POM Analyses of Carbacylamidophosphates and Sulfanylamidophosphates Tested as New Carbonic Anhydrase Inhibitors

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Abstract: Six representatives of the amidophosphate derivatives (**L**^{1}-**L**^{6}) were synthesized and evaluated for their biological activities against carbonic anhydrase enzyme. Out of the six derivatives, **L**^{1} (IC_{50} = 12.5 ± 1.35 μM), and **L**^{2} (IC_{50} = 3.12 ± 0.45 μM) showed potent activity against BCA-II, whereas (**L**^{3}, **L**^{4} and **L**^{5}) showed weak inhibitory activity with the IC_{50}

value of 24.5 ± 2.25, 24.5 ± 2.25, 55.5± 1.60, and 75.5 ± 1.25 μM respectively. They were found to be weak inhibitors of carbonic anhydrase as compared to acetazolamide (IC_{50} =0.12 ± 0.03 μM) which was used as standard inhibitor. All physic-chemical parameters were derived from the computational Petra/Osiris/Molinspiration/DFT (POM/DFT) model. They govern the bioactivity amidophosphate derivatives (**L^1-L^6**) which contain O,O-pharmacophore site. The six compounds (**L^1-L^6**) analyzed here were previously screened experimentally and now screened virtually for their anti-carbonic anhydrase activity. The highest anti-carbonic anhydrase activity was obtained for compound **L^2** which exhibited excellent bioactivity (% inhibition = 95%) when compared to acetazolamide (% inhibition = 89%). The compound **L^3** represents an increased activity as compared to its analogues (**L^4-L^6**). The increase of bioactivity from **L^3** to **L^4-L^6** could be attributed to the existence of minimum steric effect of substituents of P=O moiety which plays a crucial template role in the organization of anti-carbonic anhydrase O,O-phramacophore sites. Moreover, it is cheap, leading to fewer side effects and is possible to be included in selective anti-carbonic anhydrase agents design.

***Keywords*:** Amidophosphate derivatives . Carbonic anhydrase . Acetazolamide . Docking, Petra/Osiris/Molinspiration (POM) analyses . Pharmacophore site identification.

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1. NTRODUCTION

Carbonic anhydrase (CAs, EC 4.2.1.1) belongs to a group of zinc containing enzymes that found in vertebrates, eubacteria, and algae as well as in plants. Its function is to facilitate the physiological process *i.e.* interconversion of CO_2 to HCO_3^- by hydration. Carbonic anhydrase enzyme plays a vital role in various physiological processes like respiration and transfer of CO_2/HCO^-_3 between metabolizing tissue and lungs, to control pH as well as regulate inner environment of CO_2 and secretion of electrolytes. Furthermore, it is related with many other pathological or physiological processes for instance calcification, bone resorption and tumorigenicity. Carbonic anhydrase inhibitory properties are related with glaucoma, thyroid and some types of cancers [1, 2]. Carbonic anhydrase is substantial in diverse biosynthetic reactions such as lipogenesis, gluconeogenesis, and ureagenesis [3].

The chemistry of carbacylamidophosphates (CAPh)– compounds having functional fragment -C(O)N(H)P(=O) and sulfanylamidophosphates (SAPh) – compounds containing -$S(O)_2$N(H)P(=O) fragment was elaborated in the 1960s. Nowadays, the so-called phosphazo-reaction [4] is an extensively used technique in modern phosphororganic chemistry. These diverse biological activities could be attributed to the existance of peptide group as in CAPh or sulfonylamide group which is part of SAPh and phosphoryl groups combined together in one molecule. Many compounds of this type are already well-known as drugs, insecticides, acaricides, fungicides, herbicides, growth regulator for plants, defoliants *ect* [5-10].

Taking into account the wide spectrum of the biological activity revealed for CAPh and SAPh compounds, we decide to resynthesize a several representatives of CAPh and SAPh compounds [11] and to test their biological activities for the first time against carbonic anhydrase enzyme. This choice was supported by Petra/Osiris/Molinspiration (POM) calculations [similarity of O,O-pharmacophore sites of ligands (**L^1-L^6**) and O,N-pharmacophore sites of standard drug] (Figure 1).

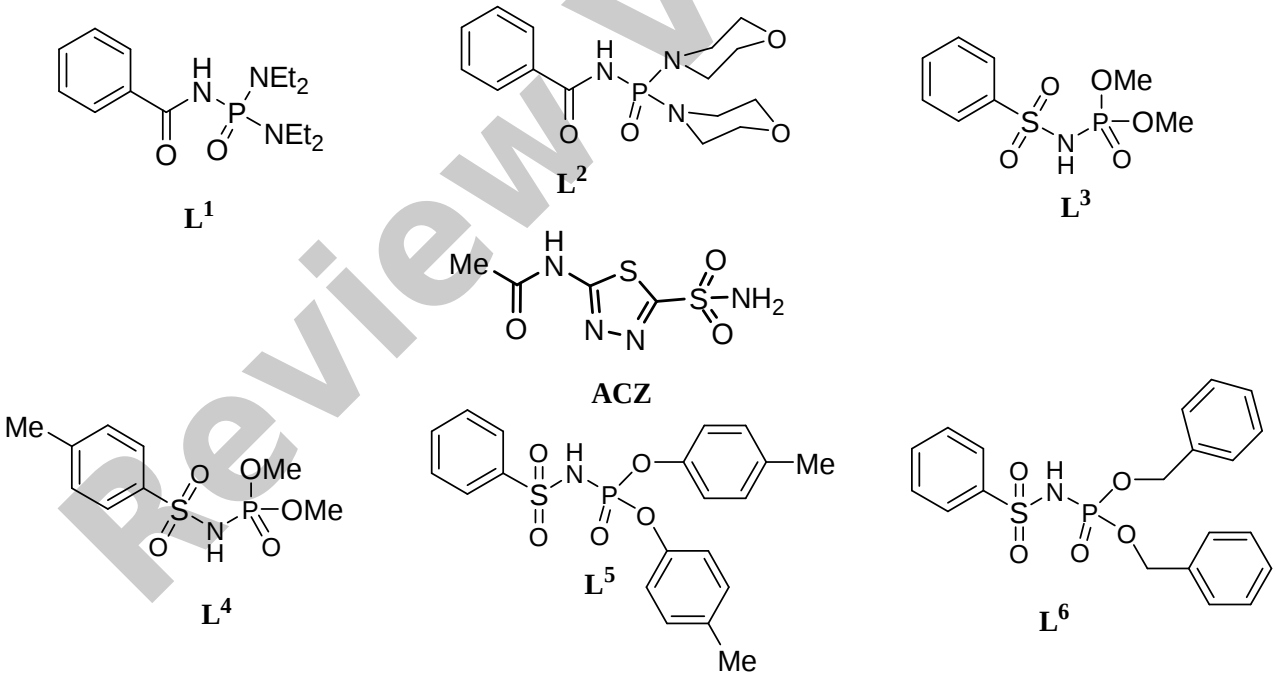


Figure-1. Molecular structure of compounds (**L^1-L^6**) and standard drug (ACZ = acetazolamide) studied.

2. RESULTS AND DISCUSSION

2.1. Synthesis of compounds (**L^1-L^6**)

A series of amidophosphate derivatives (**L^1-L^6**) were synthesized according to the schemes described in [11]. The first synthetic step for all compounds under consideration was the phosphoro–azo reaction in which the amide of corresponding organic acid interaction with phosphorus pentachloride [12]. All the initial compounds for the syntheses of amides of aromatic or sulfonic acids, amines, alcohols are commercially available. It should also be noted that the reactions by which the investigated compounds are generally characterized give by fairly high yields.

