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Graphical Abstract

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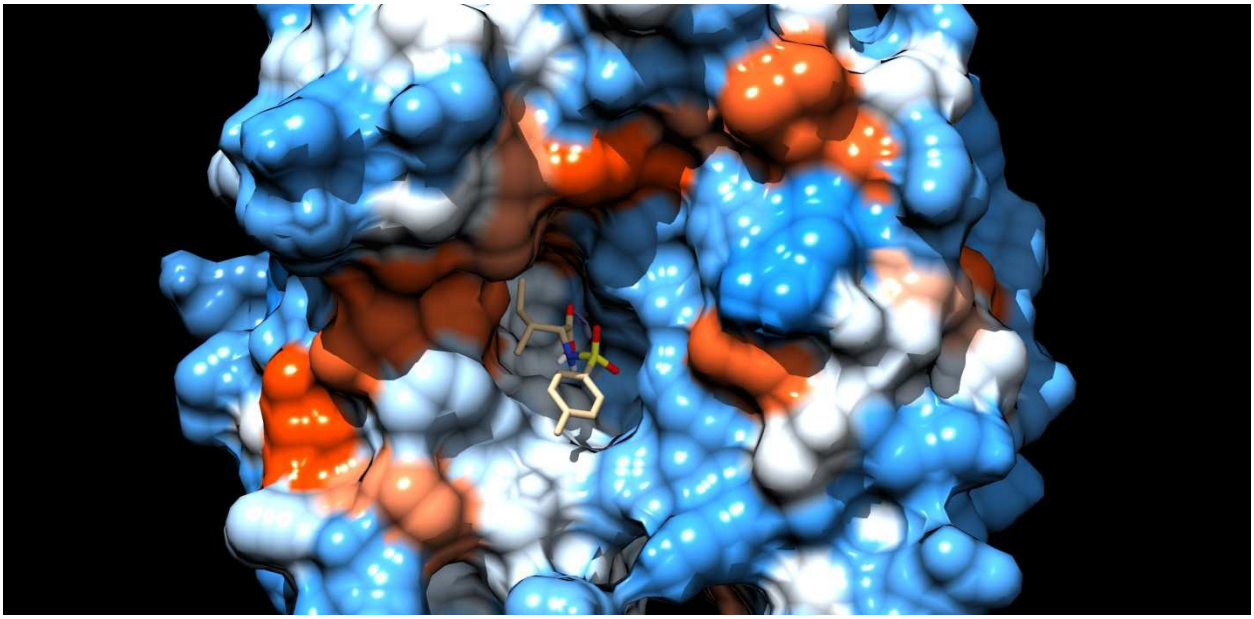

Compound **NA3** bound inside the active site of the enzyme.

Metal Complexes of Tosyl Sulfonamides: Design, X-ray Structure, Biological Activities and Molecular Docking Studies

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Abstract

The present study reports the synthesis of Zn(II) complexes of tosyl sulfonamide derivatives which were obtained by the reaction of tosyl chloride with L-amino acids. The ligands and their complexes were characterized by IR, ^{1}H and ^{13}C-NMR, GC-MS, elemental analysis and X-ray crystallography in case of **NA3**. All the compounds were screened for their carbonic anhydrase inhibitory activities. The results demonstrated that complexes are stronger inhibitors of carbonic anhydrase as compared to their parent ligands, which warrants further development of organometallics as active carbonic anhydrase inhibitors. Cytotoxicity assay on lung carcinoma (H-157) and kidney fibroblast (BHK-21) cancer cells demonstrated that compounds were potent anticancer agents. Additionally, the complexes were screened against promastigote forms of *Leishmania major* and found to be significant antileishmanial agents. Molecular docking studies

were performed against bCA II enzymes to rationalize the inhibitory properties of these compounds. The identified inhibitors showed promise for the design of interesting pharmacological agents.

Key words: Anticancer activity, Carbonic anhydrase, Leishmania major, Molecular docking, Zn(II) complexes

Introduction

The zinc metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes the prompt conversion of carbon dioxide and water into a proton and the bicarbonate ion.[1-3] Despite the fact that CO_2, bicarbonate and protons are essential molecules and ions in numerous significant physiological processes in all life forms, and absolutely they occur in bulk in various tissues of all organisms, it is not surprising that CAs evolved at least five times after consideration.[1-4]

Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is one of the most formidable afflictions in the world.[5] Over the years, the design of cancer chemotherapy has become increasingly sophisticated.[6] In the bioinorganic medicinal chemistry, numerous metal complexes have been screened for their anticancer potency.[7] The development of metal complexes with platinum central atoms such as cisplatin or carboplatin incorporated broad influence on current cancer chemotherapy.[8] Cisplatin, in particular, has become one of the most extensively used drugs and is highly effective in treating several cancers such as ovarian and testicular cancers.[7]

Leishmaniasis is one of the life-threatening diseases after malaria, which is caused by a unicellular kinetoplastid protozoan flagellate of genus *Leishmania* and is transmitted by the bite of female phlebotomine sand flies.[9] Classical antiparasitic drugs are highly toxic and most of them are no longer effective due to the emergence of drug resistance in the parasites, so there is a dire need to synthesize new antiparasitic drugs having minimum toxicity and low resistance in the parasites.[10,11]

