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Carbonic Anhydrase Inhibitors: Docking, Synthesis and Evaluation of Sulfonamide bearing Amino-Acid as CAII Inhibitors

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Abstract

A set of twelve of p-amino benzene sulfonamides derivatives containing amino acids were synthesized. The synthesized compounds were characterized and tested for their bCA II inhibitory activity, taking acetazolamide as standard. The percentage of bCAII inhibitory activity of the synthesized compounds ranged from 20-78 at a concentration of $0.1*10^3$µm. The highest inhibition was shown by compound D2 (78.53) which is comparable to acetazolamide (78.62). The lowest inhibition was shown by compound D9 (20.02) respectively.

Docking analysis of the synthesized compounds was also performed using GOLD software. The PDB ID 4HT0 was selected for docking studies. All the synthesized compounds show same interaction of nitrogen (N1 and N2) atoms with Zn^{2+}, Oxygen atom of SO_2 forms H- bonding with Thr forming tetrahedral geometry. The distance between the nitrogen (N1and N2) atoms and Zn^{2+} in the synthesized compounds is similar to that of distance between the nitrogen (N1andN2) atoms and Zn^{2+} found in standard drug acetazolamide.

Keywords: Benzenesulfonamide, Amino acid, CA II, Acetazolamide, Enzyme inhibition, Docking

1. Introduction:

Carbonic anhydrase (CA) constitutes family of enzymes that have Zn^{2+} ion as cofactor [1]. These enzymes are present in almost all living organisms ranging from prokaryotes to eukaryotes. In mammals carbonic anhydrase catalyses reversible conversion of CO_2 and H_2O to form bicarbonate, a crucial reaction for almost all the pathological and physiological processes taking place in body, like respiration, lipogenesis pH homeostasis etc [2]. About 16 different isoforms of carbonic anhydrase exist in mammals and have been classified depending on the location i.e., mitochondrial, membrane associated, and cytosolic. Within cytosol isoforms CA I and CA II [3] are present in high concentration and CA II is reported to be most active. Carbonic anhydrase II is one of the highest catalytically efficient enzyme and this widely distributed isoform covers almost every tissue and organ including

erythrocytes, eye, GI tract, bone osteoclasts, kidney, lung, testis, stomach, liver, brain and numerous biological fluids [4].

It was found that abnormal activities of CA are often linked to various diseases, which include epileptic seizures, leukemia, glaucoma etc. Inhibition of carbonic anhydrase in the ciliary processes of the eye decreases aqueous humor secretion [5], most likely by slowing the formation of bicarbonate ions with consequent decrease in sodium and fluid transport. Acetazolamide a well-known example of clinically established carbonic anhydrase inhibitor shows anti-glaucoma activity.

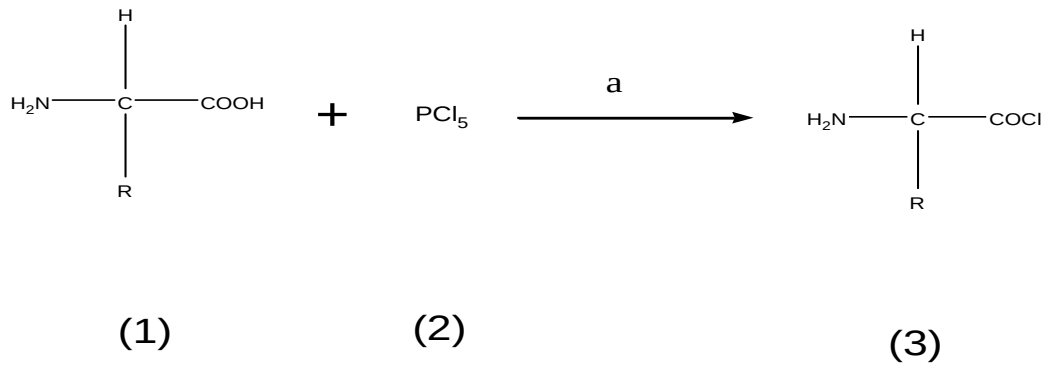
Sulfonamides are well known for their anti- CA I, diuretic and anti-malarial activity [6]. Among the broad spectrum of activity exhibited by sulfonamides, their role as inhibitors of the zinc containing metalloenzymes CA is presumably most widely studied [7]. Acetazolamide, topiramate, ethoxazolamide, brinzolamide, dorzolamide all are CA inhibitors having sulfonamide moiety. Literature shows that the benzene sulfonamide has been substituted with various substituents like 1,3,5 triazinyl[10], pyrrole- pyrrolopyrimidine, coumarine-benzocoumarine[9] moities showing various degrees of CA inhibitor activity. Recently studies on Probenecid-based amide derivatives, incorporating different natural amino acids and groups were synthesized, their interaction with some mammalian CA isozymes was studied.
In the present study p-aminobenzene sulfonamide was substituted with various amino acids and studied for its CA II inhibitory activity. A docking analysis of the synthesized compounds was also performed to study the interactions using acetazolamide as standard.