2.2. Pharmacology

Evaluation the biological activities of all derivatives [11] against bovine carbonic anhydrase.were carried out. Two compounds **L^1** and **L^2** showed potential excellent inhibition which is comparable to the standard inhibitor (acetazolamide). Compounds **L^1** and **L^2** have shown potent activity with IC_{50} value of 12.5 ± 1.35 and 3.12 ± 0.45 µM respectively. The reason for potential excellent inhbition is the presence of lone pair donor atoms such as nitrogen and oxygen. Those two have the tendency to make hydrogen bonding with the active site as well as with the allosteric site of the enzyme. However, compounds **L^3**, **L^4** and **L^5** showed very weak inhibitory activity with IC_{50} value of 24.5 ± 2.25, 55.5± 1.60, and 75.5 ± 1.25 µM respectively. Comparing the activity of these compounds, the presence of -POMe group makes the compound less active than the presence of NEt group. The introduction of aromatic system with PO group results in the decrease of the activity, whereas the inhbitory activity in compound **L^6** vanished due to the presence of the aromatic system. The aromaticity can cause steric repulsion with the active site residue of amino acide of the enzyme. The results of the potential inhbition of these compoumds are shown in Table-1.

Table-1: Inhibitory Activity of urea derivatives (**L^1-L^6**) against BCA-II.

Compound	% Inhibition	IC_{50} ± SEM[a] [µM]
L^1	89	12.5 ± 1.35
L^2	95	3.12 ± 0.45
L^3	78	24.5 ± 2.25

L^4	70	55.5± 1.60
L^5	62	75.5 ± 1.25
L^6	30	---
ACZ [b]	89	0.12 ± 0.03

[a] SEM = standard error of mean, [b] ACZ = Acetazolamide.

2.3. Molecular docking

The docking studies in general showed that compounds which give lesser docking score have good activities. It can be concluded that there are special features in the compounds which are involved in the inhibitory activities of CA-II enzyme.

The docking analysis of CA-II enzymes with compounds (**L^1-L^6**) is based upon the hydrogen bonding and hydrophobic interactions. As a general rule most of the effective compounds have the property of making hydrogen bonding and hydrophobic interactions with the enzyme binding pocket. If such a type of interacting capacity is present in the new compounds, it is in the favor of more enhancing biological activities against the targeted enzyme. The compounds (**L^1-L^6**) have proved the best docking results than the standard acetazolamide against the bCA-II.

The predicted docking poses of the compounds (**L^1-L^6**) and standard acetazolamide with a receptor bCA-II and their superimposition are presented in the Figure 2. The docking score of the compounds (**L^1-L^6**) is presented in **Table 1**. These interaction energies are lower than the docking result of the standard.

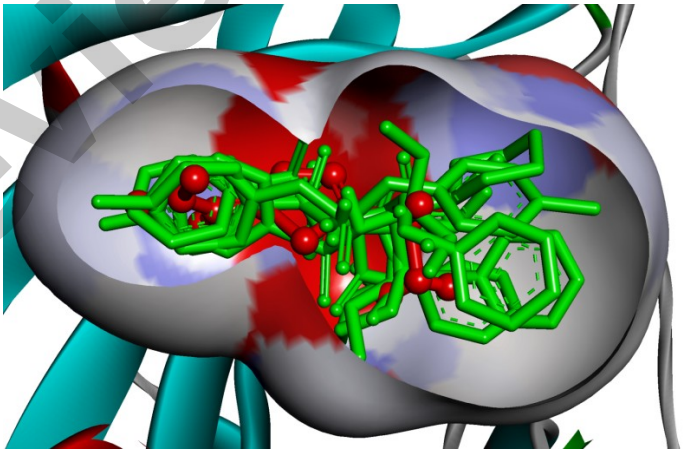


Figure-2. Docked poses and superimposition of compounds (**L^1-L^6**) (green color) and standard acetazolamide (red color) against the bCA-II.

The docking studies of compound **L**[1] with CA-II reveal that it forms one hydrogen bond interactions (Figure 3) with a Thr198 (3.15Å). The twelve hydrophobic contacts were observed from the surrounding binding residues such as His63, Asn66, Glu68, Val90, Gln91, His93, His95, Phe129, Leu196, Thr197, Pro199 and Pro200. These interactions are responsible for such low predicting interaction energies and best inhibition of the compound **L**[1] against bCA-II.

The interaction analysis of compound **L**[2] (Figure 4) revealed that it forms thirteen hydrophobic contacts with the active site of bCA-II. These contacts include, His2, Trp4, His63, Val90, Gln91, His93, His95, Phe129, Leu196, Thr197, Thr198, Pro199 and Pro200. In conclusion, these types of features in the compounds are responsible for the best mediating biological activities.

Table-2: Docking statistics of compounds (**L**[1]-**L**[6]) against the bCA-II enzyme.

Compd.	i-GEMDOCK score (kcal/mol)				Autodock vina score (kcal/mol)
	Total Energy	VDW	HBond	Elec	B. Affinity
L[1]	-72	-66	-6	0	-6.4
L[2]	-85	-76	-9	0	-6.6
L[3]	-65	-56	-9	0	-5.3
L[4]	-70	-68	-2	0	-5.5
L[5]	-62	-59	-3	0	-5.1
L[6]	-55	-53	-3	0	-4.4
ACZ[a]	-70	-54	-16	0	-6.1

[a] Standard drug (ACZ = acetazolamide).

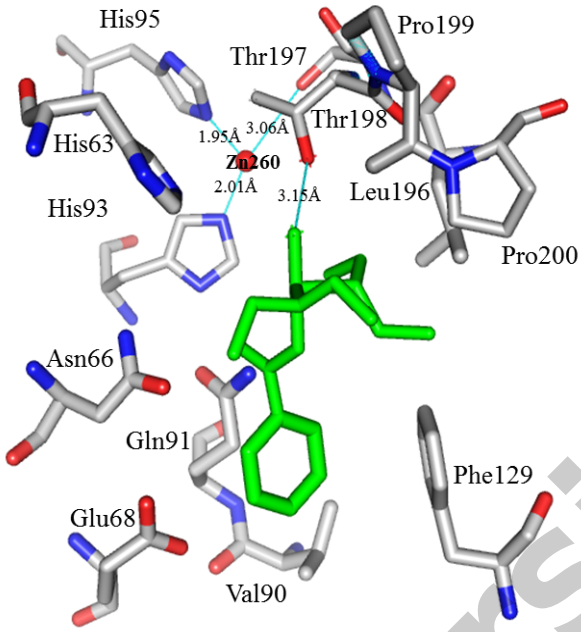


Figure-3. 3-D interaction profile of compound **L**[1] with the active site of bCA II.

