ejpmr, 2025, 12(3), 180-186

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STRUCTURE-GUIDED SYNTHESIS AND EVALUATION OF N- (4-CETAMIDOPHENYL SULFONYL) - AMINO ACID CONJUGATES AS CARBONIC ANHYDRASE II INHIBITORS

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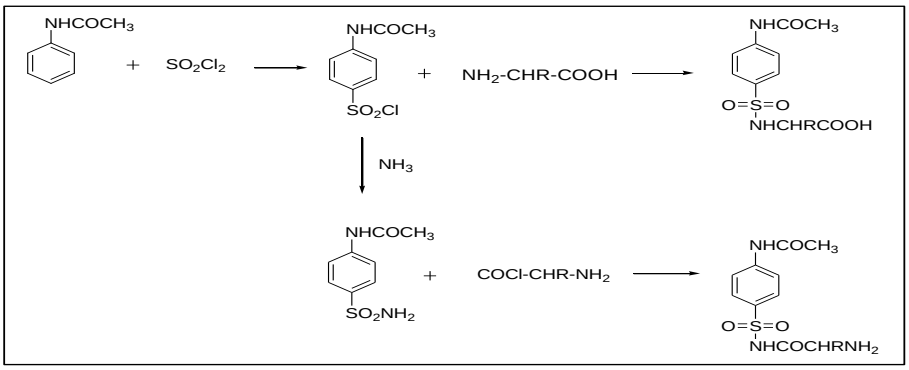
Article Received on 01/01/2025 **Article Revised on 21/01/2025** **Article Accepted on 10/02/2025**

GRAPHICAL ABSTRACT

Carbonic anhydrases are important enzymes that catalyze interconversion of CO_2 and water into bicarbonate. They are crucial for maintaining pH homeostasis and water balance. Apart from this they are also involved in biological processes like respiration, ureagenesis, gluconeogenesis etc. Inhibition of CA can produce a number of therapeutic effects in the body. CA inhibitors are used in treatment of diseases like glaucoma, epilepsy, hypoxic tumors. Lack of specificity, low potency and poor pharmacokinetic properties of the existing drugs suggests the need of new inhibitors. In the present work N-(4-Acetamidophenyl sulfonyl)-Amino acid conjugates with carboxyl/amino end were synthesized using acetanilide as starting material. Inhibition activity of these compounds was evaluated against bovine CA II. Compound G13 showed highest inhibition (80.13%) comparable to the standard inhibitor acetazolamide (78.91%) Docking studies were performed to study the amino acid interaction and the binding mode of ligands within the active site.

KEYWORDS: Carbonic anhydrase, amino acid, Acetazolamide, Inhibition, Docking.



INTRODUCTION

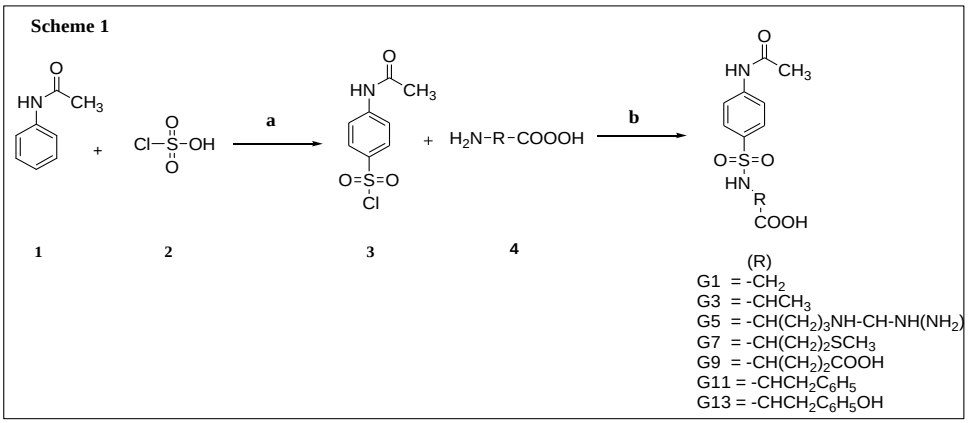
Carbonic anhydrases (CAs) are one of the most widely distributed enzymes in the living beings. They are metalloenzyme (Mollica, A. Imran, S. et al 2015) consisting of zinc as a cofactor that efficiently catalyzes reversible conversion of CO_2 to bicarbonate Mojzyh, M. et al 2015). CA isoforms are found in many tissues where they participate in several important biological processes such as, respiration, acid base balance, carbon dioxide and ion transport, bone reabsorption, ureagenesis, (Zolnowaska, B.et al 2015) gluconeogenesis, lipogenesis and electrolyte secretion.[5] Several isoforms of carbonic anhydrase are targets for designing inhibitors for various therapeutic applications like epilepsy glaucoma, obesity.[6] and tumorigenesis.[7,8] They are also used as diuretics as an adjuvant therapy for hypertension, for treatment of high altitude sickness, memory functions, duodenal and gastric ulcers, osteoporosis as well as a diagnostic tool in photon emission tomography and magnetic resonance imaging. To date, several moieties like sulfonamides[9], imidazole, hydroxyurea, phenol, organic phosphate shown to have anti-CA properties have been developed as potential inhibitors. These inhibitors act by replacing the hydroxide ion attached to Zn^{2+} within the active site, making it unavailable to attack on CO_2 and thereby