Synthesis and biological investigations of amino acid derivatives and their complexes have also been reported,[12,13] which are among the most biologically active organic molecules. The complexes of amino acids with transition metal ions are very important class of compounds specifically in terms of biological response. The use of amino acids as different models to study

the pharmacodynamics and pharmacokinetics is becoming the interest of the medicinal chemists which ultimately have a number of advantages, for instance, providing dietary supplement and reducing the side effects. Complexes of the different metals with amino acids from D- or L-isomers of proline, lysine, histidine, tryptophan and arginine etc, have earned larger importance to treat diabetes, malaria and also served as an important co-enzyme in reversible oxidation-reduction systems.[14-17] The medicinal and clinical importance of sulfonamides is well known due to the presence of sulfonamide moiety ($–SO_2NH_2$) exhibiting a variety of pharmacological actions i.e; antimalarial antimicrobial, anti-HIV, antidiabetic, high ceiling diuretic, antithyroid, and antitumor.[18-20]

The Cu(II) and Mn(II) complexes with amino acids have been shown to possess anti-inflammatory, antiviral activities, used to treat a number of allergies, various anemia and heart diseases.[21-26] The organometallic compounds with antimony are used against the leishmanial protozoan for many decades. Recently, our group has reported Zn(II) metal complexes with antimicrobial evaluation of tosyl sulfonamide derivatives by the reaction of *p*-toluene sulfonyl chloride with amino acids as precursors.[12]

We already had reported the anti-diabetic activity of some novel thiohydantoins and hydantoins starting from tosyl amino acids.[27] Based on the literature findings and our current interest in the exploration of metal complexes as possible new remedies for several targets, we have modified our procedure to prepare the tosyl sulfonamide incorporated Zn(II) complexes. We have also demonstrated the biological potential of these complexes as carbonic anhydrase inhibitors, cytotoxic and antileishmanial agents.

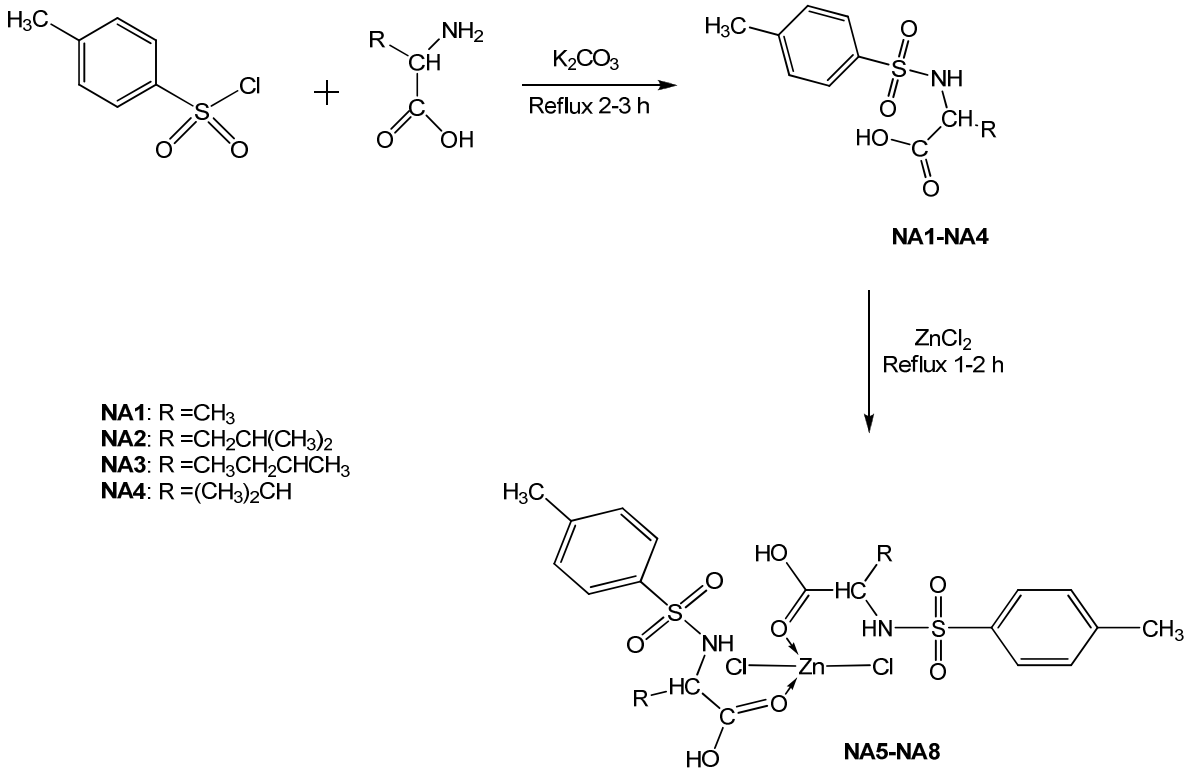
Results and Discussion

Chemistry

The general pathway leading to the synthesis of Zn(II) complexes (**NA5-NA8**) is given in Scheme 1. A series of tosyl sulfonamide containing amino acid moieties (**NA1–NA4**) were synthesized by a simple and facile condensation reaction of L-amino acids with *p*-toluene sulfonyl chloride (dissolved in 1,4-dioxane) using distilled water in the presence of catalytic amount of potassium carbonate.[27] The spectroscopic data obtained for the ligands was consistent

with the literature reports.[27] The ligands (**NA1-NA4**), and the Zn(II) chloride were dissolved in methanol to afford the desired metal complexes.[21]

The structures of the newly synthesized metal complexes were established by using spectroscopic techniques including GC-MS, IR, ^{1}H and ^{13}C-NMR spectroscopy. Single crystal X-ray diffraction analysis of **NA3** further confirmed the structural elucidation of the synthesized ligands. The structural data and refinement parameters are given in Table S1, whereas, a thermal ellipsoid plot at 50% probability level is presented in Figure 1.