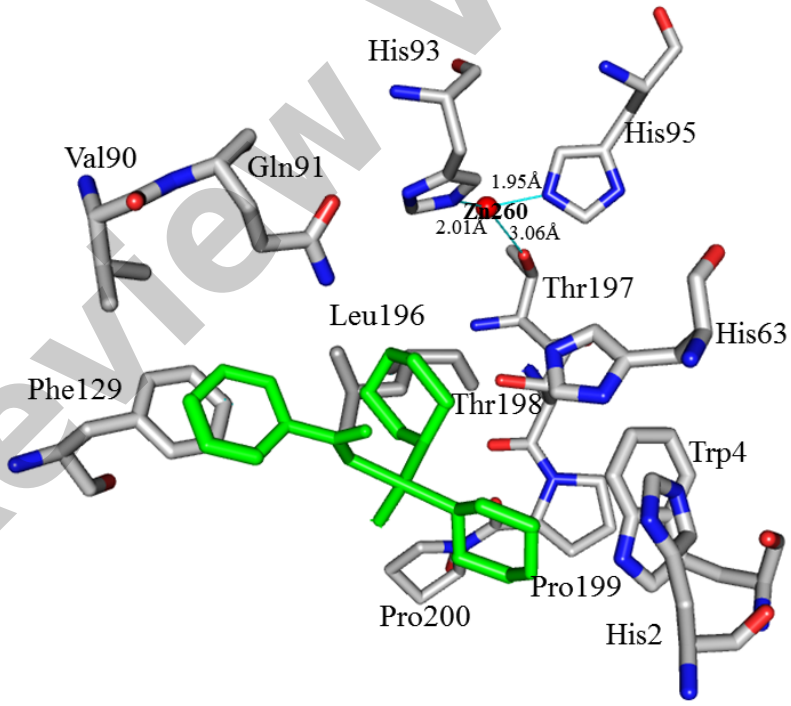
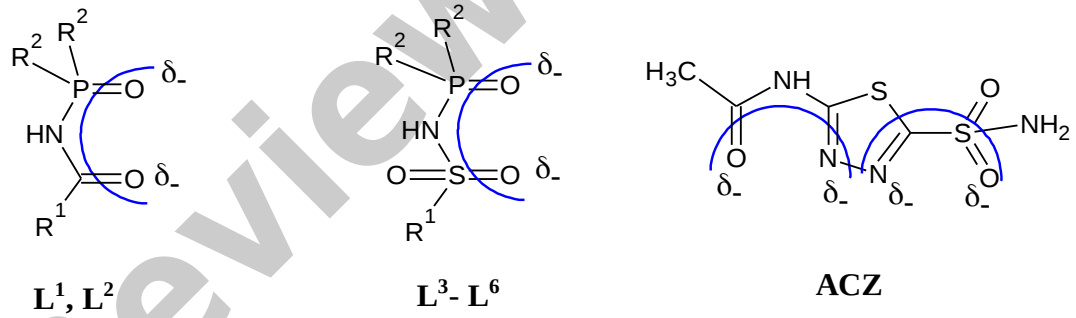


Figure-4. 3-D interaction profile of compound **L**[2] with the active site of bCA II.

2.4. POM analyses of compounds (L^1-L^6)

The analysis of the theoretical toxicity risks for the series (**L^1-L^6**) using the Osiris program showed that compound **L^6** and probably only **L^5** of the rest of the series (**L^1-L^6**) (Table-3) are less toxic than standard clinical drug acetazolamide (CCZ). It also showed that compound **L^6** can be used as antibiotics with some pharmacomodulation (% Inhib. = 30%). From the data depicated in Table-2, 3/6 the structures are assumed to be non-mutagenic when run through the mutagenicity estimation of the coordinated system. As far as irritating and reproductive effects are concerned, all the compounds (**L^3-L^6**) are at low risk comparable with the used standard drug used. The cLogP value has been used to express the hydrophilicity character of each compound. It has been assured that the permeation or absorption is extremely influenced by the hydrophilicity (value of cLogP). Accordingly, when cLogP is more than 5, the permeation or absorption decreases. On this basis, most of the compounds (**L^1-L^6**) have cLog*P* values within the acceptable criteria but another crucial parameter should be taken into consideration. This is related to the geometrical conformation of pharmacophore site (Figure-5) because it is flexible for all compounds (**L^1-L^6**). The distribution characteristics, absorption, and bioactivity were demonstrated to be dependent on the aqueous solubility and the geometrical parameter of each compound.



L^1, L^2 **L^3- L^6** **ACZ**

Figure-5. Identification of pharmacophore sites.

Further, Table-3 shows drug-likeness (DL) of the compounds (**L^1-L^6**) is not in the comparable zone with the used standard drug. We have calculated the overall drug-score (DS) for the compounds (**L^1-L^6**) and we have compared it with that of standard drug **ACZ** (**ACZ** = acetazolamide). The DS combines drug-likeness, cLog*P*, cLog*S*, molecular weight, and toxicity risks in one handy value that may be used to judge the compound's overall ability to

qualify for a drug. The reported compounds (**L**^{1}-**L**^{6}) showed low capacity to moderate DS as compared with the used standard drug SD (Tables 3 & 4).

Table-3. Osiris calculations of toxicity risks of compounds (**L**^{1}-**L**^{6}).

Compd.	MW	Toxicity Risks [a]				Osiris calculations [b]			
		MUT	TUM	IRRI	REP	cLog*P*	cLog*S*	DL	DS
L^{1}	311	---	---	+	+++	2.33	-2.71	-7.51	0.13
L^{2}	339	---	---	+	+++	0.36	-1.64	-5.39	0.31
L^{3}	265	+++	+++	---	---	-0.15	0.88	-21.8	0.17
L^{4}	279	+++	+++	---	---	0.20	0.54	-20.6	0.17
L^{5}	417	+++	+	+++	+	3.88	-3.03	-25.8	0.14
L^{6}	445	+++	+++	+++	+++	3.238	-2.45	-26.1	0.37
ACZ [c]	222	+++	---	+++	---	-0.54	-1.64	3.50	0.34

Higly toxic: (---), Slightly toxic: (+), Not toxic (+++). [a] MUT: Mutagenic, TUM: Tumorigenic, IRRIT: Irritant, RE: Reproductive effective. [b] Sol: Solubility, DL: Druglikness, DS: Drug-Score. [c] ACZ = acetazolamide.

Table-4. Molinspiration calculations of compounds (**L**^{1}-**L**^{6}).

