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ORIGINAL RESEARCH ARTICLE

QSAR of a Group of Sulphonamides as Carbonic Anhydrase Inhibitor I

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ABSTRACT

A quantitative structure-activity relationship (QSAR) study is presented for carbonic anhydrase inhibitors (CAIs), these compounds are powerful inhibitors of several isozymes of the enzyme carbonic anhydrase. Activity was found to depend on electrostatic potential-based charges on the atoms of sulfonamide groups, dipole moments and lipophilicities. The conclusion of the study is that activity is greatly modulated by electronic effects on the pyridinium ring. The most significant effects were enhancement of activity with increased positive charge on this ring, weakening of activity with increasing HOMO energy. These results are compared with those from other studies.

Key words: QSAR, carbonic anhydrase inhibitor, charge, sulphonamides, carbonic anhydrase inhibitor.

INTRODUCTION

Aromatic sulfonamides such as sulfanilamide, have been shown to act as inhibitors of the zinc enzyme carbonic anhydrase in 1940 by Mann and Keilin, 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. A very large number of sulphanilamide derivatives had been investigated in that period in the search for more effective antibacterial derivatives, among which were also homosulfanilamide or the N-4-substituted derivatives of type. Although none of these derivatives led to clinical applications. It was thereafter shown by Krebs that the aromatic sulphonamides behave as weaker CA inhibitors as compared to the heterocyclic compounds. This led to the extensive study of compounds from the class (with the subsequent introduction of acetazolamide and methazolamide in clinical medicine. The above mentioned facts and the lack of CA inhibition studies with sulfonylamido-derivatives of sulphanilamide and related amino-sulfonamides prompted to prepare a series of such derivatives and to investigate them for the inhibition of the physiologically relevant isozymes CA I.

Sulfonamide inhibitors of the zinc enzyme carbonic anhydrase have been extensively studied due to their use as clinical agents in the treatment of glaucoma, gastric and duodenal ulcers, epilepsy and possibly other disorders associated with electrolyte secretion. QSAR calculations in this class of pharmacological agents were reported for aromatic as well as heterocyclic sulphonamides. Thus , the early work of Kakeya et al stressed that the electronic properties of the sulphonamides group are important for the CA inhibitory effects of a series of 2-, 3- or 4-substituted benzenesulfonamides.

Their general conclusion was that electron-withdrawing groups (such as halogen, alkoxy, nitro etc) by decreasing the pKi, of the sulfonamido group led to stronger CA inhibitors, a fact established experimentally since the beginning of research in this field .

More recently, Menziani *et al* have reported a molecular mechanics and QSAR study of benzenesulfonamides, showing that the discrimination of an enzyme towards its inhibitors is dominated by short-range van-der-Waals forces. De Benedetti's group also reported some important QSAR studies on heterocyclic sulfonamides, such as 1,3,4-thiadiazole-2-sulfonamide 3; 1,3-thiazole- 2-sulfonamide 6; 1,3-thiazole-5sulfonamide 7 and thiophene-2-sulfonamide 8 derivatives. The conclusions of these studies are quite similar to those already mentioned for the aromatic derivatives, both for

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the electronic properties of the sulfonamido group, as well as the hydrophobic moieties present in such molecules.

Somewhat similar results were also reported by Kishida who also developed a QSAR model for explaining the CA inhibitory properties of 1,3,4-thiadiazole-2-sulfonamide derivatives, but this study has also shown that a (formally) positive charge on the endocyclic nitrogen atom in position 5 is correlated with increased inhibitory properties for the respective compounds. The objective of the QSAR study was to discover the physical characteristics of the drug molecules responsible for their activity, and hence the nature of the principal forces between the drug and its receptor. This should also throw light on the characteristic of a good inhibitor.

MATERIALS AND METHODS

QSAR study is performed on sulfonamide derivatives as carbonic anhydrase inhibitor:

In this paper we present a QSAR study for 62 such derivatives. The QSAR study reported in the following pages was done by considering the IC_{50} values towards isozyme CA I. A number of quantum chemical and other properties of the drugs were calculated, and related to the drug activity by multiple linear regression analysis (MLR). Rigorous precautions were taken to ensure that the results were not invalidated by collinearity, or by chance effects.

