Bioorganic Chemistry 86 (2019) 624–630

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis of sulfonamide, amide and amine hybrid pharmacophore, an entry of new class of carbonic anhydrase II inhibitors and evaluation of chemo-informatics and binding analysis

Attique Ahmed[a], Pervaiz Ali Channar[a], Aamer Saeed[a,*], Markus Kalesse[b], Mehar Ali Kazi[c], Fayaz Ali Larik[a], Qamar Abbas[d], Mubashir Hassan[e], Hussain Raza[e], Sung-Yum Seo[e]

[a] Department of Chemistry, Quaid-I-Azam University, Islamabad 45320, Pakistan
[b] Institut für Organische Chemie, Schneiderberg 1 B, 30167 Hannover, Germany
[c] Institute of Biochemistry, University of Sindh, Jamshoro 76080, Pakistan
[d] Department of Physiology, University of Sindh, Jamshoro 76080, Pakistan
[e] Department of Biological Sciences, College of Natural Sciences, Kongju National University, 56 Gongjudehak-Ro, Gongju, Chungnam 32588, Republic of Korea

ARTICLE INFO

Keywords:
Carbonic anhydrase II inhibition
Synthesis
Hybrid pharmacophore
Binding analysis
Chemo-informatics

ABSTRACT

Selective inhibition of carbonic anhydrase (CA) enzyme is an active area of research for medicinal chemists. In the current account, a hybrid pharmacophore approach was employed to design sulfonamide, amide and amine containing new series of potent carbonic anhydrase II inhibitors. The aromatic fragment associated with pharmacophore was altered suitably in order to find effective inhibitors of CA-II. All the derivatives **4a-4m** showed better inhibition compared to the standard acetazolamide. In particular, compound **4l** exhibited significant inhibition with IC_{50} value of 0.01796 ± 0.00036 μM. The chemo-informatics analysis justified that all the designed compounds possess < 10 HBA and < 5 HBD. The ligands-protein binding analyses showed that **4l** confined in the active binding pocket with three hydrogen bonds observed with His63, Asn66 and Thr197 residues.

1. Introduction

The carbonic anhydrases (CAs, E.C. 4.2.1.1) are a group of zinc metal containing metalloenzymes pervasive in nature. The three most prevalent isoforms in nature include, α-CAs (present in vertebrates, eubacteria, algae and cytoplasm of green plants), the β-CAs (predominantly in eubacteria, algae and chloroplasts of both mono- as well as dicotyledons) and the γ-CAs (mainly in archaea and some eubacteria). In higher vertebrates including humans, the different carbonic anhydrase isozymes include cystolic forms (CA I-III, CA VII), four membrane bound isozymes (CA IV, CA IX, CA XII and CA XIV), one mitochondrial form (CA V) and one secreted CA isozyme, CA VI [1–6]. The carbonic anhydrase catalyze an apparently simple physiological interconversion ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$) yet this simple reaction is involved in the crucial process of respiration and transport of CO_2/bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, and tumorigenicity [7–10]. The CA isozymes have been the active area of research among medicinal chemists because designing of inhibitors of CA play important role in the treatment of epilepsy, glaucoma, idiopathic intracranial hypertension and altitude sickness.

Sulfonamides possess many types of biological activities, and representatives of this class of pharmacological agents are widely used in clinic as antibacterial, hypoglycemic, diuretic, anti-hypertensive and antiviral drugs among others [11–17]. Sulfonamides ($R\text{-}SO_2NH_2$ possess great significance in the designing of CA inhibitors mainly due to the unique and tailor ability of binding with CA protein which is driven by coordination of the deprotonated sulfonamide nitrogen to the catalytic zinc ion (Zn^{2+}), additional interactions with the hydrophilic and/or hydrophobic region of the active site may take place, depending on the nature of the substituent group. After the first report of the scientific evidence of sulfanilamide as inhibitor of CA enzymes [18], it was suggested that unsubstituted aromatic sulfonamides of type $ArSO_2NH_2$ act as strong CAIs (carbonic anhydrase inhibitors) which led to the discovery of several new drug candidates. Currently, a number of research groups are actively involved in the design and discovery of sulfonamides as carbonic anhydrase inhibitors [19–23]. Despite substantial synthetic efforts, it is rather difficult to achieve high isozyme

* Corresponding author. Tel.: +92-51-9064-2128; fax: +92-51-9064-2241.
E-mail addresses: asaeed@qau.edu.pk (A. Saeed), fayazali@chem.qau.edu.pk (F.A. Larik).

https://doi.org/10.1016/j.bioorg.2019.01.060
Received 25 September 2018; Received in revised form 12 January 2019; Accepted 27 January 2019
Available online 10 February 2019
0045-2068/

selectivity *in vitro* with classical designs incorporating aromatic/heterocyclic primary sulfonamides and sulfamates due to sequence homology and thus structural similarities that exist between different CA isozymes in their active sites.

Selective inhibition of carbonic anhydrase II constitutes a viable approach to fight against the disorders caused by harmful effects of CA II enzyme [24]. Building on these premises and taking into account the fact that the amide functionality also possesses the ability to interact with active site of CA II through hydrogen bonding, besides sulfonamide, and incorporation of amine in the target compounds can further enhance their aptitude to bind effectively. We herein report a hybrid pharmacophoric approach involving coupling of sulfonamide, amide and amine as three distinct pharmacophoric entities possessing intriguing structural features such as C═O, S═O, NH_2 and NH group. Furthermore we altered the aromatic part with different groups and also used commercial drugs like amantadine. In addition, we explored the potential of synthesized compounds against carbonic II anhydrase enzyme and also provided close *in silico* insights of binding analysis and chemo-informatics.

2. Experimental

2.1. All chemicals

reagents, were purchased from Sigma-Aldrich Chemical Co. or Merck, Germany, and were used without further purification. The solvents were dried and distilled prior to use. The R_f values were determined using aluminium pre-coated silica gel plates Kiesel $60F_{254}$ from Merck (Darmstadt, Germany). The melting points of the compounds were measured in open capillaries using a Stuart melting point apparatus (SMP3) and are uncorrected. The FT IR spectra were recorded on an FTS 3000 MX, Bio-Rad Merlin (Excalibur Model) spectrophotometer as pure compounds. The ^1H and ^{13}C NMR spectra were acquired on a Bruker NMR spectrometer at 300 MHz and 75.5 MHz using TMS as an internal standard. Mass spectra were recorded on an Agilent Technologies 6890N gas chromatograph equipped with an inert mass selective detector (5973 mass spectrometer), and elemental analyses were conducted using a LECO-183 CHNS analyser.