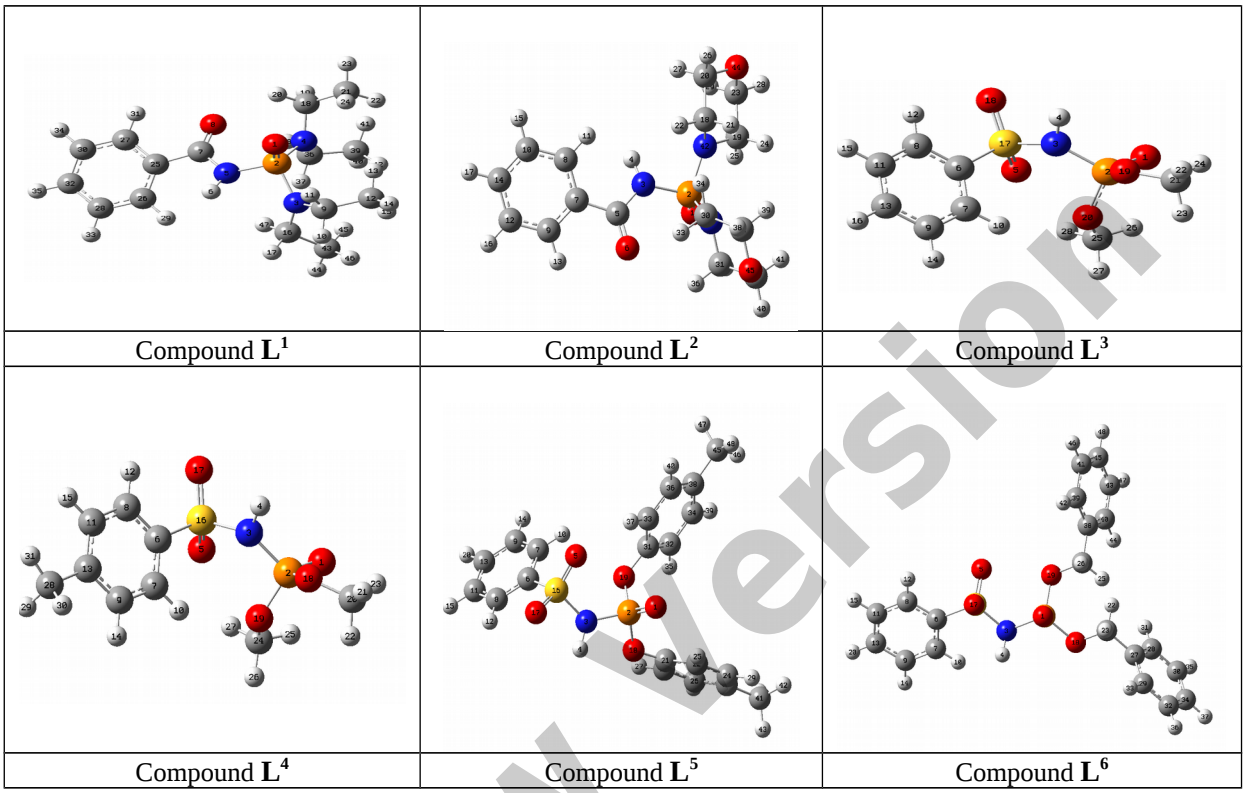
Compd.	Molinspiration calculations [a]				Drug-likeness [b]					
	TPSA	NONH	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
L^{1}	53	1	0	303	0.01	-0.14	-0.14	-0.23	0.31	0.11
L^{2}	71	1	0	300	0.01	-0.17	-0.07	-0.21	0.35	0.15
L^{3}	82	1	0	207	-0.35	-0.32	-0.78	-0.86	0.02	0.16
L^{4}	82	1	0	224	-0.33	-0.39	-0.74	-0.78	0.00	0.10
L^{5}	82	1	0	350	-0.07	-0.41	-0.14	-0.09	0.31	0.06
L^{6}	82	1	0	383	0.02	-0.11	-0.15	-0.17	0.35	0.20
ACZ [b]	115	3	0	157	-1.80	-1.29	-1.28	-2.02	-1.20	-0.13

[a] TPSA: Total molecular polar surface area; NONH: number of OH---N or O---NH interaction, NV: number of violation of five Lipinsky rules; VOL: volume. [b] GPCRL: GPCR ligand; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor. [c] ACZ = acetazolamide

2.5. Frontier Molecular orbitals

To shed some light on the chemical reactivity [13,14], optical properties [15] and biological activity [16], the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) give complete picture on them The energy gap

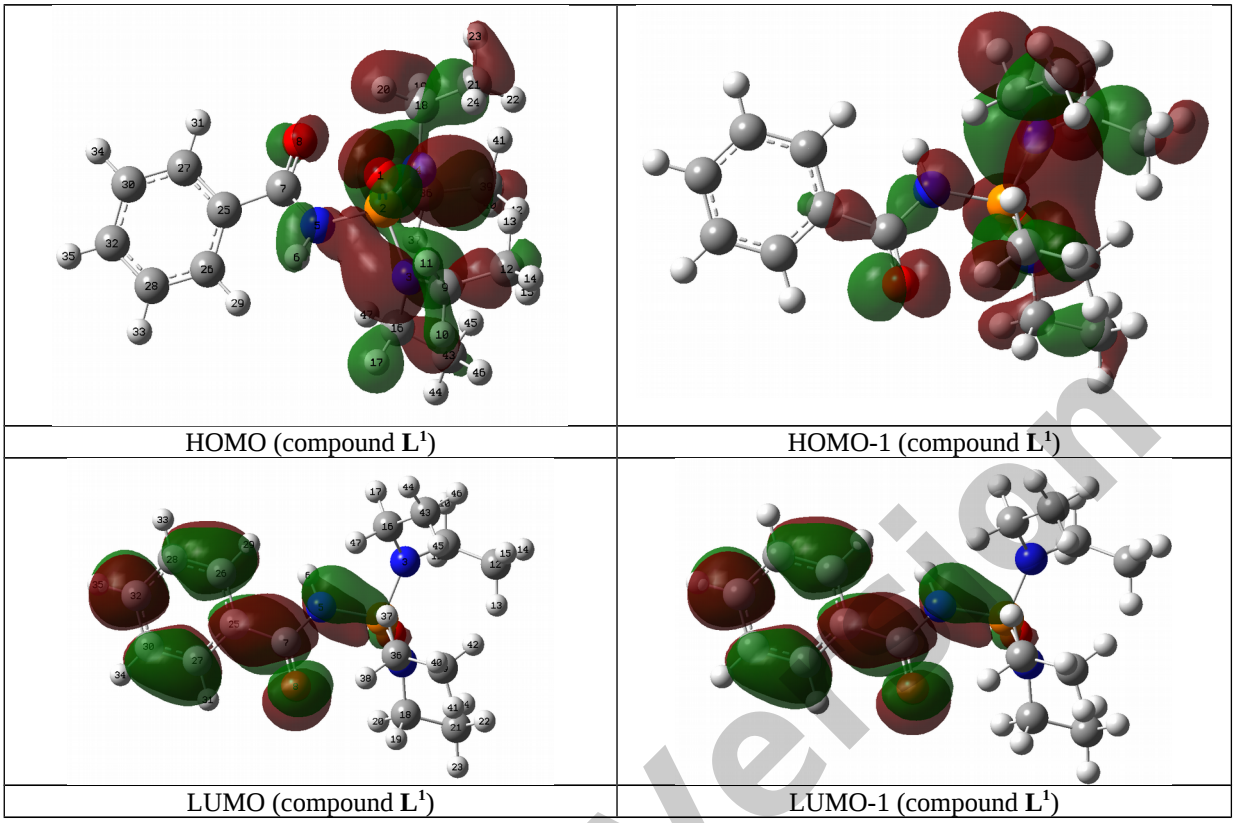
between HOMO and LUMO is obtained by the first optimization of structures (L^1-L^6) as illustrated in Figure 6.



Compound L^1	Compound L^2	Compound L^3
Compound L^4	Compound L^5	Compound L^6

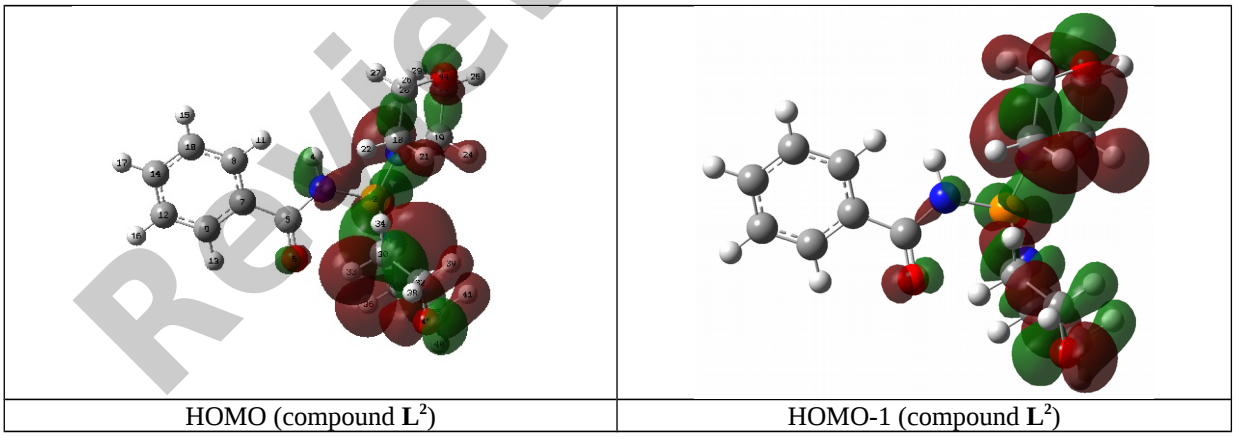
Figure-6. Optimized geometrical structure of compounds (L^1-L^6).