Methodology used for QSAR analysis:

The QSAR study was performed on a series of benzene sulfonamides. The series consist of 62 compounds. The biological activities were expressed in terms of IC_{50} and these values were reported in terms of micromoles (µm). These compounds are powerful inhibitors of several isozymes of the enzyme carbonic anhydrase.

The compounds in the series were sketched using Chem.Draw, module of Chemoffice. The structures were then placed on chem. 3-D module, and then energy was minimized for each compound to calculate electronic descriptors. MM2 server was used for energy minimization, minimum RMS gradient used was 0.01. Then subsequently MOPAC server was used for energy minimization, minimum RMS gradient used was 0.001. Properties were computed for each molecule and word file was made for each compound. Then these descriptors values were added on the excel sheet which were then further processed on VALSTAT to generate models.

QSAR analysis:

Descriptors were taken as independent variable and biological activity as dependent variable. The statistical measures used for evaluating the generated models are correlation coefficient(r), correlation coefficient (r2), fischer ratio and standard deviation. Equations were generated and the best QSAR models were selected on the basis of various stats parameter.

Test and training set:

In this method, compounds were divided in to two groups: one was training set and other, the test set with the remaining based on random selection criteria. The generated best model were validated for the predicted ability inside the model (Test and training set) and the validation parameters (S press Q2 and SDEP) were calculated for the generated model. Training set were 25% of the test test. The best model with the best equation is being shown here:

Validation:

Once the model is generated, its reliability and significance is determined. Leave one out method. In this method one compound is eliminated from the rest of the compound and QSAR model is generated. The activity of the eliminated compound is then predicted using the obtained model. This process is repeated by eliminating the rest of the compound one by one. Squared cross-correlation co-efficient (Q^2), standard deviation of sum of square of difference between predicted and observed values (S_{PRESS}) and standard deviation of error of prediction (S_{DEP}) were also calculated for each model to estimate the predictive potential of models with the help of VALSTAT software.

The nucleus for the series is:

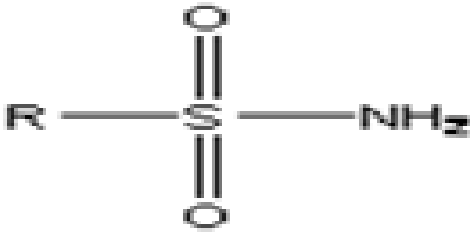
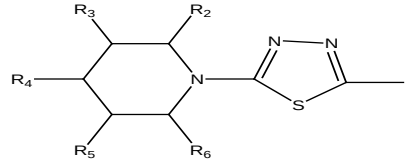
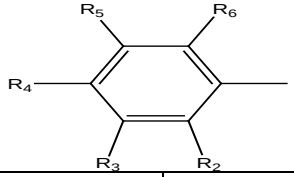


Table 1: Compounds and biological activity

MODEL R						
Drug	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**IC_{50} CA I (µm)**

1	Me	H	Me	H	Me	31
2	Et	H	Me	H	Et	24
3	i-Pr	H	Me	H	Me	21
4	t-Bu	H	Me	H	Me	19
5	i-Pr	H	Me	H	i-Pr	18
6	t-Bu	H	Me	H	t-Bu	16
7	Me	H	t-BuCH_2	H	Me	6
8	Et	H	t-BuCH_2	H	Et	6
9	i-Pr	H	t-BuCH_2	H	i-Pr	7
10	Ph	H	Me	H	Me	0.5
11	2-	H	Me	H	Me	0.8
12	Ph	H	Me	H	Ph	118
13	Me	Me	Me	H	Me	31