2.2. Synthesis of 2-chloro-N-(4-sulfamoylphenyl) acetamide (3)

To a stirred solution of 4-aminobenzene sulfonamide (4 mmol) in tetrahydrofuran, at 0–5 °C in ice jacket, 2-chloroacetyl chloride (4.5 mmol) was added drop wise over a 15-min period. The reaction mixture was further stirred for half an hour. On completion of reaction, the white precipitate appeared was filtered, washed and recrystallize with ethanol.

2.3. Synthesis of 2-(substituted phenylamino)-N-(4-sulfamoylphenyl) acetamides (**4a-4m**)

Suitably substituted aniline (2.016 mmol) were added along with anhydrous potassium carbonate and a catalytic amount of potassium iodide to the stirred solution of 2-chloro-*N*-(4-sulfamoylphenyl) acetamide (**3**) (0.5 g, 2.016 mmol) in the mixture of tetrahydrofuran and ethanol (10 mL). The reaction mixture was heated under reflux for 6–7 h. The solid products thus appeared were filtered, washed and recrystallized with ethanol: chloroform (1:1) mixture.

2-((3-chlorophenyl)amino)-*N*-(4-sulfamoylphenyl)acetamide (4a):

Light brown solid, m.p. = 261–262 °C, yield = 64%, R_f = 0.72 (Chloroform: Methanol 4:1); ^1H NMR (acetoned_6): δ (ppm) 9.69 (broad s, 1H, NH), 7.87 (s, 4H, Ar-H), 7.14 (t, 1H, *J* = 8.1 Hz, Ar-H), 6.71 (t, 1H, *J* = 1.8 Hz, Ar-H), 6.67 (d, 1H, J = 8.4, Ar-H), 6.61(d, 1H, J = 8.4 Ar-H), 6.53(s, 2H, SO_2NH_2), 5.80 (t, 1H, NH, J = 5.4), 4.0 (d, 2H, CH_2, J = 5.4). ^{13}C NMR: (75 MHz Acetone-d_6) δ (ppm) 169.32 (C═O), 149.62, 141.92, 138.79, 134.36, 130.44, 127.07, 119.04, 117.05, 112.43, 111.25, 47.91 (CH_2).

***N*-(4-sulfamoylphenyl)-2-((4-sulfamoylphenyl)amino)acetamide (4b)**:

White solid, m.p. = 290–293 °C, yield = 61%, R_f = 0.41 (Chloroform: Methanol 4:1); ^1H NMR (DMSO-d_6): δ (ppm) 8.89 (s, 1H, NH), 7.80 (broad s, 8H, Ar-H), 7.53(s, 2H, SO_2NH_2), 6.65 (s, 2H, SO_2NH_2) 5.10 (t, 1H, NH, J = 5.3), 4.0 (d, 2H, CH_2, J = 5.3). ^{13}C NMR: (75 MHz DMSO-d_6) δ (ppm) 172.22 (C═O), 158.72, 142.77, 138.99, 135.66, 133.84, 128.09, 120.03, 116.08, 113.45, 112.35, 49.31 (CH_2).

***N*-(4-sulfamoylphenyl)-2-(p-tolylamino)acetamide (4c)**:

Light brown solid, m.p. = 254–256 °C, yield = 69%, R_f = 0.63 (Chloroform: Methanol 4:1); ^1H NMR DMSO-d_6): δ (ppm) 9.22 (s, 1H, NH), 7.97 (s, 4H, Ar-H), 7.21 (d, 2H, *J* = 7.9 Hz, Ar-H), 6.50 (d, 2H, *J* = 7.8 Hz, Ar-H), 6.40 (s, 2H, SO_2NH_2), 5.92 (t, 1H, NH, J = 5.2), 4.1 (d, 2H, CH_2, J = 5.2,). ^{13}C NMR: (75 MHz DMSO-d_6) δ (ppm) 168.62 (C═O), 144.68, 141.72, 137.59, 128.62, 128.24, 118.09, 113.04, 44.61 (CH_2).

2-((4-chlorophenyl)amino)-*N*-(4-sulfamoylphenyl)acetamide (4d):

Light brown solid, m.p. = 257–258 °C, yield = 71%, R_f = 0.67 (Chloroform: Methanol 4:1); ^1H NMR (DMSO-d_6): δ (ppm) 9.19 (s, 1H, NH), 8.01 (s, 4H, Ar-H), 7.56 (d, 2H, *J* = 7.7 Hz, Ar-H), 6.64 (d, 2H, *J* = 7.7 Hz, Ar-H), 6.53 (s, 2H, SO_2NH_2), 5.70 (t, 1H, NH, J = 5.3), 4.07 (d, 2H, CH_2, J = 5.3). ^{13}C NMR: (75 MHz DMSO-d_6) δ (ppm) 169.02 (C═O), 146.70, 142.51, 136.39, 131.12, 129.14, 125.81, 117.41, 115.32, 46.71 (CH_2).

(*S*)-2-((2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)amino)pentanedioic acid (4e)

White solid, m.p. = 233–235 °C, yield = 57%, R_f = 0.46 (Chloroform: Methanol 4:1); ^1H NMR (DMSO-d_6): δ (ppm) 10.6 (broad s, 1H, COOH), 10.4 (broad s, 1H, COOH), 9.33 (broad s, 1H, NH), 7.81 (d, 2H, *J* = 7.3 Hz, Ar-H), 6.97 (d, 2H, *J* = 7.3 Hz, Ar-H), 6.63(s, 2H, SO_2NH_2), 5.70 (t, 1H, NH, J = 5.3), 3.91 (d, 2H, CH_2, J = 5.3). ^{13}C NMR: (75 MHz Acetone-d_6) δ (ppm) 175.12 (C═O), 179.23 (C═O), 167.14, (C═O), 145.52, 138.19, 131.16, 118.04, 64.08(CH), 48.17 (CH_2). 31.7, 28.32

2-((2-chlorophenyl)amino)-*N*-(4-sulfamoylphenyl)acetamide (4f):

Light brown solid, m.p. = 269–271 °C, yield = 75%, R_f = 0.75 (Chloroform: Methanol 4:1); ^1H NMR (DMSO-d_6): δ (ppm) 9.49 (s, 1H, NH), 7.91 (s, 4H, Ar-H), 7.59 (d, 1H, *J* = 7.9 Hz, Ar-H), 7.23 (t, 1H, *J* = 7.8 Hz, Ar-H), 6.81 (d, 1H, *J* = 7.8 Hz, Ar-H), 6.41 (t, 1H, *J* = 7.9 Hz, Ar-H), 6.58 (s, 2H, SO_2NH_2), 4.89 (t, 1H, NH, J = 5.1), 4.11 (d, 2H, CH_2, J = 5.3). ^{13}C NMR: (75 MHz DMSO-d_6) δ (ppm) 170.01 (C═O), 142.92, 141.67, 135.19, 132.16, 128.21, 126.51, 124.01, 122.29, 117.90, 115.07, 47.53 (CH_2).

