ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.3.P29-35

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Chemistry

Ab Initio and DFT Investigation of Effect of Substituent at the C7 Position of 4-Amino-DANA Sialidase Inhibitor

Krishnan Chandrasekaran

Sri Krishna College of Engineering and Technology, Coimbatore – 641 008, India.

Abstract: Sialic acid is the active site of neuraminidase protein, eventually it cleaves form its substrate *via* sialsyl cation intermediate and proliferates the viral infection to other cells. On account of weak binding affinity between substrate and receptor, the viral infection communicates to other cells and leads to mortality of humans. DANA is the first sialidase inhibitor formed by the dehydration of the C2 hydroxyl group of sialic acid. The replacement of hydroxyl group at C4 position of DANA by an amino group drastically increases the binding affinity and results 4-amino-DANA inhibitor, which is potent than parent DANA. Crystal structure of DANA shows that several binding sites remain free and it should be explored for more powerful sialidase inhibitors. The current study systematically investigates the effect of substituent on the C7 position of 4-amino-DANA in gas phase and solvent phase as well. X-Ray crystallographic study reveals that the C7 of glycerol side chain remains free. Hence, substituent effect at C7 analysis is carried in search of potent sialidase inhibitors. The *ab* initio and DFT investigation reveals that guanidino and methyl group at C7 position drastically increases the binding affinity between substrate and receptor. Hence further investigation of methyl and guanidine derivatives of the 4-amino-DANA could act as a promising candidate for the design and development of sialidase inhibitors. Vaccination for the H1N1 is not effective due to the new viral mutagenic strains and hence, it cannot contain the viral infection.Therefore antiviral drugs will address the limitation of vaccination. The current finding of sialidase antiviral inhibitors will effectively contain the viral infection and prevent the morbidity.

Keywords: Sialidase Inhibitors, 4-Amino-DANA, DFT, Binding Pocket

***Corresponding Author**

Krishnan Chandrasekaran , Sri Krishna College of Engineering and Technology, Coimbatore – 641 008, India.

Received On 26 March, 2021
Revised On 15 March, 2022
Accepted On 31 March, 2022
Published On 1 May, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation , Ab Initio and DFT Investigation of Effect of Substituent at the C7 Position of 4-Amino-DANA Sialidase Inhibitor.(2022).Int. J. Life Sci. Pharma Res.12(3), P29-35 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.3.P29-35

This article is under the CC BY- NC-ND Licence (https://creativecommons.org/licenses/by-nc-nd/4.0)

Copyright @ International Journal of Life Science and Pharma Research, available at www.ijlpr.com

1. INTRODUCTION

Neuraminidase is the surface protein of the influenza virus[1]. Vaccines against the influenza virus are inactive due its rapid emergence of viral mutagens and thus it paves an immense need for design and synthesis of novel potent sialidase antiviral inhibitor[2]. So, the need for antiviral drugs of neuraminidase is vital. Sialic acid is the active site of neuraminidase and its binding with the amino acid residue is very weak; thus it proliferates the viral infection to the other vicinal cells[3]. DANA is a sialic acid analogue, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and it has been reported as the first inhibitor of sialidase[4], it is shown in figure1. The replacement of C4 hydroxyl group in DANA by an amino group makes it as a 4-amino-DANA, more potent sialidase inhibitor than DANA due to its strong hydrogen bonding with the receptor and it has been shown in the figure 2.