Scheme 1: Synthesis of tosyl sulfonamides and Zn(II) complexes

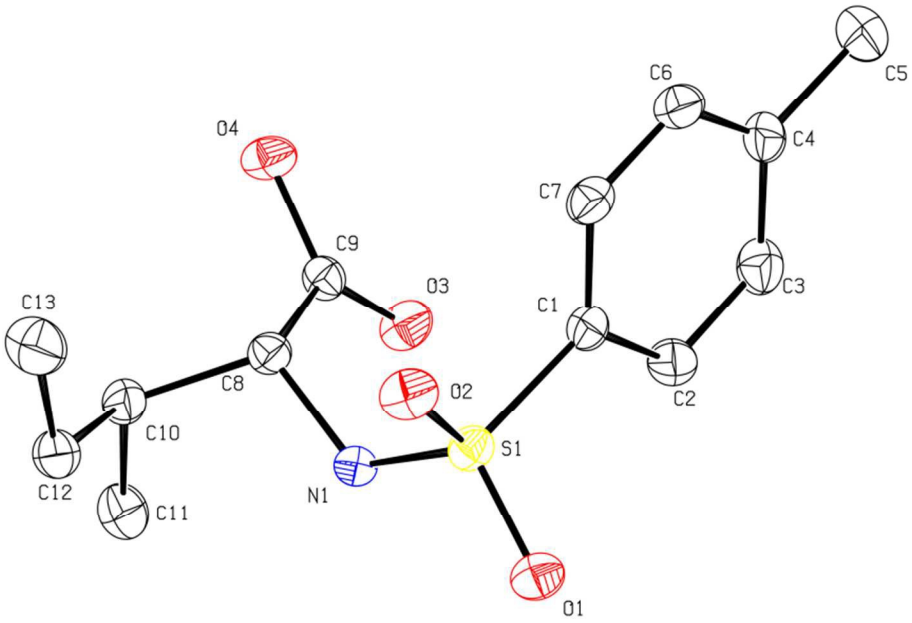


Figure 1: ORTEP diagram for compound **NA3**. Thermal ellipsoids are shown at the 50% probability level. Hydrogens are omitted for clarity.

The IR spectra have shown a strong band in the region 3475-3279 cm^{-1}confirming the presence of (OH) groups in the synthesized compounds (**NA1-NA4**). The spectra were recorded in the range of 4000-400 cm^{-1}. The characteristic vibrational frequencies were identified by comparing the spectra of the complexes with their precursors. In synthesized complexes (**NA5-NA8**), the slight shifting of NH bands further confirms the presence of metal group. In case of ligands, stretching bands of SO_2 were observed in the region of 1300-1190 cm^{-1}, while for complexes these peaks were shifted to slightly lower frequency.

^{1}H-NMR data confirmed the synthesis of newly synthesized compounds (**NA1-NA8**). The appearance of a singlet in the range of 5.1-4.91 ppm for –NH protons indicated that (NH) is not deprotonated (**NA5-NA8**). All the aromatic protons showed signals in the range of 7.78-7.41 ppm in case of compounds (**NA1-NA4**) which were slightly shielded with 7.68-7.38 ppm in case of complexes (**NA5-NA8**), indicates the attachment of metal group. It is noteworthy adding here that all the remaining signals in ^{1}H-NMR spectra agreed within the acceptable range.

The formation of ligands and their complexes is further supported by the ^{13}C-NMR data. The downfield shifting of (COO) carbon signal (C9) in the range of 172.50-168.95 ppm in ligands

(**NA1-NA4**) to 177.10-174.34 ppm in case of complexes (**NA5-NA8**).[12] All the remaining ^{13}C-NMR signals are within acceptable range. The results of elemental analysis of the newly synthesized ligands and their complexes are well complied and are within acceptable range.

Pharmacology

Carbonic anhydrase Activity

All the synthesized derivatives and their Zn(II) complexes (**NA1-NA8**) were tested for their carbonic anhydrase (CA) inhibitory efficacy against bovine CA II (bCA-II) isozyme. The inhibition data for this study is given in Table 1. Carbonic anhydrase inhibitory activities of these compounds were investigated against the standard clinically used inhibitor acetazolamide (IC_{50} = 0.96 ± 0.18 μM). The results of this study indicated that all the Zn(II) complexes were generally more active as compared to their corresponding ligands, whereas, significantly potent when compared with the standard inhibitor. The most potent compounds in the tested series were **NA5** and **NA6** representing IC_{50} values of 0.64 ± 0.07 and 0.58 ± 0.09 μM, respectively. These compounds exhibited inhibitory potency more than acetazolamide, when tested at the same concentration. Their SAR analysis revealed that these derivatives incorporate a methyl and isobutyl substituents. The replacement of these substituents with another aliphatic chain and an isopropyl group also produced good carbonic anhydrase inhibition as depicted by compounds **NA7** and **NA8**. Rest of the compounds also showed significant inhibition against carbonic anhydrase. Overall, the Zn(II) complexes were more potent as compared to the their corresponding ligands.

Table 1 Carbonic anhydrase inhibition of compounds (**NA1-NA8)**

Compounds	IC_{50} ± SEM (μM)
NA1	5.34 ± 0.11
NA2	3.73 ± 0.50
NA3	2.81 ± 0.26
NA4	1.22 ± 0.32
NA5	0.64 ± 0.07
NA6	0.58 ± 0.09
NA7	1.28 ± 0.21
NA8	1.49 ± 0.01
Acetazolamide	0.96 ± 0.18

Anticancer activity

The anticancer activity of tosyl sulfonamides and their Zn(II) complexes against lung carcinoma (H-157) cancer cell lines, kidney fibroblast (BHK-21) cell lines and vero cell lines was also evaluated. Vincristine was used as a standard anticancer agent. The results of this study are summarized in Table 2. The results depicted that all the tested compounds were significantly active against H-157 and BHK-21 cancer cells whereas exhibited low cytotoxicity at Vero cells. Vero cells are normal epithelial kidney cells extracted from African green monkey and used here as control to determine the safety. The results of the anticancer assay and SAR analysis revealed that all the compounds tested against Lung carcinoma (H-157) and kidney fibroblast (BHK-21) exhibited an effective cytotoxic behavior with significant IC_{50} values against both cancer cell lines, which extend advancements for the progress of safe and coherent anticancer agents. Generally, the metal complexes were significantly more active as compared to their corresponding tosyl sulfonamide ligands. These derivatives also showed relatively low toxicity against vero cells which could be a positive aspect of this study in the design of more active and safer analogues. These compounds may prove to be a good choice for treatment of cancer after *in vivo* and other clinical studies.