2. Result & Discussion:

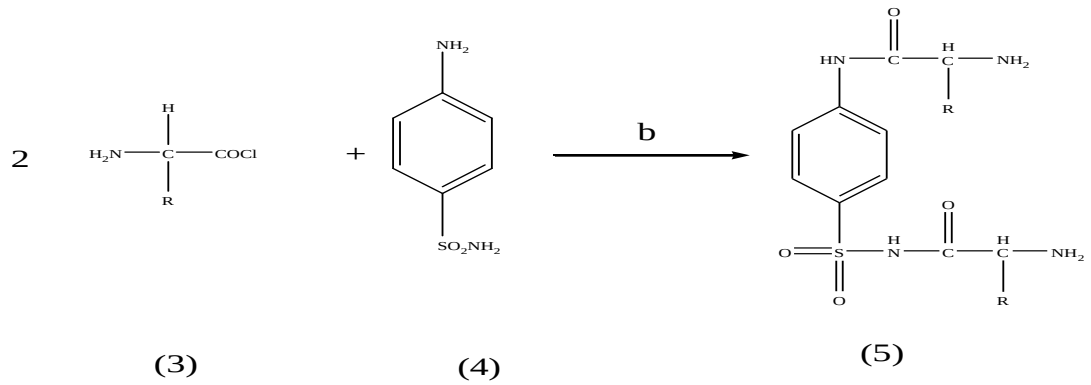
2.1 Chemistry

Benzene sulfonamide and their derivatives are reported to be effective inhibitors of CA isoforms. In the present study both the amino groups of benzene sulfonamide were substituted with amino acids. The synthesis of compounds was carried out in two steps:

1. Chlorination of amino acid by reacting amino acid with phosphrous pentachloride.
2. Addition of amino acid chloride to the solution of benzene sulfonamide in 10% aqueous NaOH with continuous stirring.



Step 1: Chlorination of amino acid



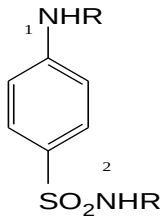
Step 2: Synthesis of di-substituted benzene sulfonamide

Step 1 and 2: Synthesis of p-aminobenzene Sulfonamide Derivative; (1) Amino acid, (2) Phosphorus pentachloride, (a) stirring for 4hr., (3) amino acid chloride, (4) p-aminobenzene Sulfonamide, (b) 10% aqueous NaOH, (5) Di-substituted Benzene Sulfonamide.