To recognize the impact of the structural properties of the amido-phosphate moieties, all HOMO, HOMO-1, LUMO and LUMO+1 energies were calculated at B3LYP/6-311+G(d,p) and LANL2DZ theory level which illustrated in Table 5. As can be seen from Figures 7-13, for most compounds, HOMO is mainly delocalized on the central carbonyl; thionyl, phosphonyl groups; however LUMO is mainly delocalized on the aryl ring and heteroatoms. According to these results; generally HOMO and LUMO orbitals are mainly delocalized on the rings that mean π-antibonding type orbitals.-



HOMO (compound $\mathbf{L^1}$)	HOMO-1 (compound $\mathbf{L^1}$)
LUMO (compound $\mathbf{L^1}$)	LUMO-1 (compound $\mathbf{L^1}$)

Figure-7. HOMO and LUMO molecular orbitals of the ligand ($\mathbf{L^1}$) using the 6-311+G(d,p) method.



HOMO (compound $\mathbf{L^2}$)	HOMO-1 (compound $\mathbf{L^2}$)

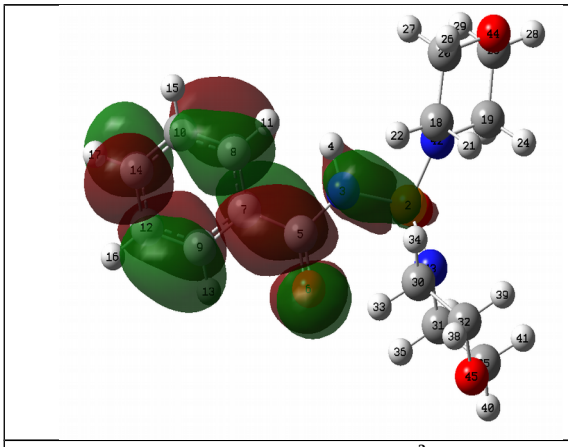
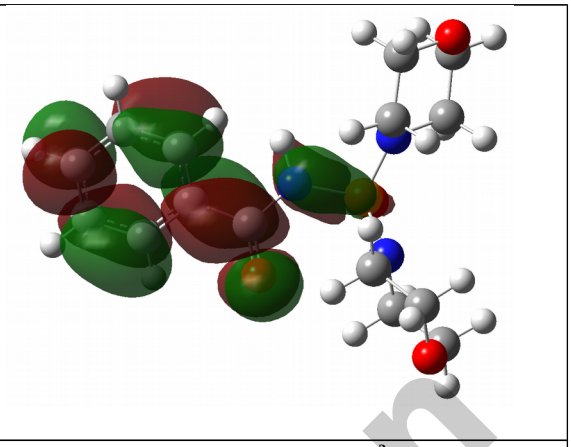
	
LUMO (compound $\mathbf{L}^2$)	LUMO-1 (compound $\mathbf{L}^2$)

Figure-8. HOMO and LUMO molecular orbitals of the ligand ($\mathbf{L}^2$) using the 6-311+G(d,p) method.

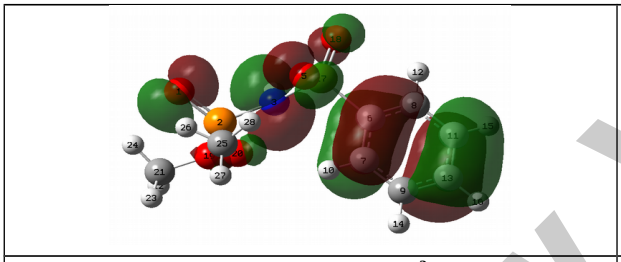
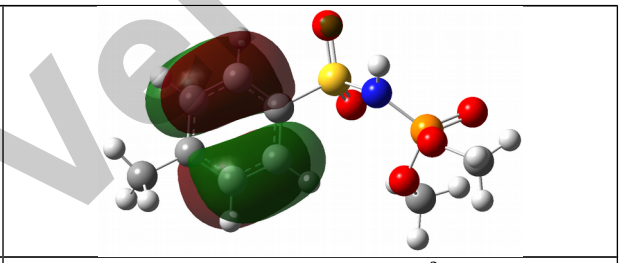
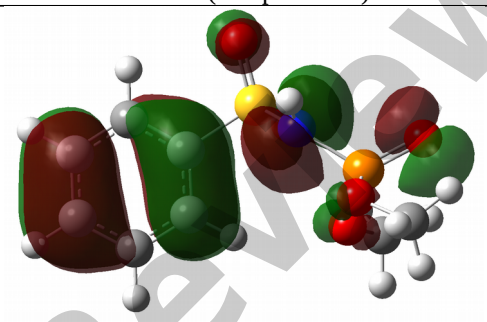
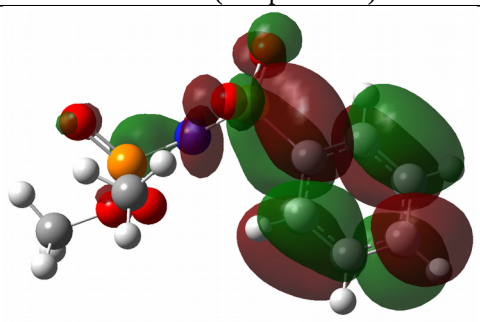
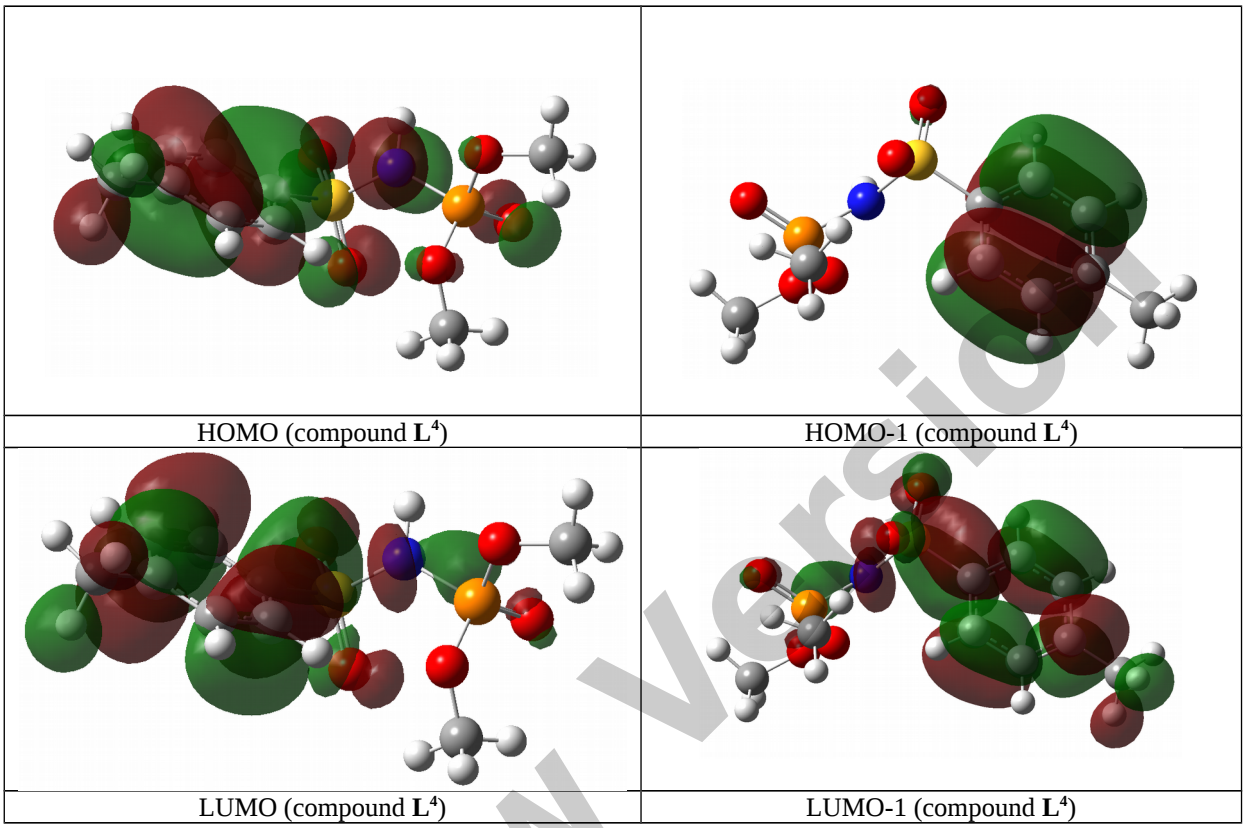
	