Table 2 Anticancer activity of synthesized compounds (**NA1-NA8**) against H-157, BHK-21 and Vero cell lines

Compounds	H-157	BHK-21	Vero cell lines
	IC_{50} ± SEM (µM)		% inhibition
NA1	4.59 ± 0.27	6.21 ± 0.30	15.48 ± 3.5
NA2	7.74 ± 0.17	5.11 ± 0.12	13.6 ± 2.9
NA3	9.49 ± 0.18	7.64 ± 0.16	17.9 ± 3.4
NA4	5.36 ± 0.14	8.29 ± 0.13	14.1 ± 2.3
NA5	1.82 ± 0.11	2.19 ± 0.15	13.7 ± 1.8
NA6	2.48 ± 0.13	3.01 ± 1.43	21.2 ± 4.6
NA7	3.17 ± 0.28	2.93 ± 0.17	11.7 ± 2.1
NA8	1.97 ± 0.17	2.74 ± 0.12	23.6 ± 1.9
Vincristine	1.08 ± 0.09	1.08 ± 0.09	11.7 ± 1.1

Antileishmanial activity

The antileishmanial activity of the synthetic derivatives (**NA1-NA8**) was measured by MTT method and IC_{50} values of all the compounds are reported in Table 3. Amphotericin B was used as a standard drug for anti-leishmanial activity. The results of this research showed the antileishmanial potential of tosyl sulfonamide derivatives and their complexes against *Leishmania major in vitro*. All the compounds screened in the series exhibited significant IC_{50} values. Among them, **NA6** showed significant antileishmanial potential with an IC_{50} value of 0.32 ± 0.08 µM which is comparable to the standard inhibitor Amphotericin B (IC_{50} = 0.29 ± 0.05 µM). This Zn(II) complex incorporates an aliphatic branched chain attached to amide functionality. The corresponding ligand for this metal complex was less active. Similarly,

compound **NA7** was also significantly active with an IC_{50} value of 0.97 ± 0.06 µM. Within the tested series, metal complexes were generally more active as compared to their corresponding tosyl sulfonamide ligands. These results indicated that these compounds may be used as therapeutic agents to treat leishmaniasis.

Table 3 Antileishmanial activity of the synthesized compounds (**NA1-NA8**)

Compounds	IC_{50} ± SEM (µM)
NA1	3.91 ± 0.25
NA2	5.73 ± 0.41
NA3	4.66 ± 0.16
NA4	8.59 ± 0.27
NA5	1.59 ± 0.39
NA6	0.32 ± 0.08
NA7	0.97 ± 0.06
NA8	1.82 ± 0.14
Amphotericin B	0.29 ± 0.05

Molecular Docking

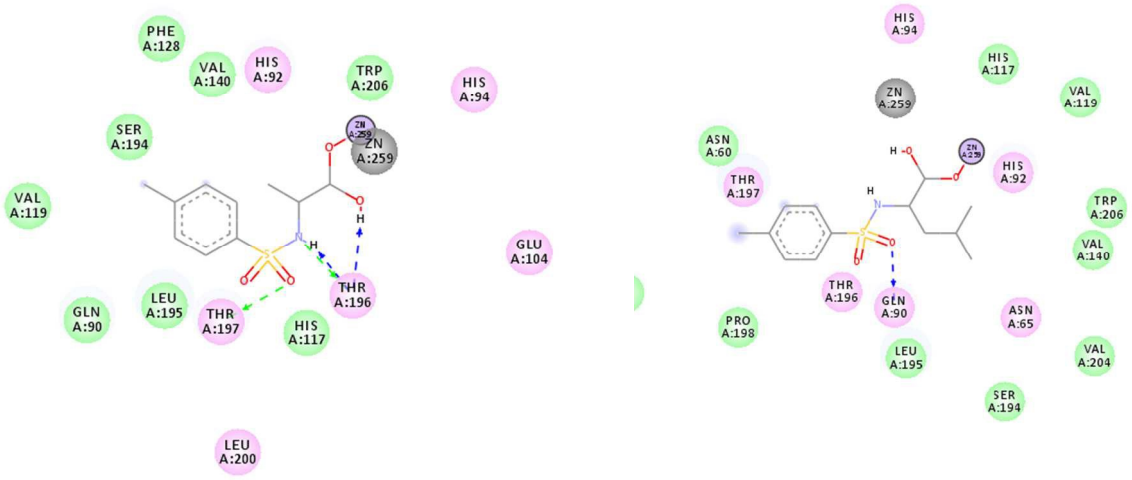
Molecular docking studies were carried out to find out the mode of binding and most favorable binding conformations of the inhibitors. Different binding conformations of same ligand differing by less than 2 Å rmsd are by default grouped together in one cluster. Careful inspection of clustering histogram (see supporting information), generated by AutoDock, provides a good *qualitative* assessment of the docking results, whereas the AutoDock generated binding free energy (ΔG) provides a good *quantitative* assessment of the docking results.[28,29] The calculated

binding free energy (docking score) for docked compounds along with their rank and sub rank (according to cluster profile) is given in Table 4.