MODEL R



Drug	R_2	R_3	R_4	R_5	R_6	IC_{50} CA I (µm)
14	H	H	$PhCH_2SO_2NH$	H	H	0.109
15	H	H	$PhSO_2NH$	H	H	0.103
16	H	H	4-Me-$C_6H_4SO_2NH$	H	H	0.096
17	H	H	2,4,6-Me_3-$C_6H_2SO_2NH$	H	H	0.069
18	H	H	4-F-$C_6H_4SO_2NH$	H	H	0.040
19	H	H	4-Cl-$C_6H_5SO_2NH$	H	H	0.038
20	H	H	4-Br-$C_6H_4SO_2NH$	H	H	0.035
21	H	H	4-MeO-$C_6H_4SO_2NH$	H	H	0.045
22	H	H	2-HOOC-$C_6H_4SO_2NH$	H	H	0.026
23	H	H	3-$H_2NC_6H_4SO_2NH$	H	H	0.168
24	H	H	4-$H_2NC_6H_4SO_2NH$	H	H	0.164
25	H	$PhCH_2SO_2NH$	H	H	H	0.150
26	H	Me_2NSO_2NH	H	H	H	0.210
27	H	$PhSO_2NH$	H	H	H	0.295
28	H	4-Me-$C_6H_4SO_2NH$	H	H	H	0.301
29	H	2,4,6-Me_3-$C_6H_2SO_2NH$	H	H	H	0.098
30	H	4-F-$C_6H_4SO_2NH$	H	H	H	0.065
31	H	4-Cl-$C_6H_5SO_2NH$	H	H	H	0.044
32	H	4-Br-$C_6H_4SO_2NH$	H	H	H	0.040
Drug	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**IC_{50} CA I (µm)**
33	H	4-MeO-$C_6H_4SO_2NH$	H	H	H	0.059
34	H	2-HOOC-$C_6H_4SO_2NH$	H	H	H	0.032
35	H	4-AcNH-C_6H_4 SO_2NH	H	H	H	0.298
36	H	3-$H_2NC_6H_4SO_2NH$	H	H	H	0.176
37	H	4-$H_2NC_6H_4SO_2NH$	H	H	H	0.135
38	H	H	$MeSO_2NHCH_2$	H	H	0.104
39	H	H	$PhCH_2SO_2NHCH_2$	H	H	0.096
40	H	H	$Me_2NSO_2NHCH_2$	H	H	0.090
41	H	H	$PhSO_2NHCH_2$	H	H	0.081
42	H	H	4-Me-$C_6H_4SO_2NHCH_2$	H	H	0.085
43	H	H	2,4,6-Me_3-$C_6H_2SO_2NHCH_2$	H	H	0.060
44	H	H	4-F-$C_6H_4SO_2NHCH_2$	H	H	0.033
45	H	H	4-Cl-$C_6H_5SO_2NHCH_2$	H	H	0.029
46	H	H	4-Br-$C_6H_4SO_2NHCH_2$	H	H	0.024
47	H	H	4-MeO-$C_6H_4SO_2NHCH_2$	H	H	0.043
48	H	H	2-HOOC-$C_6H_4SO_2NHCH_2$	H	H	0.021
49	H	H	3-$H_2NC_6H_4SO_2NHCH_2$	H	H	0.123
50	H	H	4-$H_2NC_6H_4SO_2NHCH_2$	H	H	0.109
51	**H**	**H**	**$MeSO_2NHCH_2CH_2$**	**H**	**H**	**0.093**
52	H	H	$Me_2NSO_2NHCH_2CH_2$	H	H	0.075

53	H	H	$PhSO_2NHCH_2CH_2$	H	H	0.069
54	H	H	4-Me-$C_6H_4SO_2NHCH_2CH_2$	H	H	0.070
55	H	H	2,4,6-Me_3-	H	H	0.046
Drug	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**IC_{50} CA I (µm)**
56	H	H	4-F-$C_6H_4SO_2NHCH_2CH_2$	H	H	0.028
57	H	H	4-Cl-$C_6H_5SO_2NHCH_2CH_2$	H	H	0.024
58	H	H	4-Br-$C_6H_4SO_2NHCH_2CH_2$	H	H	0.019
59	H	H	4-MeO-$C_6H_4SO_2NHCH_2CH_2$	H	H	0.038
60	H	H	2-$O_2NC_6H_4SO_2NHCH_2CH_2$	H	H	0.022
61	H	H	3-$H_2NC_6H_4SO_2NHCH_2CH_2$	H	H	0.101
62	H	H	4-$H_2NC_6H_4SO_2NHCH_2CH_2$	H	H	0.095