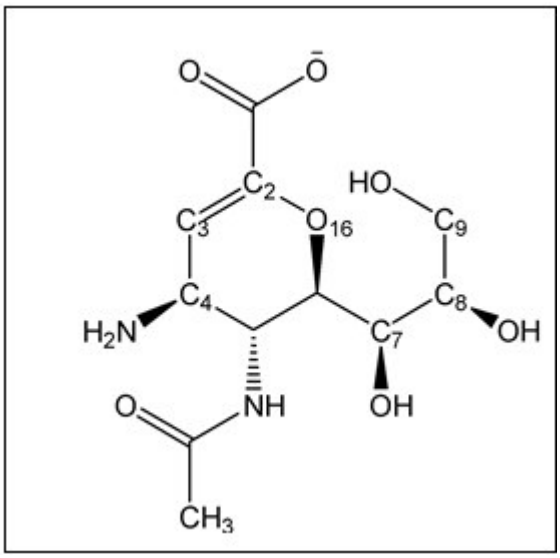

Fig 1: DANA

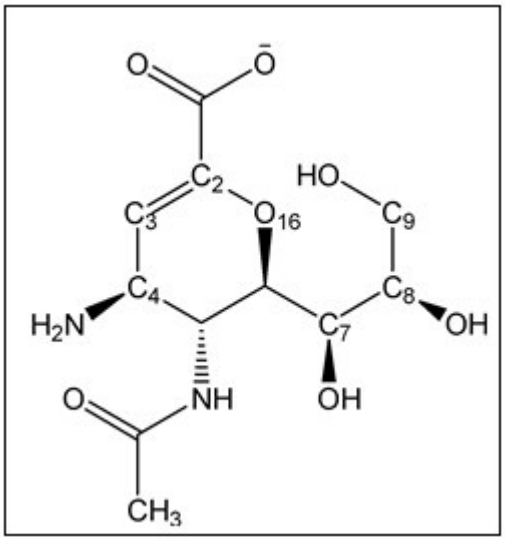

Fig 2: 4-amino-DANA

The presence of buried water molecules interlocked in the protein network as well as the presence of substituent's at the key position of substrate have substantial effect on the inhibitor binding affinity,; eventually the same will act as potent sialidase inhibitor[5,6]. The interaction between the functional groups and amino residues within the binding pockets of enzyme residue, plays a pivotal role in the design of effective sialidase inhibitors and it has been carried through the GRID software[7]. It predicts that apart from electrostatic interaction of functional groups; the non-bonding interaction between substrate and receptor also contributes to the ligand binding[8]. It is well established that the 4-substituted DANA has higher binding affinity than its parent DANA and it is predicted using the GRID software. Theory of chemical kinetics also ascertain that the compounds closely resemble the transition state intermediate have higher binding affinity towards the enzyme substrate than its respective parent compound. As DANA mimics the structure of sialosyl cation intermediate; the template of DANA modeling will have constructive effect on the development of sialidase inhibitors. The C4 position of DANA is very significant, because the C4 hydroxyl group is bonded to two amino acid residues in the three dimensional structure. It is well established that the replacement of C4 hydroxyl group in DANA by an amino group drastically increases the binding affinity between ligand and receptor[9]. Hence, various substituents have been introduced in 4-Amino DANA at its C7 position to validate its binding affinity. The current research study evaluates the binding affinity of 4-amino-DANA with various C7 substituent's in gas phase and solvent phase. Substituent with higher binding affinity will have greater impact in the design and

development of sialidase antiviral inhibitor. Likewise the extensive theoretical investigation on the substituent effect of oseltamivir reveals that the certain substituent's at the C6 and C12 position of oseltamivir increases the binding affinity[10] and hence exploration of 4-amino-DANA substituent effect could be very useful in the design of inhibitors. X-ray crystallographic studies of 4-amino-DANA and its analogues bound to neuraminidase protein, indicates that the C7 hydroxyl group of glycerol side chain did not participate in any receptor interaction and it remains free[11]. Taking into account, the current study systematically investigates the effect of substituent's at the C7 position of 4-amino-DANA and validates its binding affinity. The key substituent with higher binding affinity will act as a promising sialidase antiviral inhibitor.

2. COMPUTATIONAL METHOD

4-amino-DANA compound and its substituent derivatives have been optimized using the Hatree Fock HF/6-31G level of theory and subsequently single point energy calculations have been carried out using DFT B3LYP/6-31G (d) level of theory in gas phase and solvent phase. CPCM[12] (Conductor like Polarized Continuum model) model is employed for the solvent phase single point energy calculations and water has been used as the solvent for the calculations. All the quantum chemical calculations have been performed by using Gaussian 03 suit program[13]. The binding affinity of 4-amino-DANA and its substituted derivatives have been calculated using the following relation

$$\Delta E_{(Bind)} = Eg_{(Substrate\text{-}Ligand\ Complex)} - (E_{(substrate)} + E_{(Ligand)})$$