Table 4 Binding free energy and ranking scores for **NA1-NA4**

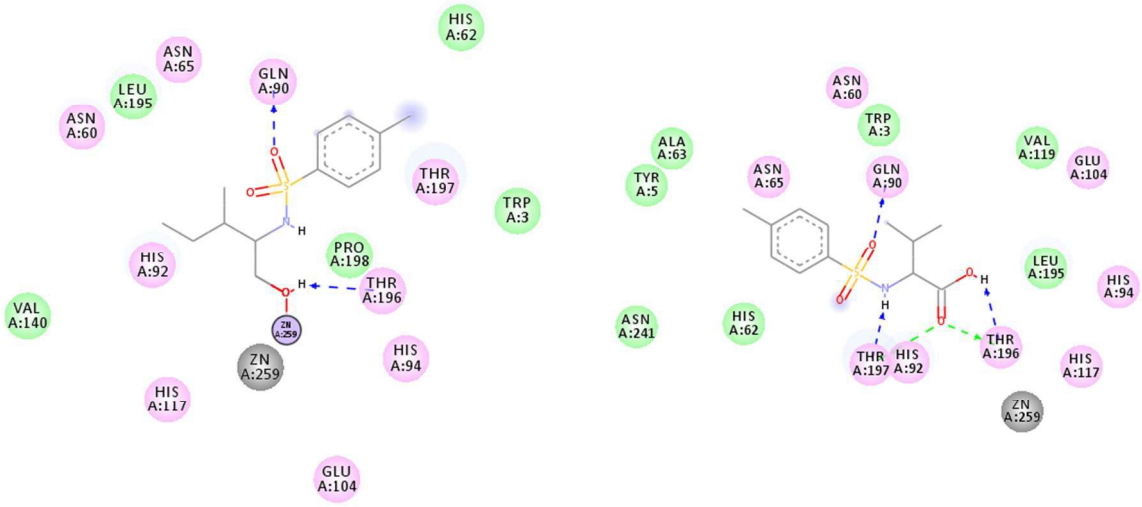
Compound	Rank Sub-Rank	Binding Free Energy ΔG (KJ/mol)
NA1	1-1	-10.29
NA2	1-2	-9.67
NA3	1-1	-10.0
NA4	1-1	-9.87

Similar mode of binding was found for all compounds reported in this study. Docking studies revealed that the sulfonamide group is not involved in making direct contact with the Zn metal ion, which is expected as the sulfonamide nitrogen atom is substituted. The most common binding mode found for most ligand conformations was one where the carboxylic acid group interacts with the positively charged zinc metal ion of the CA active site (Figures 2 and 3). The oxygen atom of the carboxylic acid group additionally makes a hydrogen bond contact with Thr196. In compounds **NA1** and **NA4** the sulfonamide nitrogen atom is involved in making hydrogen bonded contact with Thr196 and Thr197. For compounds **NA2** and **NA3** the sulfonamide oxygen atom makes a contact with amino acid residue Gln90. Ligand binding site interactions are given in Figures 2 and 3, where residues indicated with pink and green colored spheres represent electrostatic and van der Waals contacts respectively, hydrogen bond interactions are indicated with dashed lines. The docking scores are in good agreement with the experimentally determined inhibition values. Figure 4 showed **NA3**, the most active compound in the series, bound inside the active site of CAII enzyme.



(a) **(b)**

Figure 2. 2D interaction diagram; **a**) compound **NA1**; **b**) compound **NA2**



(a) **(b)**

Figure 3. 2D interaction diagram; **a**) compound **NA3**; **b**) compound **NA4**

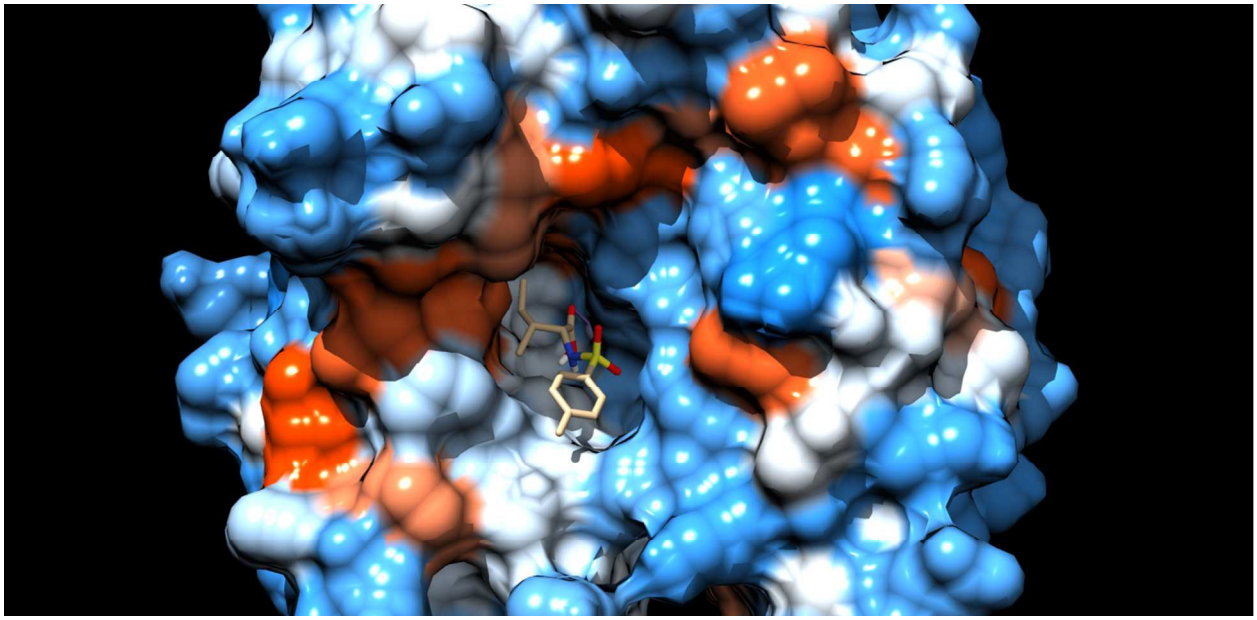


Figure 4. Compound **NA3** bound inside the active site of the enzyme. Blue colored surface indicate hydrophilic (polar) surface, white indicates neutral surface and orange is for hydrophobic or non-polar surface.