inhibiting the formation of bicarbonate. Clinically CA inhibitors like Acetazolamide, ethoxazolamide brinzolamide, dorzolamide are used as anti–glaucoma agents.[11] All these CA inhibitors have sulphonamide as primary zinc ion binding group. However, The main drawback associated with CA inhibitors is the lack of selectivity.[12] Novel inhibitors with high Inhibitory potency as well as good pharmacokinetic profile are needed as an effective anti-CA strategy. In the present work we synthesized various acetamidobenzenesulfonamide-amino acid conjugates and evaluated them for inhibition against bovine carbonic anhydrase II.[13]

2. RESULTS AND DISCUSSION

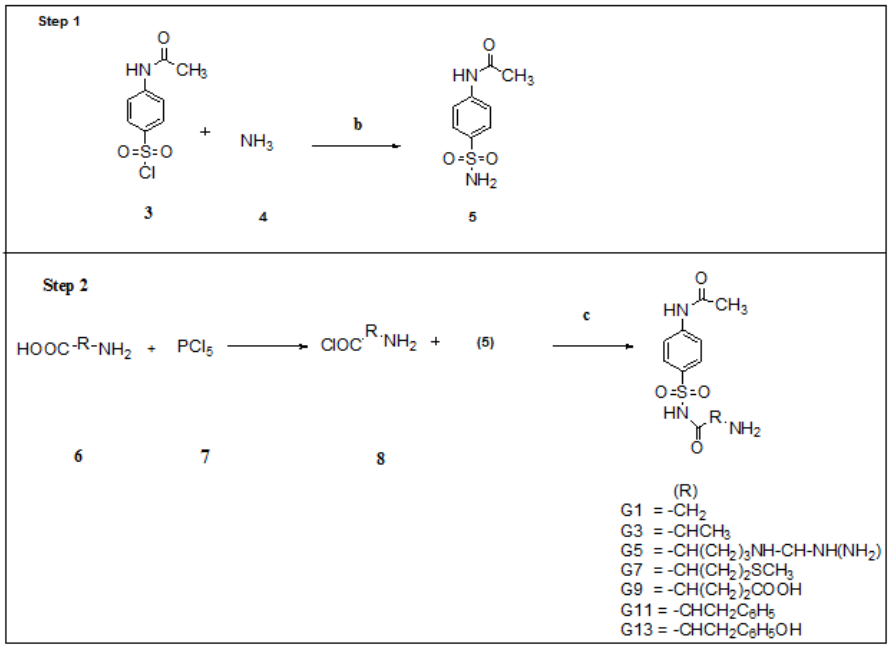
2.1 **Chemistry:** A series of N-(4-acetamidophenylsulfonyl)-amino acid conjugates with carboxyl/amino end were synthesized according to the reaction scheme 1 and 2 respectively. Acetanilide was taken as starting material.

Scheme1: Electrophilic aromatic substitution reaction was carried out to obtain p-acetamidobenzenesulfonyl chloride from acetanilide and chlorosulfonic acid, which was further reacted with the amino acid to obtain the product with carboxyl end group.



Scheme 1: (1) Acetanilide, (2) Chlorosulfonic acid, (3) p-acetamidobenzenesulfonyl chloride (4) Amino acid (a) heating for 1 hr under reflux, (b) overnight stirring at RT in basic medium (5% NaOH.

