

N = 72, $R^2 = 0.600$, $Q^2 = 0.560$, s = 0.26, F = 33.9, $P = 6 \times 10^{-13}$, $\Lambda = 1.21$

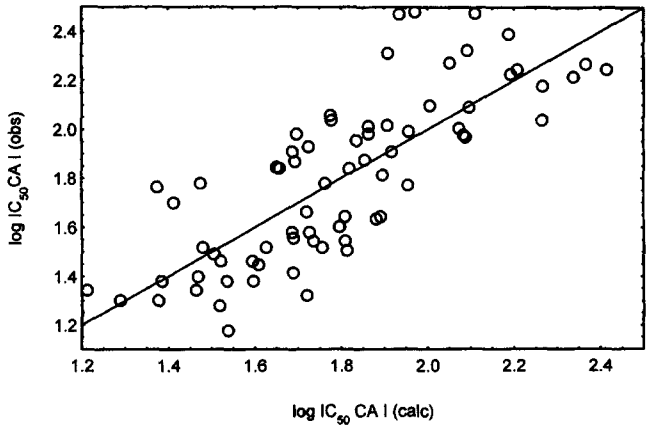



**Figure 2.** Plot of observed log $IC_{50}$ values for CA I against those calculated by equation 1 for all 72 drugs.

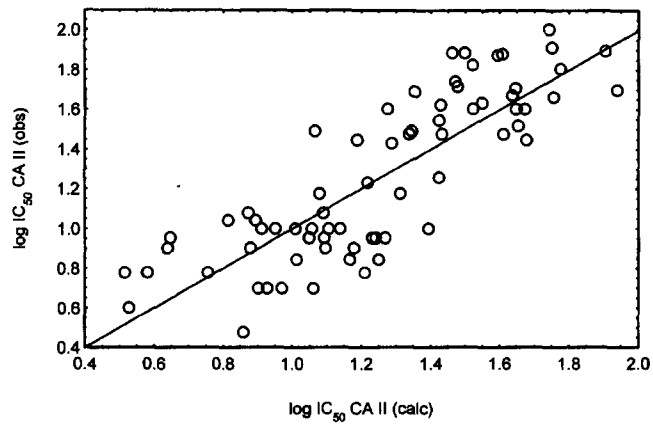



**Figure 3.** Plot of observed log $IC_{50}$ values for CA II against those calculated by equation 4 for all 72 drugs.

*3.2.4. General*

Because of the significance of the frontier orbital energy terms, it is of interest to examine the nature of these orbitals. As the molecules in this study are not even approximately linear or planar, their orbitals do not classify easily into π and σ. However, in the HOMO of all of the drugs except **C38** and **D56** there were large coefficients of the $p_z$ atomic orbitals on the carbon atoms of the primary sulfonamide ring, and usually also on the nitrogen of the secondary sulfonamide group. In **C38** and **D56** there were contributions to the HOMO on the second benzene ring. The makeup of the LUMO was more varied, but usually included a large contribution from the secondary sulfonamide group, and often from the second benzene ring when this was present. The HOMO tended to be much more π-like than the LUMO.

A test for the likelihood of chance correlation using random reassignment of the dependent variable as described previously [41, 42] and as recommended by Topliss et al. [43, 44] yielded probabilities of $7 \times 10^{-6}$, $1 \times 10^{-6}$ and $7 \times 10^{-7}$ for CA I, CA II and CA IV respectively. Plots of the best fit equation for each isozyme are shown in *figures 2–4*, and a correlation dendrogram of the most important descriptors is given in *figure 5*. Values of the calculated quantities found to correlate significantly with activity in this study may be obtained from the Internet by anonymous ftp [45].

## 4. Discussion

*4.1. Chemistry*

Reaction of alkyl/arylsulfonyl halides with aromatic sulfonamides containing an amino group, such as sulfanilamide, metanilamide, *p*-aminomethyl-benzenesulfonamide and *p*-(2-aminoethyl)-benzenesulfonamide occurred at the non-sulfonamide $NH_2$ moiety, when acetonitrile has been used as solvent and in the presence of an organic base such as pyridine or triethylamine. Mention should be made that when Schotten-Baumann reaction conditions have been used in these syntheses, in addition to the desired products of type **A–D**, the $SO_2NH_2$ moiety of the initial sulfonamide has also been derivatized and mixtures of compounds were obtained which were generally difficult to purify. This is in strong contrast with the case of the heterocyclic compounds of type **9** and **10** previously reported by us (prepared by the same reaction as the one applied in the syntheses of compounds **A–D**), case in which the Schotten-Baumann