2-((2,4-dinitrophenyl)amino)-*N*-(4-sulfamoylphenyl)acetamide (4g):

Yellow solid, m.p. = 287–288 °C, yield = 58%, R_f = 0.43 (Chloroform: Methanol 4:1); ^1H NMR (DMSO-d_6): δ (ppm) 10.09 (s, 1H, NH), 8.91 (s, 1H, Ae-H), 8.63 (d, 1H, *J* = 9.1 Hz, Ar-H), 8.01 (s, 4H, Ar-H), 7.43 (d, 1H, *J* = 9.2 Hz, Ar-H),), 6.88 (s, 2H, SO_2NH_2), 5.41 (t, 1H, *J* = 4.9 Hz, NH), 3.89 (d, 2H, *J* = 4.9 Hz, CH_2); ^{13}C NMR (75 MHz DMSO-d_6) δ (ppm) 169.35 (C═O), 146,51, 143.61, 137.25, 136.01, 135.22, 131.06, 129.66, 123.24, 120.45, 119.21, 48.07 (CH_2).

4-((2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)amino)benzoic acid (4h):

Dark brown solid, m.p. = 277–278 °C, yield = 65%, R_f = 0.61 (Chloroform: Methanol 4:1); ^1H NMR (DMSO-d_6): δ (ppm); 11.53 (broad s, 1H, COOH), 9.84 (s, 1H, NH), 7.93 (m, 4H, Ar-H), 7.78 (d, 1H, *J* = 8.1 Hz, Ar-H),), 6.72 (s, 2H, SO_2NH_2), 6.61 (d, 1H, *J* = 8.6 Hz, Ar-H,) 5.74 (t, 1H, *J* = 5.2 Hz, NH), 4.09 (d, 2H, *J* = 5.2 Hz, CH_2); ^{13}C NMR (75 MHz DMSO-d_6) δ (ppm) 169.46 (C═O), 168.15 (C═O), 149.11, 144.21, 137.25, 131.21, 129.52, 119.16, 118.72, 116.28, 49.17 (CH_2).

2-((2,3-dichlorophenyl)amino)-*N*-(4-sulfamoylphenyl)acetamide (4i):

Light brown solid, m.p. = 273–274 °C, yield = 67%, R_f = 0.52 (Chloroform: Methanol 4:1); ^{1}H NMR (DMSO-d_6): δ (ppm); 9.74 (s, 1H, NH), 7.82 (d, 2H, J = 8.1 Ar-H), 7.68 (d, 2H, *J* = 8.1 Hz, Ar-H),), 7.24 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.11 (t, 1H, *J* = 8.5 Hz, Ar-H), 6.43 (s, 2H, SO_2NH_2), 6.21 (d, 1H, *J* = 8.5 Hz, Ar-H), 5.02 (t, 1H, *J* = 5.1 Hz, NH), 4.34 (d, 2H, *J* = 5.1 Hz, CH_2); ^{13}C NMR (75 MHz DMSO-d_6) δ (ppm) 169.76 (C═O), 148.67, 145.91, 138.25, 134.45, 133.34, 130.02, 123.16, 121.92, 119.12, 117.28, 49.45 (CH_2).

2-((1R,3S,5r,7r)-adamantan-2-ylamino)-N-(4-sulfamoylphenyl) acetamide (4j)

White solid, m.p. = 243–245 °C, Yield = 58%, R_f = 0.55 62 (Chloroform: Methanol 4:1); ^{1}H NMR (DMSO-d_6, 300 MHz,); δ (ppm): 9.19 (broad s, 1H, NH), 8.17 (d, 2H, *J* = 7.7 Hz, Ar-H), 7.94 (d, 2H, *J* = 7.7 Hz, Ar-H), 6.13(s, 2H, SO_2NH_2), 5.10 (t, 1H, NH, J = 5.2), 3.36 (d, 2H, CH_2, J = 5.2), 2,28 (m, 6H, CH_2), 2.18 (m, 3H, CH), 1.56 (m, 6H, CH_2); ^{13}C NMR (75 MHz DMSO-d_6) δ (ppm) 167.88 (C═O), 141, 131.88, 129.36, 129.22, (Ar-C), 54.15, 47.23, 40.27, 36.15, 29.12

4-((2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)amino)benzenesulfonic acid (4k):

White solid, m.p. = 279–281 °C, yield = 62%, R_f = 0.39 (Chloroform: Methanol 4:1); ^{1}H NMR (DMSO-d_6): δ (ppm) 9.92 (s, 1H, NH), 7.91 (broad s, 8H, Ar-H), 6.93(s, 2H, SO_2NH_2), 5.20 (t, 1H, *J* = 5.2 Hz, NH), 4.03 (d, 2H, *J* = 5.2 Hz, CH_2). ^{13}C NMR: (75 MHz DMSO-d_6) δ (ppm) 171.12 (C═O), 157.22, 146.17, 145.29, 144.66, 135.84, 134.09, 128.13, 125.09, 120.45, 119.15, 49.51 (CH_2).