Scheme 2: p-acetamidobenzenesulfonyl chloride was converted to p-acetamidobenzenesulfonamide by reaction with ammonia, which was further reacted with amino acid chloride to obtain the product with amino end group.



Scheme 2: (3) p-acetamidobenzenesulfonyl chloride (4)Ammonia (b) heating with ammonia water mixture (1:1) (5) p-acetamidobenzensulfonamide, (6) amino acid (7) Phosphorus pentachloride, (8) amino acid chloride (c) overnight stirring at room temperature.

2.2 Carbonic Anhydrase Inhibition Activity: CA II from bovine erythrocytes was used to evaluate the inhibitory ability of the synthesized compounds. Acetazolamide a well known CA inhibitor was taken as a standard in the assay. The results are reported in Table1 and are mean of three independent experiments. The percentage inhibitions shown by synthesized compounds ranges from 13.09 to 80.13. It was observed that all the compounds exhibiting more than 50% inhibition had carboxyl end group. Compound G3, G5, G7, G11 and G13 with carboxyl end group had better inhibition then their corresponding amino end compounds. Highest inhibition was shown by compound G13 (80.13%), containing amino acid tyrosine and end group being carboxyl. Compound G12 showed least inhibition of bovine CA II, containing phenylalanine and amino end group.

General Structure

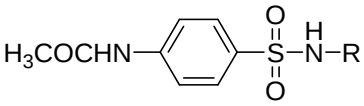
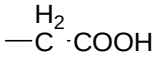
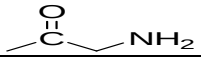
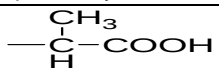
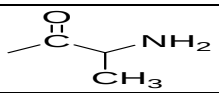
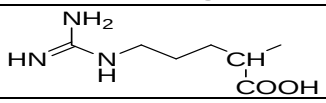
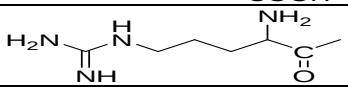
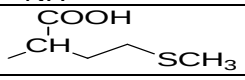
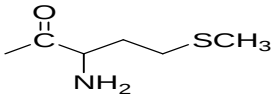
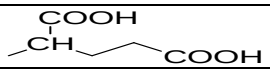
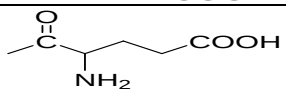
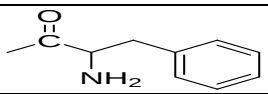
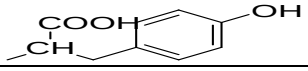
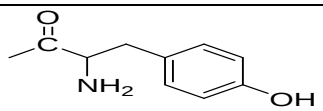


Table1: Inhibition data of carbonic anhydrase II with synthesized compounds.

S. No.	Compound code	R	% Inhibition*
1	G1		34.27
2	G2		43.90
3	G3		76.86
4	G4		42.30
5	G5		71.92
6	G6		43.97
7	G7		76.67
8	G8		44.09
9	G9		16.82
10	G10		21.95
11	G11		21.56
12	G12		13.09
13	G13		80.13
14	G14		14.18
15	Azm	-	78.91

*** % inhibition at $0.5*10^2$ µM; Azm = acetazolamide**

2.3 Docking Studies: Docking studies of all the synthesized compounds were performed with human carbonic anhydrase isozyme II (PDB ID 4HT0) to study their binding patterns with the enzyme binding site. The docking studies were validated by redocking the cocrystallized ligand. All the synthesized compounds were docked in the active site of protein showing gold score in range of 45-60. Acetazolamide interacted with active site forming H-bond with Thr 199 and coordination of its N with Zn^{2+} as shown in figure1. Similar binding pattern was observed with the compounds. The Nitrogen atom of all the synthesized

compounds coordinated with Zn^{2+}. Interaction with Thr199 was also shown by most of the compounds, in a way similar to Azm. Best score was assigned to compound G13 (60.5) which also showed highest inhibition whereas lowest gold score was shown by compound G4 and G6. Hence, the Structural contribution of standard inhibitor and synthesized compounds toward protein binding could be simulated; however more bulky substituent at the R group shows higher gold score when compared to acetazolamide. The interaction of compound G3 and Azm are illustrated in fig1. Compounds with carboxyl end showed higher gold score as compared to compounds with amino end. A correlation between the gold score and inhibitory activity was also observed. Compounds showing high gold score gave higher inhibitory activity.