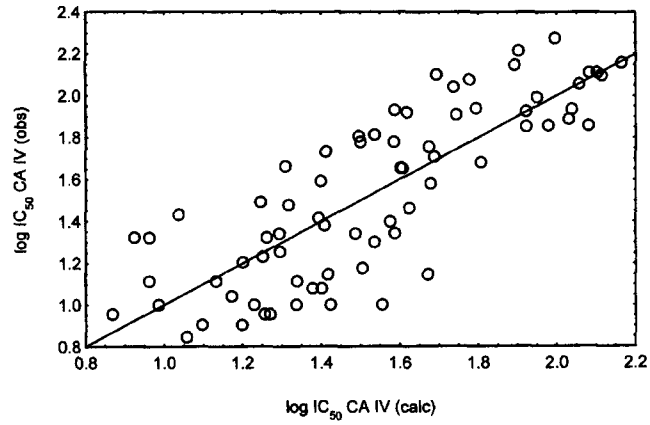



**Figure 4.** Plot of observed log $IC_{50}$ values for CA IV against those calculated by equation 7 for all 72 drugs.

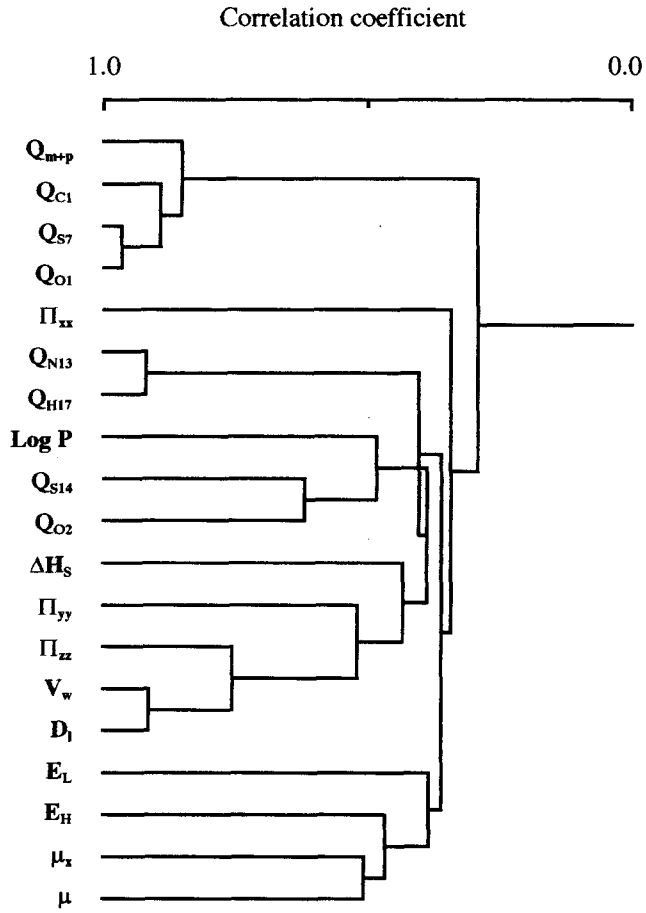



**Figure 5**. Correlations between descriptors which were found to be significant in this study.

conditions led to best yields in alkyl/arylsulfonylamido-1,3,4-thiadiazole-2-sulfonamide derivatives, without the undesired derivatization of the primary sulfonamido group [18]. Thus, the reactivity towards the sulfonyl halides of the aromatic sulfonamides investigated here is just similar to that towards the aromatic sulfonyl halides which has been recently investigated by this group [46, 47].

A large number of alkyl- and substituted-arylsulfonyl moieties has been chosen to be introduced in the molecules of the new compounds reported here, as it has been well documented that the nature of side chains present in a molecule of a CA inhibitor greatly influence the biological activity [12, 16–18, 48]. The inhibitory capacity varied generally in the following way in the four series of obtained derivatives (possessing the same moiety substituting the amino group): metanilamides **B** < sulfanilamides **A** < p-aminomethyl-benzenesulfonamides **C** < p-(2-aminoethyl)benzene-sulfonamides **D**. Moieties that led to strong inhibitors were: 4-halogenophenyl; 2-, 3- and 4-nitrophenyl; 2-carboxyphenyl and dimethylaminosulfamoyl among others. The susceptibility to inhibition of the three isozymes was generally in the following order: CA I < CA IV < CA II. Thus, as for other sulfonamide derivatives [12, 18, 47], the rapid isozyme CA II had the highest affinity for these new sulfonamides, followed by the membrane-bound isozyme CA IV, whereas the low activity isozyme CA I possessed the weakest affinity.

### *4.2. QSAR models*

For inhibition of all three isozymes, the most influential descriptors were atomic charges and frontier orbital energies. Inhibitory activity correlated negatively with the positive charge on the sulfonamide S and negatively with the negative charge on the carbon bonded to it, and also negatively with the positive charge on the H bonded to the sulfonamide N, and negatively with the negative charge on the sulfonamide N and O. With CA I the correlation was with the charge on the O atoms of the primary sulfonamide. With CA II and CA IV it was weaker, and with the charge on the O and N atoms of the secondary sulfonamide. For CA II and CA IV, activity was positively correlated with local dipole index. Thus the more the general tendency of positive and negative charge in the molecule to separate, the more active the drug.

For inhibition of CA I $E_H$ and $E_L$ were both important, and both had positive coefficients (i.e. stronger inhibitors had lower frontier orbital energy values). The coefficient of $E_L$ was in each case the smaller. Only $E_L$ was important for inhibition of CA II, and although both were significant for CA IV inhibition, they were of less importance than for the other two isozymes.