RESULTS

Results of QSAR studies: Optimized model no. 2 results are:

BA= [-0.857066(± 1.75465)] +NVDW [-0.0806035 (± 0.0699705)] +HOMO [0.221215 (± 0.163646)] +RE [-0.000235738(± 5.42633)] +TTE [-0.0025978 (±0.000361979)] Contribution of parameters to model is: NVDW:HOMO:RE:TTE::1:8.54752:20.2444:41.1056

Table 2:

S. No	Parameters	values
1	n	62
2	r	0.935924
3	r^2	0.875955
4	variance	0.13175
5	Std	0.362974
6	F	100.627
7	FIT	516.037
8	Bootstrapping r^2	0.896876
9	Q^2	0.798081
10	Spress	0.463099
11	S_{DEP}	0.444033

In the above QSAR model n is no of data points, r is correlation coefficient, r^2 is sequential, std is standard deviation or standard error of estimate, accuracy is shown by low values of std error of estimate, F- fischer ratio between the variance of calculated and observed activity. The orthogonal nature of the descriptors in the model was established by the calculation of correlation matrix, which indicates absence of any cross correlation among the descriptors used for the formulating the model. The r = 0.93 for the hCAI represents the better fit of the regression and good predictability ability and robustness of the model. Very low S_{press} and S_{DEP} of the model indicate predictivity of the models. Low std error of estimation (std =0.36 for hCAI) suggest a high degree of confidence in model.

Result of training and test set:

pKi = [-3.368(± 1.452)] -NVDW [0.071(± 0.0396)] -HOMO [0.373(± 0.129)] -RE [0.00014(± 4.452e-005)] -TTE [0.0023(± 0.00032)] : contribution of parameters to model is: NVDW:HOMO:RE:TTE::1:5.9506:5.35609:15.329

Table 3:

S. No	Parameters	Values
1	n	43
2	r	0.968
3	r^2	0.938
4	variance	0.0388
5	std	0.197
6	F	145.781
7	FIT	1023.03
8	Bootstrapping^2	0.935636
9	Q^2	0.917536
10	Spress	0.228723
11	SDEP	0.215014

Table 4: Observed activity, calculated activity, Z value and predicted activity of training set for hCAI.

S. No	Comp. No	Calculated Value	Predicted Value	Observed Activity	Z Value
1	16	6.9406	6.9332	7.017729	1.25339
2	17	7.08768	7.07132	7.161151	1.46634
3	23	6.7584	6.75663	6.774691	0.114113
4	36	6.73308	6.73037	6.754487	0.0285056
5	56	7.84811	7.90528	7.552842	-2.41506
6	49	6.75376	6.72717	6.910095	-0.246301
7	54	7.24365	7.25	7.154902	-0.100406
8	19	7.48118	7.48755	7.420216	0.222261
9	8	5.24526	5.25273	5.221849	-0.386809
10	58	7.60338	7.59153	7.721246	0.19976
11	42	7.17454	7.18029	7.070581	-0.193532
12	43	7.1507	7.14306	7.221849	0.62748
13	53	7.16055	7.16052	7.161151	0.212394
14	55	7.17809	7.14151	7.337242	1.109
15	38	6.52752	6.41195	6.982967	2.46476
16	26	6.62063	6.61664	6.677781	0.160536
17	31	7.40434	7.40839	7.356547	0.132263
18	14	6.88399	6.88084	6.962574	0.18718
19	39	6.97934	6.9768	7.017729	-0.250392
20	62	7.06932	7.07542	7.022276	-0.879864
21	20	7.32617	7.31859	7.455932	0.818701
22	41	7.07505	7.07401	7.091515	0.254657
23	27	6.80204	6.82543	6.530178	-0.555292
24	61	6.8324	6.79969	6.995679	-0.293929
25	5	5.05234	5.12727	4.744727	-1.61925
26	30	7.60883	7.6591	7.187087	-1.9217
27	59	7.61403	7.59732	7.823909	0.894542
28	47	7.51001	7.5188	7.366532	-0.900802
29	25	6.5393	6.51218	6.823909	0.194643
S. No	**Comp. No**	**Calculated Value**	**Predicted Value**	**Observed Activity**	**Z Value**
30	51	6.62901	6.56294	7.031517	2.11046
31	46	7.64957	7.65254	7.619789	-0.209947
32	50	6.88925	6.88138	6.962574	-0.407311
33	3	4.9471	5.01605	4.677781	-1.47773
34	29	6.99157	6.98717	7.008774	1.17709
35	15	6.81418	6.79702	6.987163	1.39205
36	45	7.68607	7.70156	7.537602	-0.833867
37	34	7.67051	7.70012	7.49485	-0.843983
38	1	4.59542	4.63587	4.508638	-0.78238
39	28	6.86085	6.8837	6.521434	-0.891317
40	24	6.72881	6.72066	6.785156	0.151345
41	57	7.80554	7.82362	7.619789	-0.879125
42	37	6.66743	6.63192	6.869666	0.713891
43	33	7.23362	7.23397	7.229148	0.203625