Experimental

All the chemicals used were of analytical grade and were purchased from Sigma Aldrich. Melting points were recorded using open capillary tubes on a digital Gallenkamp (SANYO) apparatus and were uncorrected. FTIR spectra were recorded using Bruker FTIR (4000-400 cm^{-1}), ^{1}H and ^{13}C-NMR spectra were recorded on Bruker AV400RG spectrophotometer using $CDCl_3$, CD_3CN and DMSO as solvents. Elemental analysis was performed on a LECO-183 CHNS analyzer. Mass spectra were recorded by Agilent GCMS.

General procedure for the synthesis of ligands (NA1-NA4)

The ligands **NA1-NA4** were prepared by using a literature reported method with slight modifications.[27] L- Amino acid (1 equiv, 2 mmol) and potassium carbonate (0.5 equiv, 1 mmol) were dissolved in distilled water (10 mL) with continuous stirring. Solution of *p*-toluene sulfonyl chloride (1.02 equiv, 2.02 mmol) in freshly distilled 1,4-dioxane (8 mL) was added to the reaction mixture. Resultant mixture was refluxed for 2.5 to 3.5 h and then cooled to room

temperature. The solution was acidified (pH 1-2) with hydrochloric acid (2N). Precipitates were filtered, washed with distilled water and recrystallized from ethanol:water (2:0.5) by slow evaporation. The data obtained for the ligands (**NA1-NA4**) was consistent with the literature reports.[27]

General procedure for the synthesis of Zn(II) complexes

A schlenk flask was charged under glove box with the corresponding ligand (**NA1-NA4**) (1.0 equiv, 2 mmol) in methanol (10 mL). Zinc chloride (0.6 equiv, 1.2 mmol) was dissolved separately in methanol (10 mL) and added to ligand solution and stirred for 2 h at 60 ^{o}C. The reaction mixture was cooled to room temperature which precipitated after 5-12 h. The solvent was evaporated under vacuum in schlenk line, filtered, washed with cold ethanol and recrystallized in chloroform-hexane (1:1) mixture.[30]

Zn(II) complex of 2-(4-methylphenylsulfonamido)propanoic acid (NA5)

Yield 70%; colorless crystalline powder; m.p. 130 ^{o}C; IR (ATR, cm^{-1}): 3415 (OH), 3242 (N-H), 1656 (C=O), 1596, 1467 (aromatic C=C), 1303 (SO_2); ^{1}H-NMR (400 MHz, CD_3CN): δ (ppm) 7.76 (d, *J* = 8.0 Hz, 4H, Ar-H), 7.42 (d, *J* = 8.0 Hz, 4H, Ar-H), 7.41 (s, 2H, OH), 5.93 (s, 2H, NH), 3.62 (q, *J* = 5.2 Hz, 2H, CH), 2.41 (s, 6H, Ar-CH_3), 1.38-1.32 (m, 6H, $CHCH_3$); ^{13}C-NMR (CD_3CN): δ (ppm) 177.10, 138.31, 135.29, 129.10, 125.01, 47.50, 21.8, 18.25; GCMS, m/z (%): Major peak, 622 $(M+H)^+$; Elemental analysis: $C_{20}H_{26}Cl_2N_2O_8S_2Zn$; Calculated (%); C 38.57; H, 4.21; N, 4.50. Found (%); C, 38.77; H, 3.99; N, 4.72.

Zn(II) complex of 4-methyl-2-(4-methylphenylsulfonamido)pentanoic acid (NA6)

Yield 83%; colorless solid; m.p. 115-121 ^{o}C; IR (ATR, cm^{-1}): 1670 (C=O), 3277 (N-H), 1545, 1473 (aromatic C=C), 1260 (SO_2), 538 (M-N), 446 (M-O); ^{1}H-NMR (400 MHz, CD_3CN): δ (ppm) 7.77 (d, *J* = 8.02 Hz, 4H, Ar-H), 7.40 (d, *J* = 8.0 Hz, 4H, Ar-H), 7.31 (s, 2H, OH), 5.92 (s, 2H, NH), 4.43-4.38 (m, 4H, CH_2), 2.41 (s, 6H, CH_3), 1.93–1.78 (m, 2H, CH), 1.45-1.41 (m, 2H, CH), 0.9 (s, 12H, CH_3); ^{13}C-NMR (CD_3CN): δ (ppm) 176.92, 138.74, 134.90, 129.55, 124.88, 49.21, 31.65, 26.57, 22.16, 21.8; GCMS, m/z (%): Major peak, 705 $(M+H)^+$; Elemental analysis for $C_{26}H_{38}Cl_2N_2O_8S_2Zn$: Calculated (%); C, 44.17; H, 5.42; N, 3.96. Found (%); C, 43.98; H, 5.56; N, 4.17.