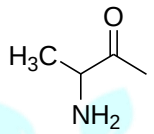
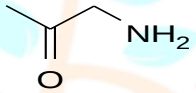
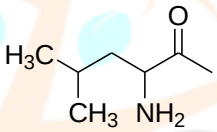
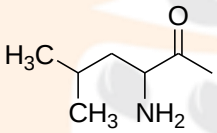
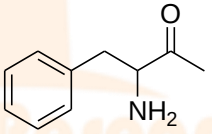
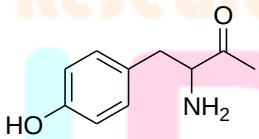
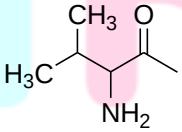
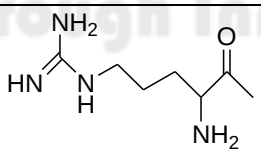
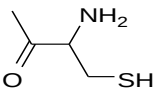
2.2 Carbonic anhydrase II inhibitory activity

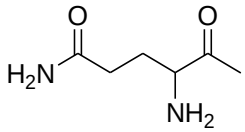
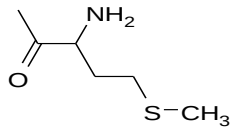
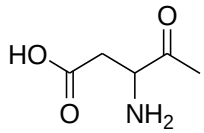
The carbonic anhydrase inhibitory activity of the synthesized compounds and the standard drug Acetazolamide was assessed against the cytosolic isoform II from bovine erythrocytes. The cytosolic isoform bCAII was inhibited by all the synthesized compounds to different extent (Table 1) the percentage inhibition ranged between 69 to 78. Compound D2 containing amino acid glycine showed highest inhibition (78.53%) against bCAII isoform comparable to acetazolamide (78.62%). Whereas, compound D9 containing amino acid cystein showed lowest inhibition (20.02%).

Table 1: Carbonic anhydrase II inhibition activity of synthesized compounds.



General Structure

S. NO.	Compound Code	R	% Inhibition*
1.	D1		**37.86**
2.	D2		**78.53**
3.	D3		**30.68**
4.	D4		**36.9**
5.	D5		**70.05**
6.	D6		**69.06**
7.	D7		**20.21**
8.	D8		**73.97**
9.	D9		**20.02**

10.	D10		**23.55**
11.	D11		**22.59**
12.	D12		**24.9**
13.	AZM		**78.62**

*Percentage inhibition at concentration $0.1*10^3$µm

2.3 Docking Studies

In the present study, docking of the synthesized compounds was also performed. The PDB ID: 4HT0 was downloaded from RCSB Protein Data Bank. For inhibitory activity of carbonic anhydrase the ligand receptor complex, interaction of ligand with zinc (co-factor) and amino acid residues (Thr, & His) is important. The standard CAI acetazolamide involves coordination of nitrogen atom with Zn^{2+} ion as well as hydrogen bond formation of oxygen of SO_2 with amino acid (Thr). The standard inhibitor acetazolamide consists of two nitrogen atoms from which N1 (NH_2) has a distance 6.40 A° Zn^{2+} while the other nitrogen atom N2 (SO_2NH_2) group has distance 3.69 A° from Zn^{2+}. Interaction of compound D8 and acetazolamide shows (figure 1) that interactions of CA-I replace hydroxide ion bound to Zn^{2+} and form a tetrahedral geometry. Compound D8 which is showing highest docking score (71.67) shows interaction of N1 (7.40) and N2 (3.71) with Zn^{2+} ion. Oxygen atom of SO2 interacts with Thr amino acid residue.(Table 2).