Fig 1: Scatter plot between the observed activity and calculated activity for model no.1 of training set for hCAI

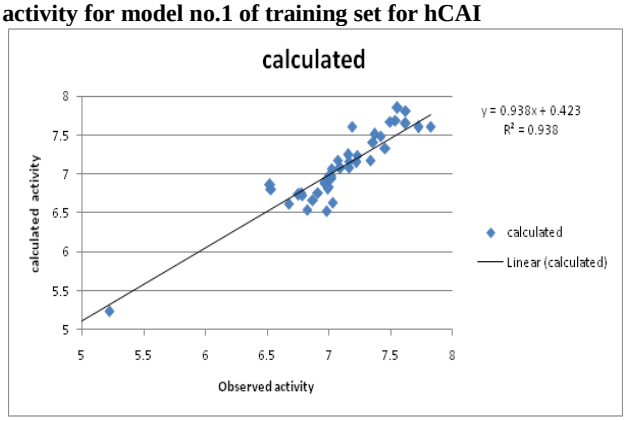


Fig 2: Scatter plot between the observed activity and predicted activity for model no.1 of training set for hCAI

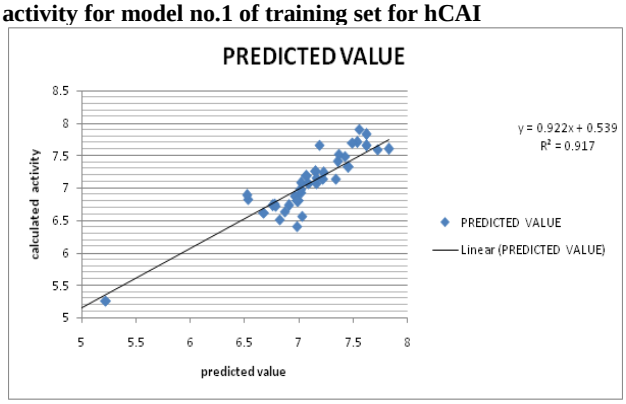
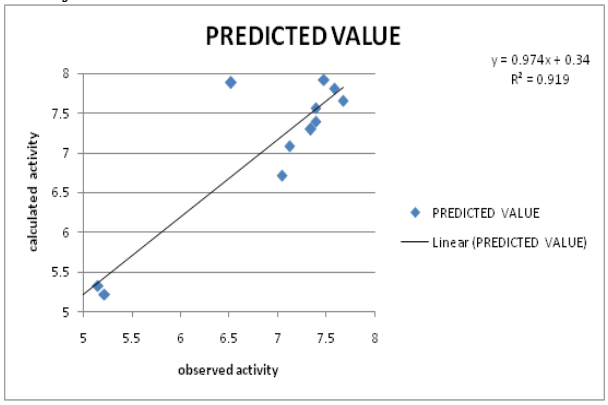