Zn(II) complex of 3-methyl-2-(4-methylphenylsulfonamido)pentanoic acid (NA7)

Yield 79%; colorless crystalline powder; m.p. 150-155 ^{o}C; IR (ATR, cm^{-1}): 3336 (OH), 3297 (N-H), 1696 (C=O), 1597, 1496 (aromatic C=C), 1336 (SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 7.79 (s, 2H, OH), 7.77 (d, *J* = 8.0 Hz, 4H, Ar-H), 7.42 (d, *J* = 8.0 Hz, 4H, Ar-H), 5.51 (s, 2H, NH), 2.41 (s, 6H, CH_3), 1.91–1.78 (m, 2H, $CHCH_2$), 1.62 (d, *J* = 6.8 Hz, 6H, CH_3), 1.54-1.46 (m, 8H, CH_2), 1.42 (d, *J* = 6.8 Hz, 6H, CH_3), 0.90 (t, *J* = 6.7 Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$): δ (ppm) 176.43, 141.29, 137.30, 129.50, 127.47, 51.28, 30.81, 27.92, 25.44, 21.8, 18.25, 16.53; GCMS, m/z (%): Major peak, 733 $(M+H)^+$; Elemental analysis: $C_{28}H_{42}N_2Cl_2O_8S_2Zn$; Calculated (%); C, 45.75; H 5.76; N, 3.81. Found (%); C, 45.32; H, 5.38; N, 3.50.

Zn(II) complex of 3-methyl-2-(4-methylphenylsulfonamido)butanoic acid (NA8)

Yield 81%; colorless crystalline solid; m.p. 170-175 ^{o}C; IR (ATR, cm^{-1}): 3472 (OH), 3286 (N-H), 1619 (C=O), 1597, 1463 (aromatic C=C), 1329 (SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 7.75 (s, 2H, OH), 7.72 (d, *J* = 8.0 Hz, 4H, Ar-H), 7.28 (d, *J* = 8.0 Hz, 4H, Ar-H), 5.13 (s, 2H, NH), 3.83-3.80 (m, 2H, CH), 2.43 (s, 6H, CH_3), 2.13–2.12 (m, 2H, CH), 1.29 (d, *J* = 4.6 Hz, 12H, CH_3); ^{13}C-NMR ($CDCl_3$): δ (ppm) 174.34, 143.50, 129.31, 128.3, 126.20, 60.20, 31.27, 21.20, 18.66; GCMS, m/z (%): Major peak, 677 $(M+H)^+$; Elemental analysis: $C_{24}H_{34}N_2Cl_2O_8S_2Zn$; Calculated (%); C, 42.45; H, 5.05; N, 4.13. Found (%); C, 42.29; H, 4.90; N, 3.99.

Biological Protocols

***In vitro* carbonic anhydrase inhibition**

A series of tosyl sulfonamide derivatives and their complexes were screened for their inhibitory activity against carbonic anhydrase (CA). The activity was measured by determining the amount of *p*-nitrophenol formed during the hydrolysis of *p*-nitrophenyl acetate by using standard protocol.[31,32] The compounds were initially screened at 1 μM concentration. Compounds which exhibited >50% inhibition were further diluted at 8-10 concentrations. Results of the active compounds in the form of IC_{50} values are presented in the Table 1.

Anticancer activity

Cell lines and cell cultures

Lung carcinoma (H-157), (ATCC CRL-5802), kidney fibroblast (BHK-21), (ATCC CCL-10) and African green monkey kidney normal cell line (Vero), (ATCC CCL-81) were kept in RPMI-1640, having heat-inactivated fetal bovine serum (10%) glutamine (2 mM), Pyruvate (1 mM), 100 U/mL penicillin and 100 µg/mL streptomycin, in T-75 cm^2 sterile tissue culture flasks in a 5% CO_2 incubator at 37 °C.[33] For experiment, 96-well plates were used for growing H-157, BHK-21 and Vero cells by inoculating 5×10^4 cells per 100 µL per well and plates were incubated at 37 °C for 24 h in a humidified atmosphere containing 5% CO_2. Within 24 h, a uniform monolayer was formed which was used for experiments.

Cytotoxicity analysis by sulforhodamine B (SRB) assay

To perform cytotoxicity assay, with H-157, BHK-21 and Vero cells, a previously described method by Skehan *et al*.,[34] was adopted with minor modifications. Briefly, cells were cultured in different 96 well plates for 24 h. The compounds in different concentrations were inoculated in test wells. Furthermore, positive control vincristine was prepared in DMSO. The well containing culture media with cells having no compound or drug was taken as blank. Vero cells were treated at 100 µM test compounds to check the toxicity against normal cell lines. The plates were then incubated for 48h. After that, cells were fixed with 50 µL of 50% ice cold trichloroacetic acid solution (TCA) and plates were incubated at 4 °C for 1 h. Subsequently, plates were washed five times with phosphate-buffered saline (PBS) and air dried. Fixed cells were further treated with 0.4% w/v sulforhodamine B dye (prepared in 1% acetic acid solution) and left at room temperature for 30 min. After that the plates were rinsed with 1% acetic acid solution and allowed to dry. In order to solubilize the dye, the dried plates were treated with 10 mM Tris base solution for 10 min at room temperature. The absorbance was measured at 490 nm subtracting the background measurement at 630 nm.[35] All experiments were repeated at least three times. Results reported are the mean value of three independent experiments (± SEM). IC_{50} values of potential inhibitors (≥50%) were determined with the help of the Graph Pad prism 5.0 Software Inc., San Diego, California, USA.

Antileishmanial activity

Parasite and culture

Leishmania major promastigotes were cultured at 25 ± 1 °C to logarithmic phase in D-MEM/F-12 medium (Gibco BRL) without phenol red, supplemented by 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin and 100 µg/mL streptomycin, then washed 3 times with phosphate-buffered saline (PBS). The cells were centrifugated at 1500 rpm, for 10 min at room temperature and re-suspended at a concentration of 2.5×10^6 parasites/mL in medium.