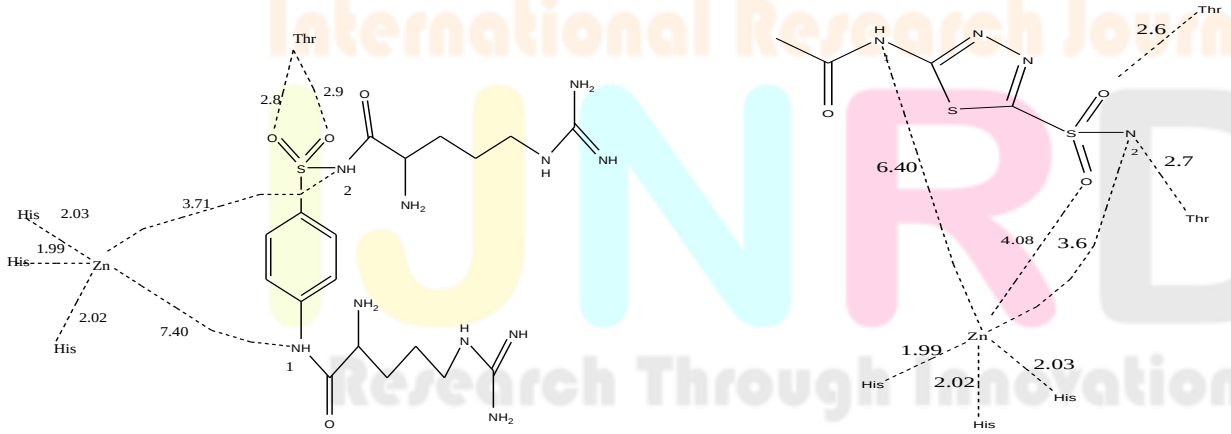


Figure 1: Compound D8 and acetazolamide showing interaction with Zinc and amino acid residues

Table 2: Docking of synthesized compounds

COMPOUND	GLOD SCORE	INTERACTING GROUP	LENGTH (A°)
AZM	42.88	Zn-NH Zn-$NHSO_2$	6.40 3.69
D1	31.89	Zn-NH	8.19

		Zn-NHSO$_2$	2.59
D2	48.92	Zn-NH Zn-NHSO$_2$	6.891 3.95
D3	47.47	Zn-NH Zn-NHSO$_2$	8.04 2.70
D4	52.41	Zn-NH Zn-NHSO$_2$	8.0 1.708
D5	54.66	Zn-NH Zn-NHSO$_2$	7.28 2.54
D6	53.89	Zn-NH Zn-NHSO$_2$	7.48 2.39
D7	49.50	Zn-NH Zn-NHSO$_2$	8.29 2.55
D8	71.67	Zn-NH Zn-NHSO$_2$	7.40 3.71
D9	51.68	Zn-NH Zn-NHSO$_2$	8.4 2.54
D10	55.03	Zn-NH Zn-NHSO$_2$	6.978 3.30
D11	52.08	Zn-NH Zn-NHSO$_2$	8.07 1.74
D12	55.95	Zn-NH Zn-NHSO$_2$	7.409 3.15

3.0 Conclusion

Twelve compounds containing p-aminobenzene sulfonamide substituted with amino acid were synthesized. Out of all the synthesized compounds four compounds D2, D5, D6 and D8 showed good inhibition of CA II whereas remaining eight compounds (D1, D3, D4, D7, D9, D10, D11, D12) showed inhibition less than 50 percent. Inhibition by compound D2 (78.53) is comparable with acetazolamide (78.62). All the synthesized compounds show amino acid interaction in a manner similar to acetazolamide with difference in distance from Zn^{2+} ion. Docking scores of the synthesized compounds range from 31 to 71.

4.0 Experimental

4.1 Material and method

Carbonic anhydrase II was purchased from sigma Aldrich 4-nitro phenyl acetate was purchased from alfa-acer. Tris buffer (reagent grade) was purchased from Bio-Rad. DMSO of reagent grade was used in the experiment. The experiment was performed in triplicate.^{1}**H** NMR spectra were recorded using JNM-ECS 400 spectrophotometer using D_2O as a solvent and TMS as internal reference from IIT Roorkee Ropar and chemical shift value expressed in ppm. Infrared spectra were recorded on FTIR Alpha Bruker by KBr disk method at S.G.S.I.T.S. Indore, and values expressed in cm^{-1}. Electron impact mass spectra (LC-MS) were recorded on Bruker micro TOF QII high resolution mass spectrometer technique using CIF mass facility IISER Bhopal. Thin layer chromatography (TLC) was performed on pre-coated silica gel plates, and visualized in UV chamber.