Table 5: Observed activity and predicted activity for model no.1 of test set for hCAI

S. No	Comp. No	Predicted Value	Observed Act
1	18	7.56504	7.39794
2	22	7.81048	7.585027
3	48	7.65936	7.677781
4	7	5.21713	5.221849
5	40	6.71064	7.045757
6	35	7.88094	6.525784
7	21	7.29158	7.346787
8	2	4.84584	4.619789
9	32	7.39911	7.39794
10	13	4.63116	4.508638
11	6	5.02919	4.79588
12	44	7.91955	7.481486
13	4	4.9471	4.721246
14	9	5.32297	5.154902
15	52	7.08546	7.124939

Fig 3: Scatter plot between the observed activity and predicted activity for model no.1 of test set for hCAI



Result of Validation:

BA = [0.570784(± 1.45841)] +NVDW [-0.226178(± 0.0670433)] +HOMO [-0.186405(± 0.135601)] +RE [-0.000156407(± 4.48258e-005)] +TTE [-0.00178596(± 0.000327137)]

Contribution of parameters to model is:NVDW:HOMO:RE:TTE::1:2.51681:4.67033:9.83888

Table 6:

S. No	Parameters	Values
1	n	46
2	r	0.969955
3	r^2	0.940813
4	variance	0.0571914
5	std	0.239147
6	F	162.929
7	FIT	1059.7
8	Bootstrapping r^2	0.939249
9	Q^2	0.918867
10	Spress	0.279995
11	SDEP	0.26434
12	r^2pred	0.891758

In which

HOMO: energy of highest occupied molecular orbital.

NVDW: non vander walls forces that is the sum of pair wise vander walls interaction energy .

RE: repulsion energy that is the total core internuclear repulsion between atoms.

TTE: total energy that is mopac electronic energy and repulsion energy.

DISCUSSION

All parameters have contributed negatively to the BA, the above result shows that subsituent with less dipole moment, less total energy, repulsion energy, and less energy of highest occupied molecular orbital will be responsible for the inhibition of energy. For inhibition of the enzyme, the most influencing descriptor may be atomic charges and frontier molecular orbital energies. The frontier energy term E_H may be indicative of the formation of a charge- transfer complex with the aromatic part of the inhibitor. The coefficient for this term is negative, implying that a large E_H leads to low activity. This suggests that the charge transfer bonding competes with the process leading to enzyme inhibition. Selectivity of the inhibitor for CA I may be improved by the use of larger, more polarizable and more electron withdrawing. Carboxy and Nitro act as electron withdrawing groups which may increase the activity. Fluoro substitution on either of benzene ring would enhance activity via lowering $E_{H.}$ Substituents which are electron donating by resonance such as phenyl, raise the HOMO energy and thus may decrease the activity. Polarizability seems to differentiate the drugs, with larger, more polarizable drugs being relatively better inhibitors of CAI, and poorer inhibitors of CAII. So it means such subsituents would increase the activities which are more polar or less non-polar. The carbon atoms of the pyridinium ring are negatively charged, except for that in position 4. All correlations involving charge suggested that

increasing charge in a positive sense on any of these atoms increased the activity of the drug. Phenyl substitution may be effective in this respect [16].

Ultimate aim of this research is to

- Increase effective designing of carbonic anhydrase inhibitor by using efficient principles of molecular modelling techniques.
- The designing of these specific carbonic anhydrase inhibitors would be less time consuming and cost effective.
- Selectivity of the drugs can be enhanced on the basis of QSAR studies.

The present study involving study of carbonic anhydrase inhibitor (CAIs) aims at:

- Observing the contribution of different descriptors to the biological activity and optimizing those parameters by optimizing the models.
- The scope for enhancement of selectively of the present class using different substituents, thus the magnitude of the activity can be increased greatly by appropriate selection of same.

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