2-((4-nitrophenyl)amino)-*N*-(4-sulfamoylphenyl)acetamide (4l):

Light yellow solid, m.p. = 284–287 °C, yield = 59%, R_f = 0.53 (Chloroform: Methanol 4:1); ^{1}H NMR (DMSO-d_6): δ (ppm); 9.94 (s, 1H, NH), 8.11 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.91 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.72 (d, 2H, *J* = 8.2 Hz, Ar-H), 6.73 (d, 2H, *J* = 8.9 Hz, Ar-H), 6.56 (s, 2H, SO_2NH_2), 5.34 (t, 1H, *J* = 5.6 Hz, NH), 4.23 (d, 2H, *J* = 5.6 Hz, CH_2). ^{13}C NMR: (75 MHz DMSO-d_6) δ (ppm), 171.32, (C═O), 151.24, 141.62, 139.79, 136.06, 135.14, 128.07, 124.04, 119.07, 47.51 (CH_2).

2-(naphthalen-1-ylamino)-N-(4-sulfamoylphenyl)acetamide (4m)

brown solid, m.p. = 271–273 °C, yield = 69%, R_f = 0.62 (Chloroform: Methanol 4:1); ^{1}H NMR (acetoned_6): δ (ppm) 9.09 (broad s, 1H, NH), 8.07 (d, 2H, *J* = 6.5 Hz, Ar-H), 7.77 (s, 4H, Ar-H), 7.43 (t, 2H, *J* = 6.4 2 Hz, Ar-H), 7.01–6.30 (5H, Ar-H), 5.40 (t, 1H, NH, J = 5.1), 3.75 (d, 2H, CH_2, J = 5.1). ^{13}C NMR: (75 MHz Acetone-d_6) δ (ppm) 167.12 (C═O), 147.62, 143.92, 137.79, 131.36, 129.44, 127.07, 126.04, 125.07, 124.99, 124.70, 118.05, 118.01, 109.43, 46.91 (CH_2).

2.4. Carbonic anhydrase assay

Carbonic anhydrase inhibition was measured as described previously with some modifications [25]. The method is based on the principle that *p*-nitrophenyl acetate is hydrolyzed by Carbonic anhydrase to form yellow colored *p*-nitrophenol which was measured spectrophotometrically. Briefly, Reaction mixture contained 120 µL of 50 mM Tris-sulfate buffer (pH 7.6 containing 0.1 mM $ZnCl_2$), 20 µL of inhibitor and 20 µL (50 U) bovine enzyme per well. Contents were well mixed and pre-incubated at 25 °C for 10 min. substrate *p*-nitrophenyl acetate was prepared (6 mM stock using < 5% acetonitrile in buffer and used fresh every time) and 40 µL was added per well to achieve 0.6 mM concentration per well. Total reaction volume was made to 200 µL. After30 min incubation at 25 °C contents were mixed and absorbance was measured at 348 nm using a microplate reader. Acetazolamide was used as a reference inhibitor and tris-sulfate buffer was used as negative control. Each concentration was analyzed in three independent experiments. IC_{50} values were calculated by nonlinear regression using GraphPad Prism 5.0.

$$\text{Inhibition } (\%) = [(B - S)/B] \times 100$$

Here, the B and S are the absorbance for the blank and samples.

2.5. Free radical scavenging assay

Radical scavenging activity was determined by modifying method by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [26]. The assay solution consisted of 100 mL of (150 mM) 2,2-diphenyl-1 picrylhydrazyl (DPPH), 20 µL of increasing concentration of test compounds and the volume was adjusted to 200 µL in each. This reaction mixture was then incubated for 30 min at room temperature. Ascorbic acid (Vitamin C) was used as a reference inhibitor. The measurements were carried out by using a micro plate reader (OPTIMax, tunable) at 517 nm. The reaction rates were compared and the percent inhibition due to the presence of tested inhibitors was calculated. Each concentration was analyzed in three independent experiments.

3. Methodology

3.1. Repossession of carbonic anhydrase II from PDB

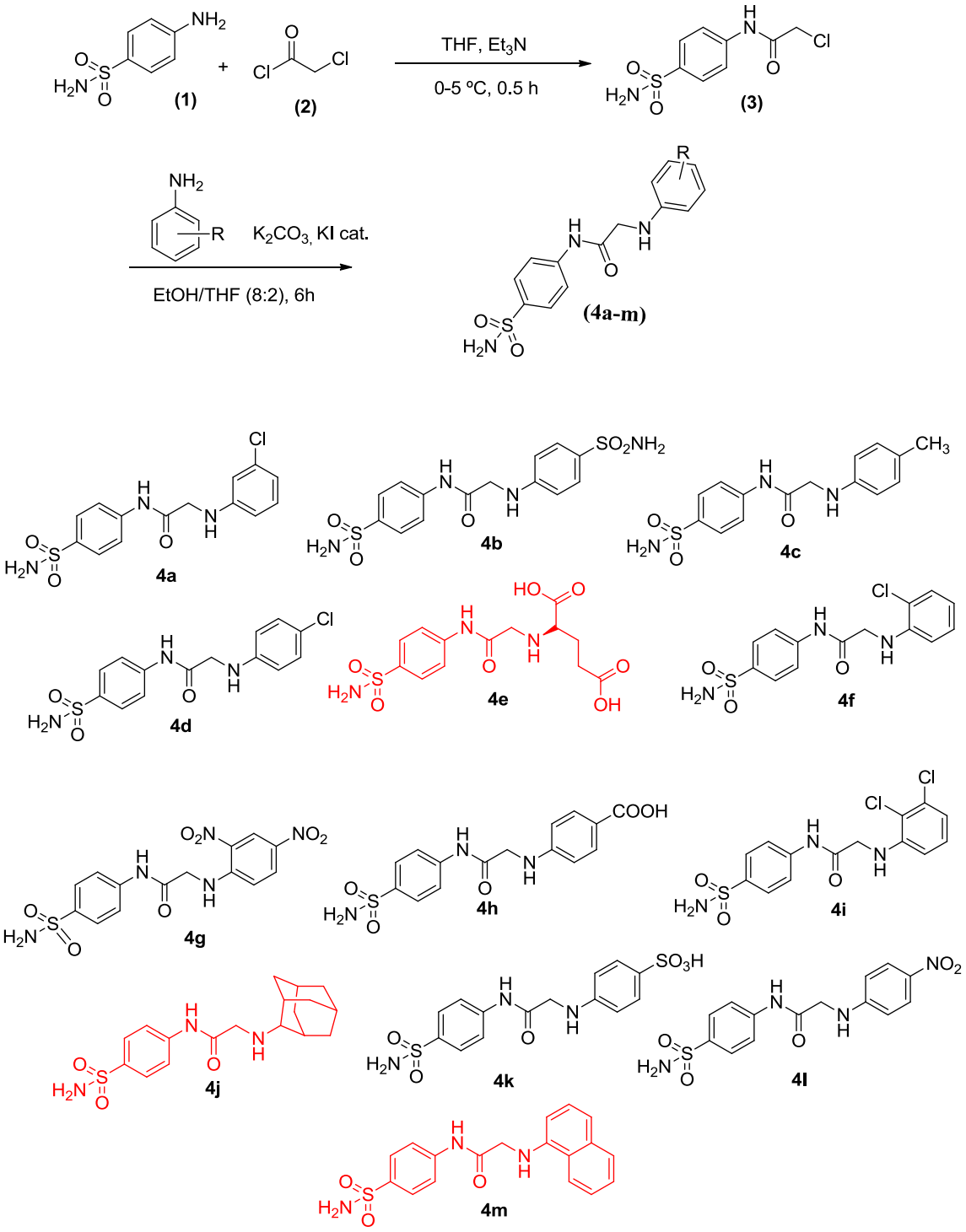
The three dimensional (3D) crystal structure of carbonic anhydrase II was retrieved form the Protein Data Bank (PDB) having PDBID 1V9E (www.rcsb.org). Energy minimization of target structure was carried out by using conjugate gradient algorithm and Amber force field in UCSF Chimera 1.10.1 [27]. The stereo-chemical properties, Ramachandran graph and values [28] of Carbonic anhydrase II structure were assessed by Molprobity server [29], while the hydrophobicity graph was generated by Discovery Studio 4.1 Client [30]. The protein architecture and statistical percentage values of helices, beta-sheets, coils and turns were accessed by using online tool VADAR 1.8 [31].