4.2 Preparation of amino-acid chlorides

A Schlenk tube, containing a suspension of PCl_5 (10mmol) in dichloromethane volume was cooled in an ice bath, and appropriate amino-acid (10mmol) was added. The resulting mixture was stirred for 15 min, then the ice bath was removed and the stirring was prolonged for 4 h. The colorless precipitate was isolated, washed with hexane (2 - 20 mL) and dried in vacuum.[11]

4.3 General procedure for synthesis of compound D1-D12

Amino acid chloride (10 mmol) was added to a solution of benzene sulfonamide (5 mmol) in 10% aqueous sodium hydroxide with constant stirring at room temperature. The reaction was completed in 8-10 hr for different amino acids. The Thin Layer Chromatography was performed to check the completion of reaction. On completion of reaction the reaction mixture was neutralized with 1 M hydrogen chloride (5-10ml). The obtained precipitate was filtered and recystalized with water. Synthesized compounds were characterized for physicochemical properties (as melting point, solubility, and partition coefficient), IR, 1H-NMR and EI-MS spectroscopy methods.

Spectral Characterization

D1: % yield 62.42; mp 168-170°C; LogP -0.806; IR (KBr Cm^{-1}): 1599.62 (C=C Aromatic), 3343.60 (N-H amide), 1638.90 (C=O amide), 1310.93 (Sulfonamide S=0); ^{1}HNMR (DMSO): δ 7.66 (4H, s, ArH), 3071 (1H, s, amide), 3.67(2H, m, methylene), 1.88(2H, s, amine), 1.43 (1H, m, methine)LC-MS m/z 314 [M^+].

D2: % yield 65.03; mp 152-155°C; LogP -0.65; IR (KBr Cm^{-1}): 1596.35(Aromatic C=C); 3259.39 (Amide N-H), 1630.10(Amide C=O); 1312.75(Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.65(4H,s,ArH), 3.46(2H,s,methylene); LC-MS m/z 287 [M+1].

D3: % yield 87.93; mp 257-260°C; LogP -0.308; IR (KBr Cm^{-1}): Aromatic C=C (1597.48); 3246.75 (Amide N-H), 1630.17 (Amide C=O), 1350.49 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.53(4H,s,ArH), 3.96(1H,s,amide), 3.40(2H,m,methylene), 1.76 (2H,m,amine), 1.60 (1H,m,methine), 0.84 (3H,m,methyl); LC-MS m/z 399 [M+1].

D4: % yield 87.93; mp 220-222; LogP -0.477; IR (KBr Cm^{-1}): 1584.50(Aromatic C=C), 3255.25(Amide N-H), 1628.46(Amide C=O), 1361.69(Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.67 (4H,s,ArH), 4.22 (1H,m,amide), 3.50 (2H,m,methylene), 1.71(2H,m,amine), 1.58 (1H,m,methine), 0.98 (3H,m,methyl); LC-MS m/z 398 [M^+].

D5: % yield 53.43; mp 200-202; LogP -0.45; IR (KBr Cm^{-1}): 1598.48(Aromatic C=C), 3343.57(Amide N-H) , 1631.58(Amide C=O), 1311.09(Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.66 (4H,s,ArH), 4.79(1H,d,amide), 3.24 (2H,m,methylene); LC-MS m/z 481 [M+1].

D6: % yield 498.55; mp 198-200; LogP -0.328; IR (KBr Cm^{-1}): 1596.62 (Aromatic C=C); 3245.47(Amide N-H), 1629.05 (Amide C=O), 1311.51 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.16 (4H,s,ArH), 6.846(1H,s,hydroxyl), 3.87(1H,m,methine), 2.99(2H,m,methylene), 4.82 (1H,d,amide), 1.886(2H,s,amine); LC-MS m/z 513 [M+1].

D7: % yield 83.24; mp 205-207; LogP -0.18; IR (KBr Cm^{-1}): 1598.29(Aromatic C=C), 3343.00(Amide N-H), 1638.25 (Amide C=O), 1358.20(Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.66 (4H,s,ArH), 3.46(2H,m,methylene), 2.17(2H,m,amine), 0.98(3H,m,methyl); LC-MS m/z 371 [M+1].