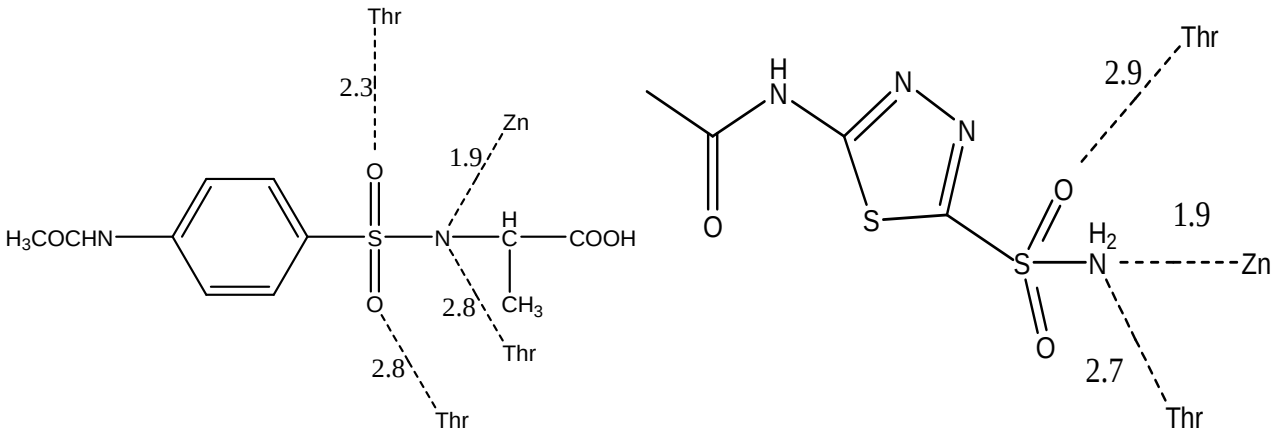


Fig. 1: Compound G3 and acetazolamide showing interaction with Zinc and amino acid residues.

3. CONCLUSION

4-acetamidobenzenesulfonyl-amino acid conjugates having different end groups were synthesized. In vitro carbonic anhydrase inhibition was performed against cytosolic isoform CA II using Acetazolamide was used as standard. Amongst the 7 compounds with carboxyl end, 4 compounds showed good inhibitory activity, comparable to standard inhibitor acetazolamide. Compounds containing tyrosine, alanine, methionine and arginine amino acids showed CA II inhibitory activity ranging from 71.92 to 80.13 (table no. 1) While remaining 7 compounds with amino end showed CA inhibitory activity less than 50 percent. Thus it can be concluded that the compounds with carboxyl end showed higher percentage inhibition as compared to amino end compounds, probably due to pH dependent ionization. Compound G13 which is tyrosine conjugate, with carboxyl end showed highest inhibition (80.13%) comparable to standard inhibitor Azm (78.91%). Compound G12 showed least inhibition (13.09%) H-bonding with Thr199 and coordination with Zn^{2+}were found to be important parameters affecting enzyme inhibition. Gold score which is sum of van der waal interaction, was found to increase with an increase in molecular weight.

4. Experimental

4.1 MATERIALS AND METHODS

CAII was purchased from Sigma Aldrich. 4-nitro phenyl acetate and acetazolamide were purchased from Alfa aeser. Tris buffer was obtained from Bio-Rad. Amino acids were purchased from LobaChemie. NMR data was recorded on JNM-ECS 400 spectrophotometer. IR spectra was determined byAPLHA (Bruker) FTIR spectrophotometer by using KBr pellet method. Mass spectra of synthesized compounds were obtained on Bruker micro TOF QII high resolution mass spectrometer. TLC was carried out on precoated silica gel plates (Silica Gel 60 F254, Merck) and visualized in UV.

4.2 Synthesis of carboxyl end conjugates (G1, G3, G5, G7, G9, G11, G13)

4.2.1. Synthesis of p-acetamidobenzenesulfonyl chloride: A 250ml two neckedround bottom flask was equipped with a dropping funnel and a reflux condenser. 0.148 mol (20gm) dry acetanilide was placed in the flask and 0.77mol (50ml) of chlorosulfonic acid was placed in the dropping funnel and a calcium chloride guard tube was inserted. Chlorosulphonic acid was added dropwise with stirring to ensure thorough mixing. When the addition was complete, the reaction mixture was heated on a water bath for 1 hr. in order to complete the reaction. The reaction mixture was allowed to cool and the oily product was poured in a thin stream with stirring into crushed ice in a beaker in fume cupboard, precipitate was obtained. The flask was rinsed with a little ice water and the rinsings were added to the contents of the beaker and stirred for several minutes in order to obtain an even suspension of granular white solid. P-acetamidobenzenesulfonyl chloride was filtered and washed with cold water.[14]