3.2. In-silico designing of synthesized compounds

The synthesized ligand molecules **4a-4m** were sketched in drawing ACD/ChemSketch tool and further minimized by visualizing software UCSF Chimera 1.10.1. The different online drug assessment tools like Molinspiration (http://www.molinspiration.com/) and Molsoft (http://www.molsoft.com/) were employed to predict the drug-likeness and biological properties of these designed candidate molecules. The number of rotatable bonds, hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) were also confirmed by PubChem (https://pubchem.ncbi.nlm.nih.gov/). Moreover, Lipinski's rule of five was analyzed using Molsoft and Molinspiraion tools.

3.3. Molecular docking

The synthesized ligands **4a-4m** were sketched in ACD/ChemSketch tool and minimized by UCSF Chimera 1.10.1 tool. The molecular docking experiments were performed using PyRx docking through VINA wizard [32]. The grid box center values were adjusted as for X = 10.48, Y = −55.22 and Z = −26.48), respectively. While, the size parameters values for X = 66.60, Y = 60.08, and Z = 56.84 were also focused to get the better conformational binding poses. The default exhaustiveness value was used to maximize the binding conformational analysis. All the synthesized ligands were docked separately against urease receptor. The Autodock VINA scoring function equation was employed as mentioned in supplementary data. The predicted docked complexes were evaluated on the basis of lowest binding energy (kcal/mol) values and structure activity relationship (SAR) analyses. The graphical depictions of all the docked complexes were accomplished by Discovery Studio (2.1.0) and LIGPLOT [33].



Scheme 1. Synthesis of 2-(substituted phenylamino)-*N*-(4-sulfamoylphenyl) acetamide **4a-4m**.

4. Results and discussion

4.1. Chemistry

The synthesis of sulfonamide bearing amides and amine functional moieties is illustrated in Scheme 1. 2-Chloro-*N*-(4-sulfamoylphenyl) acetamide (**3**) was obtained by reacting 4-aminobenzenesulfonamide (**1**) in tetrahydrofuran, with 2-chloroacetyl chloride (**2**) at 0–5 °C. Suitably substituted aniline in presence of anhydrous potassium carbonate and a catalytic amount of potassium iodide were treated with (**3**) in tetrahydrofuran and ethanol to afford the target molecules **(4a-4m)** in good yields.

The synthesized compounds were characterized through ^{1}H NMR and ^{13}C NMR spectroscopy. The broad and deshielded singlet for amidic N−H was observed around 11–12 ppm. The singlet at 2–3 ppm was assigned to CH_2 in between the amide and amine and the aromatic region appeared at 7–8 ppm. The ^{13}C NMR signal for carbonyl of amide appeared in the range 169–160 ppm; those in the range 40–50 ppm value were assigned to CH_2, while the aromatic carbons appeared in the range 120–140 ppm.

Table 1
Carbonic anhydrase II activity (IC_{50} nM) of compounds (**4a-4m**).

4a	73.6 ± 1.4
4b	194.3 ± 3.8
4c	214.6 ± 4.2
4d	166.2 ± 3.2
4e	116.9 ± 2.3
4f	182.0 ± 3.6
4g	621.0 ± 12.3
4h	21.9 ± 0.43
4i	75.8 ± 1.5
4j	84.5 ± 1.67
4k	858.0 ± 17.0
4l	17.96 ± 0.36
4m	81.3 ± 1.61
Acetazolamide	997.1 ± 19.75

For calculation of IC_{50} six to eight concentrations were used. IC_{50} values were calculated by nonlinear regression using GraphPad Prism 5.0.

4.2. Carbonic anhydrase II activity and preliminary structure activity relationship

The results of carbonic anhydrase II (CA-II) has been documented in Table 1. The three **4a**, **4d** and **4f** derivatives in the series contains chlorine atom tagged with phenyl ring at meta, para and ortho position respectively and the results of inhibition of CA-II of these three molecules are in this order; **4a** > **4d** > **4f**. This reveals that chlorine at meta position is able to polarize the molecule more effectively compared to para and ortho position. The compounds 4h and 4k possess carboxylic acid and sulfonic acid respectively and results exhibited that compound 4h showed better activity compared to **4k**. The compound **4g** and **4l** possess dinitro and mono nitro group attached at the phenyl ring and compound **4l** showed better results compared to **4g** and also compared to other derivatives of the series. However, **4j** possess amantadine drug as part of the molecule and it showed good inhibition but we assumed on the basis of hypothesis (coupling of two or more pharmacophoric units results in enhanced activity) that compound **4j** should exhibit maximum inhibition but experimental results summarized in Table depict that compound **4l** showed maximum inhibition.