The significance of $E_H$ and $E_L$ derives from Koopman's theorem, which equates them with the negative of the ionization potential (IP) and electron affinity (EA) respectively. Koopman's theorem is more valid for IP than EA [49], and this has led to criticism of the use of $E_L$ in QSAR studies. It has been found however that in spite of the unreliability of the absolute values of these quantities, $E_H$ and $E_L$ calculated by AM1 correlate reasonably well with experimental IP and EA, with correlation coefficients in a series of simple compounds better than 0.9 [50]. The correlation of the activity of drugs with $E_H$ and $E_L$ is usually attributed to the formation of charge transfer complexes between the

aromatic component of the drug with a π electron system on the receptor. Direct calculation by AM1 of the interaction energy between electron donor-acceptor pairs has shown that this energy correlates reasonably well with experimental stability constants and with $E_H$ for a series of donors [51], and that the calculated interaction energy correlates with $E_L$ for a group of acceptors [52]. Experimental EA values are not available for most compounds, and the number of π electron acceptors for which experimental stability data is available is relatively small. The sum and difference of $E_H$ and $E_L$ have also been implicated in QSARs. These quantities find theoretical interpretation in terms of electronegativity and hardness [53].

The lipophilicity was also important for CA II and CA IV inhibition, with more lipophilic drugs being better inhibitors. Volume or polarizability seems to differentiate the drugs, with larger, more polarizable drugs being relatively better inhibitors of CA I, and poorer inhibitors of CA II. Over the range of polarizabilities covered by this study, there was little influence on CA IV inhibitory activity.

Correlation of log $IC_{50}$ with $\Delta H_S$ is negative and weak with CA I. Correlation with $\mu_x$ is negative and weak for both CA I and CA II. These signs are precisely the opposite of those observed previously for a series of similar thiadiazole and thiadiazoline disulfonamides [19], but the sign of dependence on $\mu_x$ agrees with that in a series of sulfanilamide Schiff's base inhibitors of CA II (but not CA I) [20].

The positive sign of the correlation of log $IC_{50}$ with $E_H$ and $E_L$ agrees with the negative sign of that of log $K_I$ with the same parameters in our noncongeneric series [21]. As was suggested there, it is possible that interactions of a charge transfer nature tend to hold the drug in a location unfavourable for enzyme inhibition in some of these series.

The only one of the three indicator variables to become significant was $I_3$, and its coefficient was negative for all three isozymes. Thus, a two-carbon spacer between the secondary sulfonamide nitrogen and the primary benzenesulfonamide is advantageous to activity for all three isozymes. Apart from this, the differences between the four groups of drugs can be better accounted for by the other physical parameters included in the study.

There was no obvious correspondence between the more severe outliers in *figures 2–4* and any particular structural feature of the drugs, and while in general the placement of the different drugs was similar for the three isozymes, a drug which was an outlier in one of the graphs was not necessarily an outlier in the others.

It may be seen from *figure 5* that most of the intercorrelations of the descriptors were small, with the exception of three small clusters: $Q_{S7}$ and $Q_{O1}$ were highly correlated with each other, and to a lesser extent with $Q_{m+p}$ and $Q_{C1}$, $V_w$ was correlated with $D_l$, and $Q_{H17}$ with $Q_{N13}$. Only the weaker correlations of the four-member cluster appeared in any of the equations presented here, and as evidenced by the Λ values, the effect was not severe. The correlations should be born in mind however when drawing conclusions about the physical significance of the terms.

The compounds in this study are all somewhat selective for CA II. Selectivity of the inhibitors for CA I may be improved, but only to a small degree, by the use of larger, more polarizable and more electron-withdrawing substituents. These effects oppose each other with mono halogen-substitution of the second benzene ring, with the polarizability effect dominating, the bromo compound being enhanced in CA I activity relative to CA II and IV. A small further improvement may be expected with iodo substitution. Carboxy and nitro as electron withdrawing groups are better still, but in none of the patterns tried here does the CA I activity approach that for CA II. Perhaps a 2,6-disubstitution or a 2,4,6-trisubstiution with one or both of these substituents would produce a drug for which the CA I activity reached or surpassed CA II activity. Structures of type D exhibit enhanced activity, and this effect is greater for CA II than for the other two isozymes. This may possibly be enhanced by extending further the two-carbon spacer. Fluoro substitution on either of the benzene rings would enhance activity via the lowering of $E_H$ and $E_L$, and would lead to compounds with higher activity for all three isozymes, but more so with CA II and CA IV than CA I. Some compounds of this type will be described in future publications.

## 5. Conclusions

The findings of previous studies that the charges on the sulfonamide group are important in determining CA inhibitory potency have been confirmed in this group of drugs, with the additional result that the charges on a second sulfonamide group, when present, are also important. Frontier orbital energy effects are also implicated, perhaps indicating a weak charge-transfer interaction in the binding site. Some indications have been found of how selectivity of the drugs for the different isozymes might be adjusted. The scope for enhancement of selectivity of compounds of the present class using substituents of the kind we have used is however limited. In contrast, the magnitude of the activity can be increased greatly by appropriate selection of substituents.

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