HOMO (compound $\mathbf{L}^3$)	HOMO-1 (compound $\mathbf{L}^3$)
	
LUMO (compound $\mathbf{L}^3$)	LUMO-1 (compound $\mathbf{L}^3$)

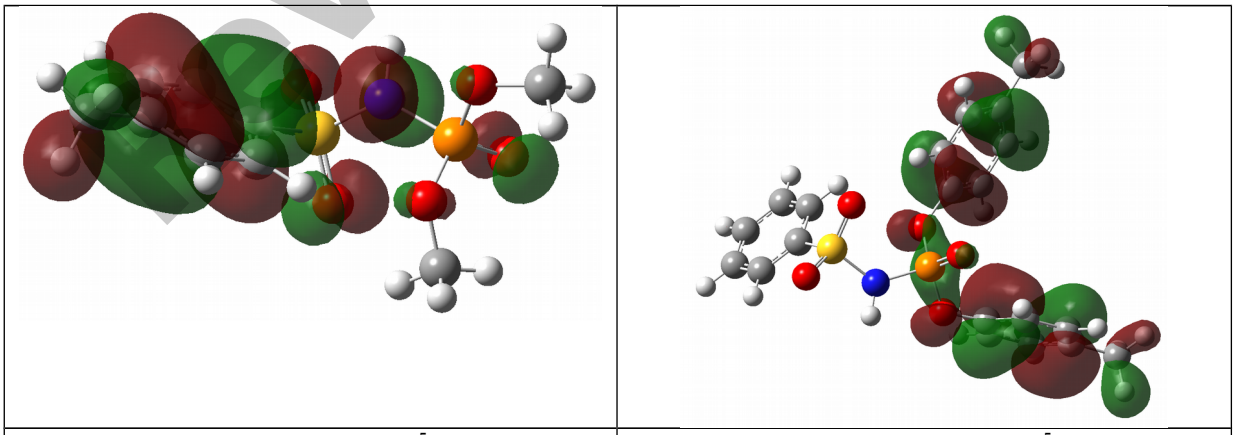
Figure-9. HOMO and LUMO molecular orbitals of the ligand (**L**^{3}) using the 6-311+G(d,p) method.



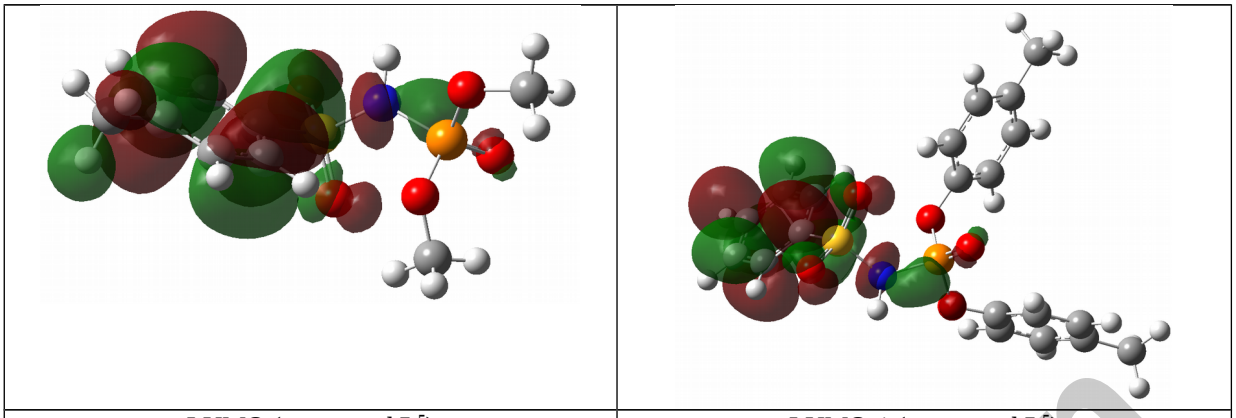
HOMO (compound **L**^{4}) | HOMO-1 (compound **L**^{4})

LUMO (compound **L**^{4}) | LUMO-1 (compound **L**^{4})

Figure-10. HOMO and LUMO molecular orbitals of the ligand (**L**^{4}) using the 6-311+G(d,p) method.