4.3. Free radical scavenging

All of the synthesized 4a-4m series compounds were evaluated for DPPH free radical scavenging ability. The compound **4f** showed excellent % scavenging potency, other compounds did not show significant radical scavenging potential even at high concentration (100 μg/mL) Fig. 1 (DPPH).

4.4. Chemo-informatic properties and Lipinski Rule (RO5) evaluation of ligands

The designed ligands were analyzed computationally to predict the best ligand on the basis of chemical and bio-molecular properties and RO5. The predicted chemo-informatics properties like Log*P*, HBD, HBA, molar volume, polar surface area (PSA) and drug likeness values of ligand molecules are mentioned in Table 1. It has been confirmed from previous research data that the standard values for molecular weight (MW) and polar surface area (PSA) are (160–480 g/mol) and (< 89 $Å^2$) respectively [34,35]. The predicted results of compounds showed good MW and PSA values which are comparable with standard values. RO5 also confirmed the therapeutic potential of all the ligands. Hydrogen-bonding capacity has been identified as an important parameter for describing drug permeability. Research data revealed poor permeation is more likely to be observed when the HBA and HBD are exceeded then 10 and 5 respectively [36]. The chemo-informatics analysis justified that all the designed compounds possess < 10 HBA and < 5 HBD. Moreover, their log*P* values were also comparable with standard value. However there are plenty of examples available for RO5 violation amongst the existing drugs [37,38]. The predicted chemo-informatics values of all the designed ligand are mentioned in Table 2.

4.5. Molecular docking and binding energy analysis

The docked complexes of all the compounds **4a-4m** against carbonic anhydrase II were analyzed separately and evaluated on the basis of minimum energy values and ligand interactions pattern. Results showed that all compounds **4a-4m** showed good binding energy value and exhibited in the active region of target protein (Table 3). Prior research showed that the standard error for Autodock is testified as 2.5 kcal/mol. However, in all docking complexes the predicted energy values difference was less than standard energy value. Although, the basic nucleus of all the synthesized compounds was similar, therefore most of ligands possess good efficient energy values and have no big energy fluctuations difference. The comparative docking analysis and inhibition constant (IC_{50}) value 0.01796 ± 0.00036 μM justified that **4l** has good therapeutic potential as compared to all other compounds.

4.6. Binding analyses of synthesized compounds against carbonic anhydrase II

The ligands-protein binding analyses showed that **4l** confined in the active binding pocket of target protein as mentioned in Fig. 2. The CA II

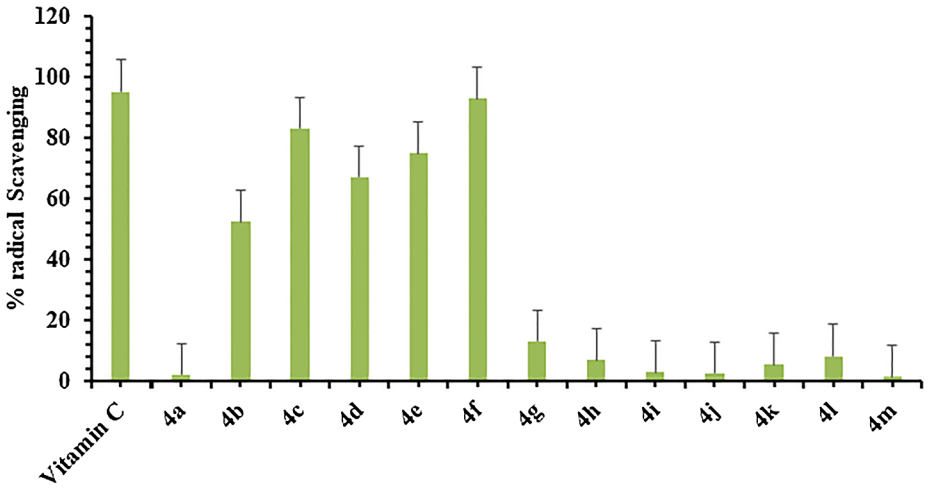


Fig. 1. Free radical % scavenging activity of synthetic compounds values was represented as mean ± SEM. All compounds concentrations were 100 μg/mL.

Table 2
Chemo-informatics analysis of designed chemical compounds.

Ligands	Mol. Wt (g/mol)	No. HBA	No. HBD	Mol. Log*p* (mg/L)	PSA (A^2)	Mol. Vol (A^3)	Drug Score
4a	339.04	4	4	2.08	84.23	279.51	0.12
4b	384.06	7	6	0.19	134.83	309.23	0.76
4c	319.10	4	4	1.77	84.23	285.52	0.31
4d	339.04	4	4	2.08	84.23	279.44	1.10
4e	359.08	9	6	−1.79	142.24	309.58	0.29
4f	339.04	4	4	1.96	83.53	278.32	0.33
4g	395.05	8	4	0.70	159.75	316.79	−1.18
4h	349.07	6	5	1.08	112.63	297.68	1.24
4i	373.01	4	4	2.56	83.53	293.99	0.61
4j	363.16	5	4	1.81	85.89	346.17	1.17
4k	385.04	7	5	−0.47	126.64	305.15	0.65
4l	350.07	6	4	1.10	122.49	290.21	−0.18
4m	355.10	4	4	2.70	83.26	314.81	0.03

Abbreviation: HBA = No of hydrogen bond acceptor, HBD = No of hydrogen bond donor, Log*P* = lipophilicity of partition coefficient, LogS = lipophilicity of water, PSA = polar surface area, MR = Molar refractivity, PZ = polarizability.

Table 3
Docking results of synthesized compounds.

Docking complexes	Binding Affinity
4a	−6.7
4b	−7.5
4c	−6.7
4d	−6.6
4e	−7.6
4f	−6.8
4g	−6.9
4h	−7.1
4i	−6.9
4j	−6.8
4k	−7.2
4l	−6.7
4m	−7.6

has an active site cleft (15 Å in diameter and 15 Å deep), and contains a Zinc ion that is coordinated in a tetrahedral geometry with three histidine residues (His94, His96 and His119) and a water molecule/hydroxide ion. The **4l** receptor docked complex reveals the good conformational state with hydrogen bond interactions within the receptor binding pocket. The docking result of **4l** receptor docked complex showed that three hydrogen bonds were observed at His63, Asn66 and Thr197 residues, respectively. The amino carbonyl moiety of functional group in A12 interacts with His63 with bond distance 2.49 Å while other form another hydrogen bond against Asn66 with bond length 1.92 Å, respectively. Similarly, another hydrogen bond was observed between amino group of **4l** and Thr197 having bond length 2.36 Å. Single hydrophobic interaction was also seen between His93 and oxygen moiety of **4l** with bond length 3.06 Å. No π-π stacking interactions were observed between ring structure of ligands and aromatic residues of target protein.