Antileishmanial activity assay (MTT assay)

In vitro antileishmanial activity of the compounds was evaluated against the promastigote forms of *Leishmania major* using a MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide). The MTT assay used was originally described by Mosmann (1983)[36] and later on modified by Niks and Otto (1990).[37] A stock solution of MTT (Sigma Chemical Co., St. Louis, Mo.) was prepared by dissolving in PBS at 5 mg/mL and storing in the dark at 4 °C for up to 2 weeks before use. For the antileishmanial activity assay, 100 µL/well of the culture which contained 2.5×10^6 cells/mL promastigotes was seeded in 96-well flat-bottom plates. Then, 10 µL/well from various concentrations of compounds were added to triplicate wells and plates were incubated for 72 h at 25 ± 1°C. The well containing only 100 µL culture medium without any compound, drug or parasite, was taken as blank. Amphotericin B was used as a standard drug. At the end of incubation, 10 µL of MTT was added to each well and plates were again incubated for 3 h at 25 ± 1 °C. Enzyme reaction was then stopped by the addition of 100 µL of 50% isopropanol and 10% sodium dodecyl sulfate (0.1 N HCl). The plates were incubated for an additional 30 min under agitation at room temperature. Relative optical density (OD) was then measured at a wavelength of 570 nm using a 96-well microplate reader (Bio-Tek ELx 800™, Instruments, Inc. Winooski, VT, USA). The background absorbance of plates was measured at 690 nm and subtracted from 570 nm measurement. The absorbance of the formazan produced by the action of mitochondrial dehydrogenases of metabolically active cells was correlated with the number of viable cells.[36,37] All experiments were repeated at least three times. Results reported were the mean of three independent experiments (± SEM). IC_{50} values of potential inhibitors (≥50%) were determined with the help of the Graph Pad prism 5.0 Software Inc., San Diego, California, USA.

Molecular Docking

High resolution crystal structures of hCA II (PDB ID: 3K34, 0.9 Å resolution) and bCA II (PDB ID: 1V9E) were downloaded from the RCSB protein data bank. We have previously reported the structural comparison and percent similarity (87.3%) and percent identity (80%) between these two enzymes. The RMSD with respect to the active site residues of the two proteins was found to be 0.22Å.[38] The validity of the docking methodology was assessed by re-docking the bound ligand extracted from the active site of hCA II (3K34). The docking methodology successfully reproduced the experimentally bound ligand conformation with an rmsd of 0.65. Method validation could not be carried out for bCA II, since it did not crystallize with any ligand. On this basis hCA II was selected to carry out molecular docking studies. The structures of all ligands used for docking were drawn using ACD/ChemSketch.[39] Gasteiger charges were added on each ligand using ANTECHAMBER[40] and the energy of each molecule was minimized through 100 steepest descents and 100 conjugate gradient steps using a step size of 0.02 each using Chimera.[41] AutoDock 4.2 was used to carry out the docking studies.[42] For visualization of docked results Discovery Studio Visualizer 3.5 was used.[43]

The enzyme hCA II was prepared for docking using DockPrep utility of Chimera, this includes the standard preparation steps such as adding hydrogen atoms, adding charges (using ANTECHAMBER utility incorporated in Chimera), deleting all hetero and solvent molecules. Charge of +2 was added on the zinc metal of the CA active site. The appropriate files (.pdbqt) for docking of both receptor (hCA II) and ligand were generated using ADT.[42] AutoGrid was used to calculate the affinity maps; each point on the grid has pre-calculated affinity potentials for all atom types of the ligand. By using these grid maps AutoDock allows ligand to be flexible, whereas the enzyme is held fixed during docking. Grid box had dimensions 60x90x60 with a resolution of 0.375 Å. The grid box was centered at the active site of the enzyme and was large enough to allow free movement of the ligand. Lamarckian Genetic Algorithm (LGA) was used as docking search parameter. Genetic algorithms apply multiple cycles of genetic operators (mutation and crossover, for this study, set at rate of 0.02 and 0.8 respectively) to a population of initially randomly placed ligand conformations giving rise to new generations with improved "*solution*" that is, binding free energy.[44] Maximum number of evaluations was 5000000 and number of docking generations was 27000, GA runs was 20.

X-ray structure determination

Single crystal was obtained directly from isolation of the product as described above. Diffraction data were collected with Cu K radiation on a Bruker APEXII with a Cu microsource/Quazar MX optics using the APEX2 software package.[45] Data reduction was performed with SAINT,[46] absorption corrections with SADABS.[47] Structures were solved with direct methods (SHELXS97).[48] All non-hydrogen atoms were refined anisotropic using full-matrix least-squares on *F*2 and hydrogen atoms refined with fixed isotropic U using a riding model (SHELXL97).[48]

Conclusion

In summary, the newly synthesized Zn(II) complexes of sulfonamide derivatives were observed to be more active against carbonic anhydrase than their parent ligands. Most of the investigated compounds exhibited excellent CA inhibitory activity in the lower micromolar range. **NA5** and **NA6** were most potent inhibitors having IC_{50} values of 0.64 ± 0.07 and 0.58 ± 0.09 μM, respectively. Furthermore, these finding suggest that novel Zn(II) complexes of sulfonamide derivatives may be used for further investigation on a wide range of CA isozymes which are important targets in drug development. Due to synthetic and biological versatility of tested compounds, they may be used as therapeutic agents having antiproliferative and antileishmanial activities.

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Conflict of interest

The authors have declared no conflict of interest.

Supporting Information

Complete details of the X-ray analysis for compound **NA3** have been deposited at the Cambridge Crystallographic Data Centre (CCDC) and can be retrieved with the following reference number:

981321. These data can be obtained free of charge via www.ccdc.cam.ac.uk/ data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, U.K. (Fax: +44 1223 336033).

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