4.2.2 Synthesis of Amino acid conjugates: To a stirred solution of 0.01 mol of amino acid in 5% aqueous NaOH (50ml) at, 0.01 mol of p-acetamidobenzenesulfonyl chloride was addedroom temperature. After overnight stirring of the reaction mixture at room temperature it was acidified with Hydrochloric acid. The precipitate obtained was filtered and recrystallized with water.[15]

4.2.3 Spectral data

2-(4-acetamidophenylsulfonamido) acetic acid (G1): Yield: 80.14%; M.P: 165-170; Log P: -0.55; IR (KBrcm^{-1}):1632.42 (C=O, amide) 1547.52 (C=C, aromatic), 3453.03 (N-H, amide), 2924.67 (O-H, carboxylic acid), 1319.40 (S=O, sulfonamide); ^{1}H NMR (D_2O): 7.90-9.91(1H, s, Amide), 2.19(3H, s, methyl), 7.46-7.49(4H, d, ArH), 3.73(2H, d, methylene); LC-MS: m/z (M^+ - 59).

2-(4-acetamidophenylsulfonamido) propanoic acid (G3): Yield: 68.53%; M.P: 185-188; Log P: -0.9; IR (KBr cm^{-}):1632.31 (C=O, amide), 1547.60 (C=C, aromatic), 3442.65 (N-H, amide), 2925.31 (O-H, carboxylic acid), 1319.22 (S=O, sulfonamide); ^{1}H NMR (D_2O):7.80(1H, s, amide), 7.33-7.35(4H, d, ArH), 3.81-3.86(1H, s, methyne), 4.79(1H, d, sec. amine), 1.45-1.47(3H, d, methyl); LC-MS: m/z (M^+ - 73).

2-(4-acetamidophenylsulfonamido)-5-guanidinopentanoic acid (G5): Yield: 79.62%; M.P: 150-155; Log P: -0.583; IR (KBr cm^{-1}):1631.52 (C=O, amide), 1547.65 (C=C, aromatic), 3451.75 (N-H, amide), 2924.40 (O-H, carboxylic acid), 3063.87 (N-H, amine), 1319.40 (S=O, sulfonamide); ^{1}H NMR (D_2O):7.87-7.89(1H, s, amide), 7.44-7.46(4H,d, ArH), 3.85-3.89(1H, methyne), 2.71-3.26(2H, methylene); LC-MS: m/z (M^+ - 158).

2-(4-acetamidophenylsulfonamido)-4-(methylthio) butanoic acid (G7): Yield: 78.64%; M.P: 185-188; Log P: -01.596; IR (KBr cm^{-1}):1631.54 (C=O, amide), 1547.66 (C=C, aromatic) 3454.50 (N-H, amide), 2922.93 (O-H, carboxylic acid), 1319.29 (S=O, sulfonamide); ^{1}H NMR (D_2O):7.81-7.83(1H, s, amide), 7.33-7.35(4H, s, ArH), 3.90-3.92(1H, d, methyne), 2.63(2H, s, methylene), 2.61(2H, s, methylene), 2.11-2.18(3H, s, methyl), 4.7-4.82(1H, d, sec. amine); LC-MS: m/z (M^+ - 133).

2-(4-acetamidophenylsulfonamido) pentanedioic acid (G9): Yield: 85.83%; M.P: 208-212; Log P: -0.92; IR (KBr cm^{-1}):1631.98 (C=O, amide), 1546.25 (C=C, aromatic), 3450.97 (N-H, amide), 2921.48 (O-H, carboxylic acid), 1318.61 (S=O, sulfonamide); ^{1}H NMR (D_2O):8.04-8.10(1H, s, amide), 2.54-2.57(1H, methyne), 2.13-2.15(2H, methylene), 2.17(2H, methylene), 2.19(3H, s, methyl), 4.82(1H, d, sec amine); LC-MS: m/z (M^+ - 131).