5. Conclusions

To test the hypothesis that coupling of two more pharmacophoric units results in the enhancement of biological activity, we envisioned coupling of sulfonamide, an amide and amine can result in the higher biological activity compared to the already known commercial drugs. In this connection, a new series of compounds **4a-4m** was synthesized and characterized through spectroscopic techniques and subjected to carbonic anhydrase II inhibition. Among the series compound **4l** exhibited highest activity (0.01796 ± 0.00036 μM) compared to the other members of series as well as the standard acetazolamide. The Lipinski's rule and chemo-informatics evaluation revealed that molecules possess significant hydrogen bonding sites which can enable them to bind in the active site of target protein. The binding analysis of ligand–protein that compound **4l** possess minimum binding energy (−6.7 KJ/mol) and shows hydrogen bonding interaction with His63, Asn66 and Thr197

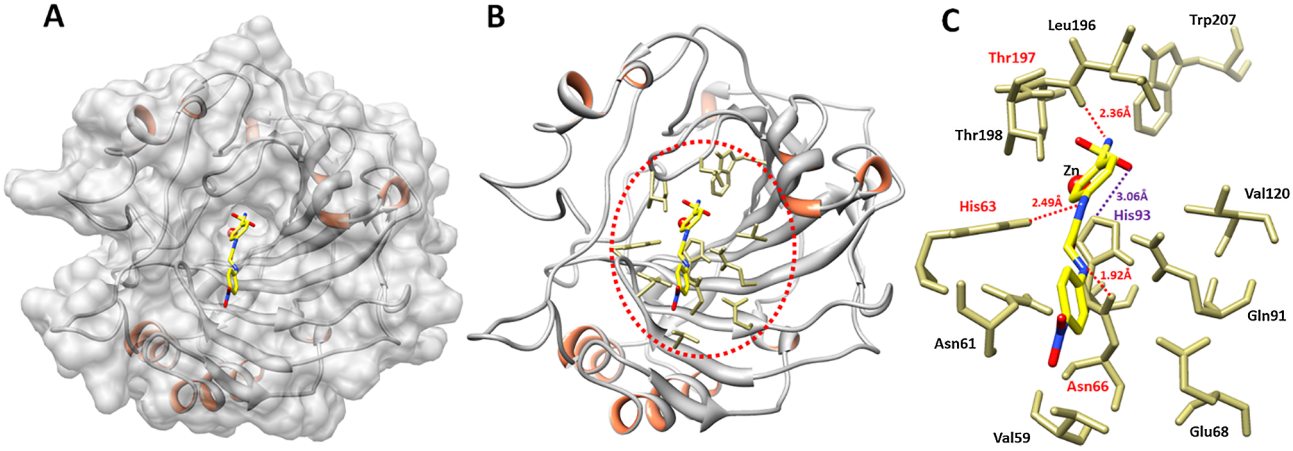


Fig. 2. Docking interaction **4l** with receptor molecule. (A) The protein structure is represented in grey color in surface format to show the binding pocket of target protein. (B) Docking complex A12 is shown in grey ribbon with interior brown color, while the interacted residues are justified in light dark khaki color. (C) The closer view of binding interaction. The ligand molecule is depicted in yellow color while their functional groups such as oxygen, nitrogen and sulphur are showed in red, blue and yellow colors respectively. Amino acids are highlighted in dark khaki color and red and purple dotted lines justifies the hydrogen and hydrophobic bindings with distance mentioned in angstrom (Å). Zinc metal is represented in grey circle.

amino acid residues. To sum up, present investigation provides an incentive to further explore the structure activity relationship and design safe and effective inhibitors of carbonic anhydrase II enzyme.

Conflict of interest

Authors declare no any conflict of interest

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.060.