D8: % yield 61.08; mp 140-143; LogP -0.448; IR (KBr Cm^{-1}): 1595.21(Aromatic C=C), 3328.00(Amide N-H), 1632.21 (Amide C=O), 1312.78 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.67 (4H,s,ArH), 3.52 (1H,m,amide), 3.22 (2H,t,methylene),1.77(2H,m,amine),1.61(1H,m,methine); LC-MS m/z 486 [M+1].

D9: % yield 55.82; mp 190-193; LogP -0.205; IR (KBr Cm^{-1}): 1597.65 (Aromatic C=C); 3247.65 (Amide N-H), 1632.48(Amide C=O), 1311.58 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.67 (4H,s,ArH), 3.99 (1H,m,amide), 3.28 (2H,m,methylene), 1.891(2H,s,amine); LC-MS m/z 379 [M+1].

D10: % yield 95.7; mp 180-183; LogP -0.56; IR (KBr Cm^{-1}): 1595.14 (Aromatic C=C), 1629.59(Amide C=O), 1312.75 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 8.45-7.67(4H,s,ArH), 4.17 (2H,s,amide), 3.69 (2H,m,methylene), 1.91(1H,s,methine), 2.20 (2H,s,amine); LC-MS m/z 429 [M+1].

D11: % yield 50.68; mp 194-196; LogP -0.82; IR (KBr Cm^{-1}): (1597.38) Aromatic C=C; 3320.78 (Amide N-H), 1626.86(Amide C=O), 1311.17 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.66 (4H,s,ArH), 6.87 (1H,d,amide), 3.81 (2H,m,methlene),2.62 (1H,m,methine), 1.884(2H,s,amine); LC-MS m/z 434 [M^+].

D12: % yield 40.78; mp 196-198; LogP -0.284; IR (KBr Cm^{-1}): (1595.23) Aromatic C=C; 3233.20 (Amide N-H), 1595.28 (Amide C=O), 1314.52 (Sulfonamide S=O); ^{1}HNMR (DMSO): δ 7.66 (4H,s,ArH), 3.84 (1H,m,amide), 2.75 (2H,m,methylene); LC-MS m/z 402 [M^+].

4.3 In vitro carbonic anhydrase inhibition

In- vitro carbonic anhydrase inhibition studies were performed using 4-nitrophenyl acetate. The CA-catalyzed hydrolysis of 4-NPA result in the generation of a yellow 4-nitrophenolate anion with an isosbestic point at 348nm that is readily detected with a spectrophotometer. Assay condition were optimized for substrate concentration, enzyme concentration, and time. Linearity and repeatability with standard inhibitor acetazolamide was performed. In vitro assay was carried out in 96 well microplates (Thermo scientific bioLite 96 well mutidish). Each individual well consists of 60µL of 50mM tris buffer (pH 7.6, containing 0.1mM ZnCl2), 10 µL of test compounds in 1% DMSO and enzyme (50 U). The plates were pre-read at 348nm after incubating at 25°C for 10 minutes. Freshly prepared 4-NPA substrate (6mM stock using 4.5% acetonitrile in buffer) was added per well. Final reaction volume in each well was made upto 100 µL. after incubating for 30 minutes at 25°C, the plates were read at 348nm. Suitable controls with DMSO and standard inhibitor acetazolamide (AZM) were included in the assay. The (%) inhibition was calculated using formula

$$\% \text{ inhibition} = A_{control} - A_{test} / A_{control} * 100$$

4.4 Molecular docking

The docking studies were carried out with the Herms 1.4 version of Gold suit software. The PDB ID 4HT0 was downloaded from a protein data bank having a resolution of 1.6 A° used for docking studies. The docking method involves the ligand preparation in Chem Draw Ultra 8.0 v, protein preparation was performed in CHIMERA version 1.7s, and energy minimization was performed in CemBio 3D ultra (MM2). The binding site was defined for carbonic anhydrase II at XYZ coordinates (X=-6.205, Y=4.809, Z=16.121) selecting all atoms at 10 A°. The compounds were docked in the active site of the protein. The gold score was analyzed according to the fitness and orientation of the ligands in the binding pocket.

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