2-(4-acetamidophenylsulfonamido)-3-phenylpropanoic acid (G11): Yield: 64.81%; M.P: 205-209; Log P: -0.133; IR (KBr cm^{-1}):1637.15 (C=O, amide), 1549.07 (C=C, aromatic), 3461.91 (N-H, amide), 2638.28 (O-H, carboxylic acid), 1311.79 (S=O, sulfonamide); ^{1}H NMR (D_2O):7.38-7.41(1H, s, amide), 7.34-7.36(1H, d, ArH), 4.79-4.82(1H, d, sec. amine), 3.15-3.28(1H, d, methylene), 4.11-4.13(1H, methyne); LC-MS: m/z (M^+ - 149).

2-(4-acetamidophenylsulfonamido)-3-(4-hydroxyphenyl) propanoic acid (G13): Yield: 82.55%; M.P: 183.88; Log P: -0.27; IR (KBr cm^{-1}): 1686.86 (C=O, amide), 1531.98 (C=C, aromatic), 3425.68 (N-H, amide), 3654.07 (O-H, carboxylic acid), 1329.14 (S=O, sulfonamide); ^{1}H NMR (D_2O):76-7.78(1H, s, amide), 6.57-6.87(1H, d, ArH), 3.65-3.91(1H, s, phenol), 3.09-3.19(1H, methyne), 2.52-2.58(2H, d, methylene), 4.75-4.86(1H, d, sec. amine) 2.15-2.18(1H, s, methyl); LC-MS: m/z (M^+ - 165).

4.3 Synthesis of amino end conjugates (G2, G4, G6, G8, G10, G12 and G14)

4.3.1 Synthesis of p-acetamidobenzenesulfonamide: Crude p-acetamidobenzensulfonyl chloride was transferred to a round bottom flask and a mixture of 70 ml ammonia and 70 ml water was added dropwise. The contents of the flask were mixed thoroughly and the mixture was heated with occasional swirling to just below the boiling point for about 15 minutes and the product was obtained. It was cooled in ice and neutralized with sulphuric acid until just acid to congo red paper. The product was collected on a Buchner funnel, washed with cold water and drained completely.

4.3.2 Synthesis of amino acid conjugates: To a stirred solution of 0.01 mol p-acetamidobenzenesulfonamide in 5% aqueousNaOH (50ml), amino acid chloride[16] was slowly added. When the addition was completed reaction mixture was left overnight with stirringand acidified with hydrochloric acid.The precipitate obtained was filtered and recrystallized with water.

4.3.4 Spectral data

N-(4-acetamidophenylsulfonyl)-2-amino acetamide (G2): Yield: 60.62%; M.P: 200-205; Log P: -0.42; IR (KBrcm^{-1}):1656.97 (C=O, amide), 1535.17 (C=C, aromatic), 3476.52 (N-H, amide), 3476.52 (N-H, amine), 1314.02 (S=O, sulfonamide); ^{1}H NMR (D_2O):1.8(2H, amine), 2.09(1H, s, methyl), 3.4(2H, t, methylene), 7.5(1H, s, amide),7.75 (1H, d, ArH); LC-MS: m/z (M^+ - 58)

N-(4-acetamidophenylsulfonyl)-2-aminopropanamide G4: Yield: 43.52%; M.P: 170-174; Log P: -0.292; IR (KBr cm^{-1}):1657. 54 (C=O, amide), 1534.58 (C=C, aromatic), 3372.15 (N-H, amide), 1317.09 (S=O, sulfonamide); ^{1}H NMR (D_2O):1.4(3H, d, methyl), 1.9(2H, d, amine), 2.1(3H, s, methyl), 3.64-3.70(1H, m, methyne), 7.6(1H, s, amide), 7.83-7.85(4H, d, ArH); LC-MS: m/z (M^+ - 72).

N-(4-acetamidophenylsulfonyl)-2-amino-5-guanidinopentanamide (G6): Yield: 92.85%; M.P: 192-197; Log P: -0.0817; IR (KBr cm^{-1}):1657.53 (C=O, amide), 1535.61 (C=C, aromatic), 3374.58 (N-H, amide), 1315.49 (S=O, sulfonamide); ^{1}H NMR (D_2O):1.60-1.61(1H, m, sec. amine), 1.78-1.87(2H, m amine), 2.16(3H, s, methyl), 3.17-3.21(2H, m, methylene), 3.60-3.63(2H, m, methylene), 6.8(1H, s, amide amine), 7.84-

7.86(4H, d, ArH); LC-MS: m/z (M^+ - 157).