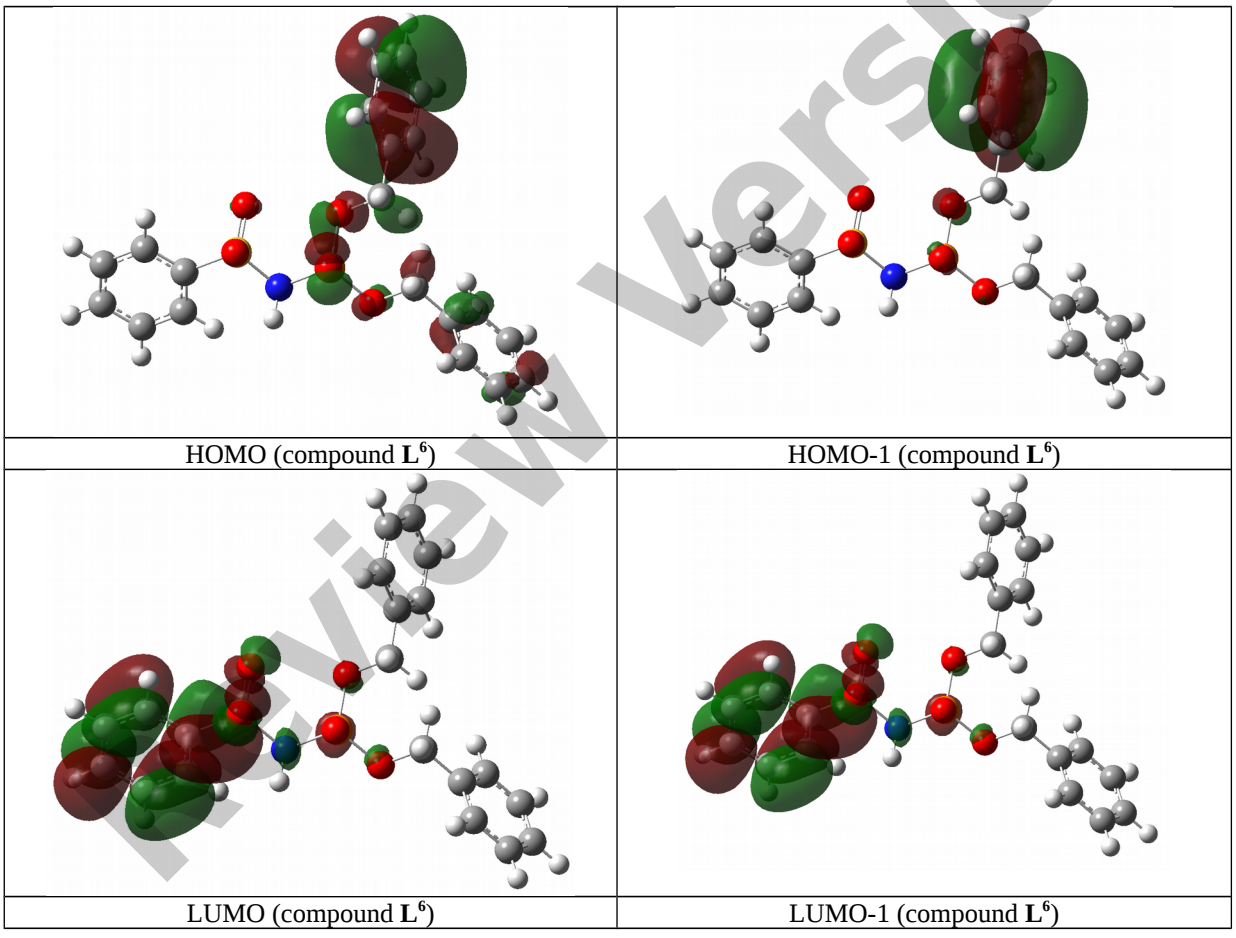


HOMO (compound **L**^{5}) | HOMO-1 (compound **L**^{5})



LUMO (compound $\mathbf{L}^5$)	LUMO-1 (compound $\mathbf{L}^5$)

Figure11. HOMO and LUMO molecular orbitals of the ligand ($\mathbf{L}^5$) using the 6-311+G(d,p) method.



HOMO (compound $\mathbf{L}^6$)	HOMO-1 (compound $\mathbf{L}^6$)
LUMO (compound $\mathbf{L}^6$)	LUMO-1 (compound $\mathbf{L}^6$)

Figure-12. HOMO and LUMO molecular orbitals of the ligand ($\mathbf{L}^6$) using the 6-31G(d) method.

Table-5. HOMO, HOMO-1, LUMO, LUMO+1 and Δ*E* energies (a.u.)

Compd.	HOMO	LUMO	HOMO-1	LUMO+1	HOMO-LUMO	HOMO-1-LUMO+1
L[1]	-0.22177	-0,04545	-0,23585	-0,01336	0,17632	0,22249
L[2]	-0,22834	-0,05200	-0,24165	-0,01776	0,17634	0,22389
L[3]	-0,26789	-0,04252	-0,27313	-0,02242	0,22537	0,25071
L[4]	-0,25868	-0,03841	-0,26952	-0,01969	0,22027	0,24983
L[5]	-0,23257	-0,04322	-0,24067	-0,02197	0,18935	0,21870
L[6]	-0,24579	-0,04614	-0,24722	-0,03641	0,19965	0,21081
ACZ [a]	-0.26493	-0.07367	-0.28819	-0.02464	0.19112	0.26355

[a] Standard drug (ACZ = acetazolamide).

2.6. Natural atomic charge

Allocation of charges (-ve and +ve) has indispensable role because of atomic charges affect molecular polarizabilit, dipole moment, acidity-basicity, electronic structure behavior of molecular system. These electronic properties have strong relations to the biological activity of compound. The calculated natural charges (NAC) at various atomic sites are cited in Table 6. The carbonyl, phosphonyl and sulphonyl O−atoms are the most electronegative atomic sites in the molecules. The calculated natural charge densities at these atoms are in the range -0.4311 to -0.5733. The two oxygen atoms (O1 and O2) of the C=O and P=O groups of **L[1]** are less electronegative than those of **L[2]**. Also, the two O-atoms of **L[2]** represent less electrostatic repulsion. So the C=O and P=O are in less transoidal geometry. In contrast to **L[2]**, the compounds **L[3]**- **L[5]** show the S=O and P=O groups have more important negative natural charge (-0.5355 to -0.5678). So the compounds **L[3]**- **L[5]** have an O1,O2-pharmacophore site which tends to have a transoidal geometry more than cisoidal geometry leading to a decrease in bioactivity. In contrast to **L[3]**- **L[5]**, compound **L[6]** represents a good O1,O2-pharmacophore site with less negative natural charges but the steric effect of the two benzyloxy substituents masque the pharmacophore site and lead to less bioactivity. These sulphonyl and phosphonyl oxygen have the low negative NAC values.

Table-6. Electronic impact on anti-Carbonic anhydrase activity of (OCNHCO) and (OCNHSO) pharmacophore sites of compounds (**L[1]**- **L[6]**).

Compd	IC_{50} (μM)	O=C-NH-P=O Pharmacophore Site			O=S-NH-P=O Pharmacophore Site		
		C=**O**-1	N-**H**	P=**O**-2	S=**O**-1	N-**H**	P=**O**-2
L[1]	12.5	-0.5032	-0.4324	-0.5773	---	---	---

L²	3.12	-0.4972	-0.4341	-0.5648	---	---	---
L³	24.5	---	---	---	-0.5386	-0.4324	-0.5454
L⁴	55.5	---	---	---	-0.5402	-0.4395	-0.5426
L⁵	75.5	---	---	---	- 0.5355	-0.4290	-0.5277
L⁶	---	---	---	---	-0.4311	-0.4870	-0.5678
ACZ [a]	0.12	- 0.5333	-0.2712	-0.4517 [b]	---	---	---

[a] Standard drug (ACZ = acetazolamide). [a] C=N1 instead of P=O2..

3. CONCLUSIONS

By using a combined bioinformatic DFT-POM platform, we have identified a new series of amidophosphate derivatives as potential candidates to inhibit carbonic anhydrase. The DFT analysis showed *transoidal* conformation of the studied compounds **L¹-L⁶**. The molecular structure of the compounds **L¹-L⁶** was optimized using the DFT/B3LYP method and 6-311G(d,p) basis set. The calculated drug score and the physic-chemical properties showed a good agreement with our reported X-ray crystal structure. The carbonic anhydrase potential suppression of the deliberated compound has been calculated using the same level of theory. The HOMO/LUMO energy gap (DE) rates proved that the deliberated compounds are better inhibitor substances than the reference drug. In accordance with the experimental data, the POM−DFT calculated atomic charge showed intense O,O-pharmacophore interaction with enzyme. The potent carbonic anhydrase inhibitory activity of the series of compounds **L¹-L⁶** indicated its potential use as a treatment. It could lead to the treatment of the carbonic anhydrase associated health disorders. Further studies towards the asymmetric synthesis of this compound are in process.