References

[1] C.T. Supuran, A. Scozzafava, Applications of carbonic anhydrase inhibitors and activators in therapy, Exp. Opin. Ther. Patents 12 (2002) 217–242.
[2] C.T. Supuran, A. Scozzafava, Carbonic anhydrase inhibitors and their therapeutic potential, Exp. Opin. Ther. Patents 10 (2000) 575–600.
[3] D. Hewett-Emmett, Evolution and distribution of the carbonic anhydrase gene families, in: W.R. Chegwidden, Y. Edwards, N. Carter (Eds.), The Carbonic Anhydrases-New Horizons, Birkha¨user Verlag, Basel, 2000, pp. 29–78.
[4] C.T. Supuran, C.W. Conroy, T.H. Maren, Is cyanate a carbonic anhydrase substrate? Proteins Struct. Funct. Genet. 27 (1997) 272–278.
[5] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discovery 7 (2008) 168–181.
[6] T. Fattah, S. Bua, G. Aamer Saeed, C.T. Shabir, Supuran, 3-Aminobenzenesulfonamides incorporating acyl thiourea moieties selectively inhibit the tumor-associated carbonic anhydrase isoform IX over the off-target isoforms I, II and IV, Bioorg. Chem. 82 (2019) 123–128.
[7] C.T. Supuran, Carbonic anhydrases as drug targets – an overview, Curr. Top Med. Chem. 7 (2007) 825–833.
[8] S.U. Mahmood, A. Saeed, S. Bua, A. Nocentini, P. Gratteri, C.T. Supuran, Synthesis, biological evaluation and computational studies of novel iminothiazolidinone benzenesulfonamides as potent carbonic anhydrase II and IX inhibitors, Bioorg. Chem. 77 (2018) 381–386.
[9] A. Innocenti, S.B. Öztürk Sarıkaya, I. Gülçin, C.T. Supuran, Carbonic anhydrase inhibitors. Inhibition of mammalian isoforms I-XIV with a series of natural product polyphenols and phenolic acids. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine, Bioorg. Med. Chem. 18 (2010) 2159–2164.
[10] M. Ceruso, S. Antel, D. Vullo, et al., Inhibition studies of new ureidosubstituted sulfonamides incorporating a GABA moiety against human carbonic anhydrase isoforms I-XIV. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine, Bioorg. Med. Chem. 22 (2014) 6768–6775.
[11] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem. Rev. 112 (2012) 4421–4468.
[12] C.T.C.T. Supuran, How many carbonic anhydrase inhibition mechanisms exist? J. Enzyme Inhib. Med. Chem. 31 (2016) 345–360.
[13] C.T. Supuran, Structure and function of carbonic anhydrases, Biochem. J. 473 (14) (2016) 2023–2032.
[14] A. Innocenti, I. Gülçin, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I-XV, Bioorg. Med. Chem. Lett. 20 (2010) 5050–5053.
[15] F. Abbate, C.T. Supuran, A. Scozzafava, P. Orioli, M.T. Stubbs, G. Klebe, The sulfonamide group as an ideal anchor for the design of potent human carbonic anhydrase inhibitors: role of hydrogen-bonding networks in ligand binding and drug design, J. Med. Chem. 45 (2002) 3583–3587.
[16] R. Qamar, A. Saeed, M. Saeed, Z. Ashraf, M. Hassan, F. Albericio, Synthesis, carbonic anhydrase inhibitory activity and antioxidant activity of some 1,3-oxazine derivatives, Drug Develop. Res. 79 (2018) 352–361.
[17] C.T. Supuran, A. Scozzafava, A. Casini, Carbonic anhydrase inhibitors, Med. Res. Rev. 23 (2) (2003) 146–189.
[18] T. Mann, D. Keilin, Sulphanilamide as a specific carbonic anhydrase inhibitor, Nature 146 (1940) 164–165.
[19] F. Abbate, A. Casini, T. Owa, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: E7070, a sulfonamide anticancer agent, potently inhibits cytosolic isozymes I and II, and transmembrane, tumor-associated isozyme IX, Bioorg. Med. Chem. Lett. 14 (1) (2004) 217–223.
[20] C.T. Supuran, Diuretics: from classical carbonic anhydrase inhibitors to novel applications of the sulfonamides, Curr. Pharm. Des. 14 (7) (2008) 641–648.
[21] T. Gokcen, I. Gulcin, T. Ozturk, A.C. Goren, A class of sulfonamides as carbonic anhydrase I and II inhibitors, J. Enzyme Inhib. Med. Chem. 31 (2016) 180–188.
[22] S. Zaib, A. Saeed, K. Stolte, U. Flörke, M. Shahid, J. Iqbal, New aminobenzene sulfonamide-thiourea conjugates: synthesis and carbonic anhydrase inhibition and docking studies, Eur. J. Med. Chem. 78 (2014) 140–150.
[23] A. Saeed, Mariya al-Rashida, M. Hamayoun, A. Mumtaz, J. Iqbal, Carbonic anhydrase inhibition by 1-aroyl-3-(4-aminosulfonylphenyl)thioureas, J. Enz. Inhib. Med. Chem. 29 (6) (2014) 901–905.
[24] T.A. Fattah, A. Saeed, P.A. Channar, F.A. Larik, M. Hassan, H. Raza, Q. Abbas, S.Y. Seo, Synthesis and molecular docking studies of (*E*)-4-(substituted-benzylideneamino)-2*H*-Chromen-2-one derivatives: entry to new carbonic anhydrase class of inhibitors, Drug Res. 68 (07) (2018) 378–386.
[25] M. Al-Rashida, M. Ashraf, B. Hussain, S.A. Nagra, G. Abbas, Discovery of new chromone containing sulfonamides as potent inhibitors of bovine cytosolic carbonic anhydrase, Bioorg. Med. Chem. 19 (11) (2011) 3367–3371.
[26] F.A. Larik, A. Saeed, P.A. Channar, U. Muqadar, Q. Abbas, M. Hassan, S.Y. Seo, M. Bolte, Design, synthesis, kinetic mechanism and molecular docking studies of novel 1-pentanoyl-3-arylthioureas as inhibitors of mushroom tyrosinase and free radical scavengers, Eur. J. Med. Chem. 141 (2017) 273–281.
[27] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera – a visualization system for exploratory research and analysis, J. Comput. Chem. 25 (13) (2006) 1605–1612.
[28] S.C. Lovell, I.W. Davis, W.B. Arendall 3rd, P.I. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson, D.C. Richardson, Structure validation by Calpha geometry: phi, psi and Cbeta deviation, Proteins. 50 (3) (2002) 437–450.
[29] V.B. Chen, W.B. Arendall 3rd, J.J. Headd, D.A. Keedy, R.M. Immormino, G.J. Kapral, L.W. Murray, J.S. Richardson, D.C. Richardson, MolProbity: all-atom structure validation for macromolecular crystallography, Acta Crystallogr. D Biol. Crystallogr. 66 (Pt1) (2010) 12–21.
[30] Studio, Discovery. "version 2.1." Accelrys, San Diego, CA, 2008.
[31] L. Willard, A. Ranjan, H. Zhang, H. Monzavi, R.F. Boyko, B.D. Sykes, D.S. Wishart, VADAR: a web server for quantitative evaluation of protein structure quality, Nucl. Acids Res. 31 (2003) 3316–3319.
[32] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, Methods Mol. Biol. 1263 (2015) 243–250.
[33] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, Protein Eng. 8 (1996) 127–134.
[34] R.U. Kadam, N. Roy, Recent trends in drug-likeness prediction: a comprehensive review of in silico methods, Indian J. Pharmaceut. Sci. 69 (2007) 609–615.
[35] A.K. Ghose, T. Herbertz, R.L. Hudkins, B.D. Dorsey, J.P. Mallamo, Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery, ACS Chem. Neurosci. 3 (2012) 50–68.
[36] M.A. Bakht, M.S. Yar, S.G. Abdel-Hamid, S.I. Al-Qasoumi, A. Samad, Molecular properties prediction, synthesis and antimicrobial activity of some newer oxadiazole derivatives, Eur. J. Med. Chem. 45 (2010) 5862–5869.
[37] S. Tian, J. Wang, Y. Li, D. Li, L. Xu, T. Hou, The application of in silico drug-likeness predictions in pharmaceutical research, Adv. Drug Deliv. Rev. 86 (2015) 2–10.
[38] Prerana B. Jadhav, Akshay R. Yadav, Megha G. Gore, Concept of drug likeness in pharmaceutical research, Int. J. Pharm. Bio Sci. 6 (4) (2015) 142–154.