N-(4-acetamidophenylsulfonyl)-2-amino-4-(methylthio) butanamide (G8): Yield: 84.46%; M.P: 210-215; Log P: -0.42; IR (KBr cm^{-1}):1627.37 (C=O, amide), 1504.45 (C=C, aromatic), 3476.41 (N-H, amide), 3384.31 (N-H, amine), 1310.84 (S=O, sulfonamide); ^1H NMR (D_2O):1.9(2H, d, amine), 2.08(3H, s, methyl), 2.2(3H, s, methyl), 2.5(2H, t, methylene), 3.5-3.6(1H, m, methyne), 6.8(1H, s, amide amine), 7.63-7.65(4H, d, ArH); LC-MS: m/z (M^+ - 132).

5-(4-acetamidophenylsulfonamido)-4-amino-5-oxopentanoic acid (G10): Yield: 66.99%; M.P: 220-226; Log P: -0.41; IR (KBr cm^{-1}):1637.15 (C=O, amide), 1548.78 (C=C, aromatic), 3461.91 (N-H, amide), 2638.28 (O-H, carboxylic acid), 1311.79 (S=O, sulfonamide); ^1H NMR (D_2O):7.63-7.65(1H s, amide), 6.83-6.86(4H, d, ArH), 3.69-3.74(1H, methyne), 2.31-2.34(2H, methylene), 2.09-2.29(2H, methylene), 1.43-1.45(1H, s, amine), 2.00-2.08(1H, s, methyl); LC-MS: m/z (M^+ - 130).

N-(4-acetamidophenylsulfonyl)-2-amino-3-phenylpropanamide (G12): Yield: 20.09%; M.P: 215-218; Log P: -0.88; IR (KBr cm^{-1}):1663.86 (C=O, amide), 1538.07 (C=C, aromatic), 3362.61 (N-H, amide), 3298.07 (N-H, amine), 1316.80 (S=O, sulfonamide); ^1H NMR (D_2O):1.87(2H, d, amine), 2.16(3H, s, methyl), 3.2(2H, d, methylene), 3.96(1H, m, methyne), 6.8(1H, s, amide), 7.6(8H, d, ArH); LC-MS: m/z (M^+ - 148).

N-(4-acetamidophenylsulfonyl)-2-amino-3-(4-hydroxyphenyl) propanamide (G14): Yield: 54.86%; M.P: 195-200; Log P: -0.149; IR (KBr cm^{-1}):1658.39 (C=O, amide), 1522.10 (C=C, aromatic), 3454.27 (N-H, amide), 3208.15 (O-H, carboxylic acid), 3023.62 (N-H, amine), 1331.39 (S=O, sulfonamide); ^1H NMR (D_2O):1.8(2H, d, amine), 2.1(3H, s, methyl), 3.7-3.8(1H, m, methyne), 3.1(2H, d, methylene), 6.8(1H, s, amide), 7.8(4H, d, ArH), 7.6(4H, d, ArH); LC-MS: m/z (M^+ - 164).

4.5 Assay for carbonic anhydrase inhibition: In-vitro carbonic anhydrase inhibition studies were performed using4-nitro phenyl acetate.[17] The CA-catalyzed hydrolysis of 4-NPA results in the generation of a yellow 4-nitrophenolate anion with an isosbestic point at 348 nm[18] that is readily detected with a spectrophotometer.In vitro assay was carried out in 96 well microplates (Thermo scientific BioLite 96 well multidish).Optimizationwas carried out for incubation time, substrate, enzyme and inhibitor concentration. Linearity and repeatability with the standard inhibitor acetazolamide was also performed. Each individual well contained 60µL of tris buffer (pH 7.6, containing 0.1mM $ZnCl_2$), 10µL of synthesized 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. Acetazolamide and DMSO were included asstandard and control respectively. Percentage inhibition was calculated by formula: % Inhibition = $A_{control}$-$A_{test}/A_{control}$*100

4.6 Molecular Docking studies: The docking studies were carried out with Hermes v1.4, a component of GOLD suite. PDB ID 4HT0 (CA II) was downloaded from RCSB protein data bank with resolution of 1.6Å and employed for present study. Protein preparation was done using CHIMERA. The energy minimization was performed with MM2 (ChemBio 3D Ultra). The water molecules were removed and hydrogens were added. The binding pocket was defined with all the residues within 10Å of the centroid (X=-6.205, Y=4.809, Z=16.121). The co-crystallized ligand was extracted and the compounds were docked in the active site of the protein.