4. EXPERIMENTAL

4.1. Material and Method

All reagents for compounds (**L¹-L⁶**) syntheses were purchased from commercial sources and used without further purification. ^{1}H and ^{31}P NMR spectra were recorded on Varian Mercury 400 NMR spectrometer at 25 °C. Chemical shifts are reported with reference to $SiMe_4$ (^{1}H) and H_3PO_4 (^{31}P). IR samples were prepared as KBr pellets and spectra were recorded with a Perkin–Elmer Spectrum BX spectrometer in the range of 4000–400 cm^{-1}. Elemental analysis (C, H, N, S) were performed using EL III Universal CHNOS Elemental Analyzer.

4.2. Carbonic anhydrase inhibition assay

Carbonic anhydrase inhibition screening was performed according to standard method [17] with slight amendment. 200 µL of total mixture volume, contained 140 µL of 20 mM HEPES-Tris buffer of pH 7.4 (bioworld: cat#40820000-1) (Invitrogen: cat# 15504-020), 20 µL of enzyme (sigma Aldrich, C2624, PCode: 1001584424) (0.1 - 0.2 mg⁄ mL in deionized water), 20 µL (0.5 mg⁄mL in DMSO) of test compound was mixed and incubated at 25 ˚C for 15 minutes. After incubation pre-read was taken at 400 nm and 20 µL of substrate (4-nitrophenol acetate, sigma Aldrich, N8130, lot#BCBK4587V) (0.7 mM in methanol) was added and re-incubated at same condition for 30 minutes and read was taken at 400 nm. Acetazolamide was taken as positive control. Result was measured by giving formula. IC_{50} was calculated by making serial dilution of original concentration.

%inhibition = 100-(absorbance of test compound ÷ absorbance of control) 100

4.3. Docking Studies

The crystal structure of bovine carbonic anhydrase-II (bCA-II) was obtained from the protein data bank (PDB) with four letter codes of 1V9E. The receptor structures were energy minimized through Swiss pdb viewer v4.1.0 program [18]. The 2D structures of the compounds (**L^1-L^6**) and standard acetazolamide were drawn in the Chem sketch software [19]. The 2-D structures were saved in mol format followed by reduction and energy refinement through Avogadro's software, then saved in pdb format [20]. Autodock Vina [21] and i-GEMDOCKv 2.1 softwares were utilized to the docking simulation [22]. The predicted docked poses of the CA-II enzyme with the compounds (**L^1-L^6**) were analyzed by Discovery studio visualizer version 4.0 (Umamaheswari et al., 2011), PyMOL version 1.7.2 [23] softwares. These studies were performed to understand the mechanism of carbonic anhydrase-II inhibition and the binding modes of the compounds (**L^1-L^6**) in the active site pocket of carbonic anhydrase-II. The crystal structure of bovine carbonic anhydrase-II with Zinc metal bound (PDB id: 1V9E) was used. The structure of bovine carbonic anhydrase-II which was reported with 1.95 Å resolutions [24] was used for the docking of the compounds (**L^1-L^6**) (as these compounds inhibited carbonic anhydrase-II with IC_{50} in low micro-molar range). The active site of carbonic anhydrase-II lies at the bottom of a deep cleft where a zinc

atom is bound. Nitrogen atoms of two highly conserved Histidines (numbered 93, 95) and Thr197 directly coordinate with zinc [25].

4.4. POM Analyses

One of the actual problems related to the synthetic drugs is the presence of diverse side effects. Besides having a perfect biological activity, a molecule must have good pharmacokinetic characterization in biological systems so as to a potent drug. To access the better pharmacokinetic profile of the synthesized compounds, we used well assured in silico tools such as POM (Petra, Osiris and Molinspiration). This tool has been supported with about 7000 commercial drugs available on the market. POM analyses offer more insight on the nature of pharmacophore sites (antibacterial, antiviral, antifungal, antitumor and anti-parasite) [26-37] in comparison with Lipinski five rules. The latter is limited to bioavailability prediction [38].

4.5. Synthesis and characterization of compounds (**L^1-L^6**)

4.5.1. N,N'-tetraethyl-N''-benzoylphosphortriamide (L^1)

It was synthesized according to known methods [39], the structure is described in [40].

NMR Data [ppm]: ^{1}H 1.03 (CH_3), 3.05 (CH_2), 7.47, 7.57, 7.86 (C_6H_5), 9.01 (NH); ^{31}P 13.69, J_{P-H} = 11.2 Hz. IR data [cm^{-1}] (selected): 3060 (ν(NH)), 1665 (ν(CO)), 1240 (ν(PO)), 1030 (ν(CN)), 940 (ν(PN)), 670 (ν_{as}(CCl)) , 640 (ν_s(CCl)).

4.5.2. Dimorpholido-N-benzoylphosphorylamide (L^2)

It was synthesized according to [41] and in this publication is also described the structure of named compound.

NMR data [ppm]: ^{1}H 3.24 and 3.36 m, CH_2; 7.49, 7.56, 7.90, m, C_6H_5 (8:8:5); ^{31}P 13.2, J_{P-H} = 12 Hz. IR data [cm^{-1}] (selected): 3100 (ν(NH)), 1685 (ν(CO)), 1200 (ν(PO)), 1030 (ν(CN)), 980 (ν(PN)).

4.5.3. Dimethyl(phenylsulfonyl)amidophosphate (L^3) and dimethyl [(4-methylphenyl) sulfonyl]amidophosphate (L^4)

They were synthsized according to [42].

L^3: NMR data [ppm]: ^{1}H 3.57 d, CH_3; 7.58, 7.65, 7.91, m, C_6H_5; ^{31}P 2.17, J_{P-H} = 11.6 Hz. IR data [cm^{-1}] (selected): 3000 (ν(NH)), 1335 ($\nu_{as}(SO_2)$), 1255 (ν(PO)), 1175 ($\nu_s(SO_2)$).

L^4: NMR data [ppm]: ^{1}H 3.50, 2.50 CH_3; 7.50, 7.81 d, C_6H_5; IR data [cm^{-1}] (selected): 2967 (ν(NH)), 1342 ($\nu_{as}(SO_2)$), 1234 (ν(PO)), 1168 ($\nu_s(SO_2)$).

4.5.4. bis(4-methylphenyl)(phenylsulfonyl)amidophosphate (L^5) and L^6 – dibenzyl (phenylsulfonyl) amidophosphate (L^6)

They were synthsized according to [43].

L^5: NMR data [ppm]: ^{1}H 2.31 m CH_3; 6.99 d, 7.10 d, 7.49 dd, 7.60 t, 7.84 m C_6H_5; ^{31}P 13.6 m. IR data [cm^{-1}] (selected): 2975 (ν(NH)), 1345 ($\nu_{as}(SO_2)$), 1255 (ν(PO)), 1180 ($\nu_s(SO_2)$).

L^6: NMR data [ppm]: ^{1}H 4.93 ddd CH_2; 7.31 m, 7.52 dd, 7.61 t, 7.89 m C_6H_5; ^{31}P 4.33 m. IR data [cm^{-1}] (selected): 2950 (ν(NH)), 1335 ($\nu_{as}(SO_2)$), 1245 (ν(PO)), 1175 ($\nu_s(SO_2)$).

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