ACKNOWLEDGEMENT

Authors are thankful to All Indian Council for Technical Education (8023/BOR/RID/RPS-125/2009-2010).

REFERENCES

1. Mollica, A.; Costante, R.; Akdemir, A.; Simone, C.;Stefanucci, A.; Macedonio, A. Bioorg. Med. Chem., 2015; 23: 5311-5318.
2. Imran, S.; Taha, M.; Ismail, N.H.; Fayyaz, S.; Khan, K.M.; Choudhary, M.I. Bioorg. Chem., 2015; 62: 83-93.
3. Mojzyh, M.; Ceruso, M.; Bielawska, A.; Bielawski, K.; Fornal, E.; Supuran, C.T.J. Bioorg. Med. Chem. 2015; 23: 3674–3680.
4. Zolnowaska, B.; Slawinski, J.; Pogorzelska, A.; Chojnacki, J.; Vullo, D.; SupuranC.T. Eur. J. Med. Chem., 2013; 71: 135-47.
5. Capkauskaite, E.; Baranauskiene, L.; Golovenko, D.; Manakova, E.; Grazulis, S Tumkevicius, S. Matulis, D. Bioorg. Med. Chem., 2010; 18: 7357-7364.
6. Esirden, I.; Ulus, R.; Aday, B.; Tanç, M.; Supuran. C.T.; Kaya, M. Bioorg. Med. Chem., 2015; 23: 6573-6580.
7. Florent, T.R.; Meignan, S.; Foulon, C.; Six, P.; Gros, A.; Bal-Mahieu, C.; SupuranC.T.; Scozzafava, A.; Frédérick, R.; Masereel, B.; Depreux, P.; Lansiaux, A.; Goossens, J.F.; Gluszok, S.; Goossens, L. Bioorg. Med. Chem., 2012; 21: 1451–1464.
8. Brzozowski, Z.; Slawinski, J.; Gdaniec, M.; Innocenti, A.; Supuran, C.T. Eur. J .Med. Chem., 2011; 46: 4403-4410.
9. Hany, S.; Ibrahim, H.S.; Abou-Seri,; S.M.; Tanc, M.; Elaasser, M.M.; Abdel-Aziz, H.A.; Supuran, C. T Eur. J. Med. Chem., 2015; 103: 583-593.
10. Zaib, S.; Saeed, A.; Stolte, K.; Flörke, U.; Shahid, M.; Iqbal, J. Eur. J. Med. Chem., 2013; 78: 140-150.
11. Sethi, K.K.; Vullo, D.; Verma, S.M.; Tanc, M.; Carta, F.; Supuran, C. TJ. Bioorg. Med. Chem.,

2013; 23: 5973–5982.
12. Carta, F.; Maresca, A.; Scozzafava, A.; Vullo, D.; Supuran, C.T. Bioorg. Med. Chem.m 2009; 17: 7093–7099.
13. Boriack-Sjodin, P.A.; Zeitlin, S.; Chen, H.H.; Crenshaw, L.; Gross, S.; AnuraDantanarayana, A.; Delgado, P.; May, J.A.; Dean, T.; Christianson, D.W. The Protein Society, 1998; 7: 2483-2489.
14. Furniss, B.S.; Hannaford, A.J.; Smith, P.W.G.; Tatchell, A.R. (1989). Vogel'stextbook of Practical organic chemistry, John Wiley and sons: New York, 883-884.
15. Fidan, I.; Salma, R.E.; Arslan. M.; Senturk, M.; Durdagi, S.; Ekinci, S.; Şentürk, E.; Cosgun, S.; Supuran, C.T. J. Bioorg. Med. Chem., 2015.
16. Bortoluzzi, M.; Marchetti, F.; Murrali, M.G.; Pampaloni, M. (2015). Inorganica Chimica Acta, 2015; 427: 150-154.
17. Rashida, M.; Hussain, S.; Hamayoun, M.; Altaf, A.; Iqbal, J. HindawiPublishing Corporation BioMed Research International, 2015.
18. Barrese, A.A.; Genis, C.; Fisher, S.Z.; Orwenyo, J.N.; Kumara, M.T.; Dutta, S.K.; Phillips, E.; Kiddle, J.J.; Tu, C.; Silverman, D.N.; Govindasamy. L.; McKenna, M.A.; McKenna, R.; Tripp, B.C. Biochem., 2007; 47: 3174–3184.