3. RESULTS AND DISCUSSION

The binding affinity of parent 4-amino-DANA on interaction with methyl guanidino provides binding energy of 112.62 kcal/mol. The amino group at the C4 position favors the effective electron delocalization between C4 and C2 atoms and thus improves the binding affinity significantly due to the partial planarity of the cyclohexane ring. Substitution of the amino group at the C7 position produces 4,7-di-amino-DANA; which on interaction with methyl guanidino provides binding energy of 109.65 kcal/mol. It is apparent that the introduction of amino group at the C7 position decreases the binding affinity, the amino group at the C7 position forms an intra molecular hydrogen bond 2.305Å with the carbonyl oxygen, its shown in figure 3 and this bond has no effect on the binding affinity. In addition, the C8 hydroxyl group forms an intra molecular hydrogen bond 2.247Å with the carboxylate group and this bond penalizes the binding affinity of the substrate and therefore decreases binding affinity. Hydrogen bonding cooperative effect[14] is the major reason for the low binding affinity of 4,7-diamino-DANA derivative.

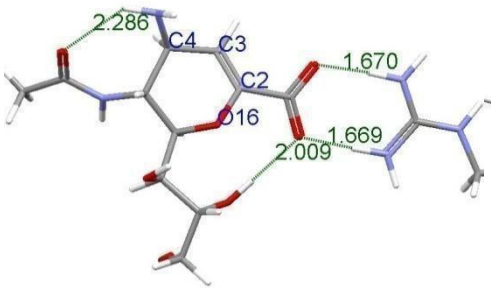

Fig 3: 4-amino-DANA

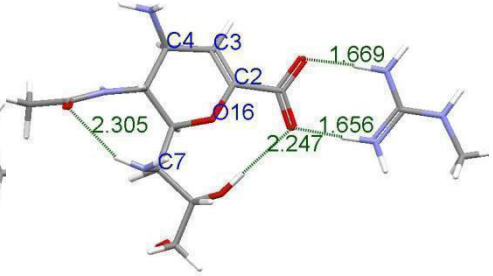

Fig 4: 4,7-di-amino-DANA

Next to the amino group, the methoxy group is introduced at the C7 of 4-amino-DANA. The 7-methoxy-4-amino-DANA on interaction with the model amino acid residue provides binding energy of 109.25 kcal/mol. The methoxy group at the C7 position cannot delocalize electron between C7 and C4 group and as a result it cannot increase the binding affinity and remains same with the parent 4-amino-DANA compound.

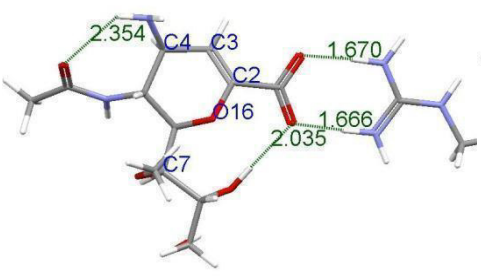

Fig 5: 4-amino-7-methoxy-DANA

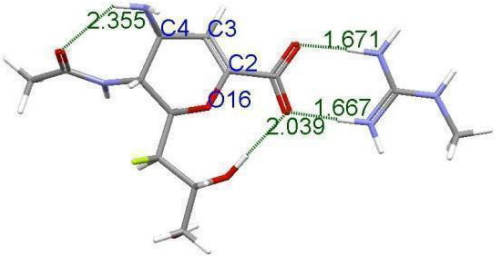

Fig 6: 4-amino-7-fluoro-DANA

Amino group is replaced by the methoxy group at the C7 of 4-amino-DANA, it has been shown in figure 5. The 7-methoxy-4-amino-DANA on interaction with the model amino acid residue provides binding energy of 109.25

kcal/mol. The methoxy group at the C7 position retards the electronic transfer between C7 and C4 group and as a result it cannot increase the binding affinity and remains same with the parent 4-amino-DANA compound[15]. Introduction of fluorine at the C7 position of 4-amino-DANA yields binding energy of 109.35 kcal/mol; which is lower than the parent binding energy and its structure is shown in figure 6. Fluorine is an electronegative substituent and hence its presence in the vicinity of highly polarized oxygen atom O16 acts as a barrier for the delocalization of electrons between C2 and C7 atoms. So, the electrical effect of fluorine is retarded and hence, it accounts for limited binding energy on par with the parent 4-amino-DANA. Chlorine is introduced at the C7 position of 4-amino-DANA and it undergoes interaction with arginine model amino acid residue, shown in figure 7. It attains the binding affinity of 108.15 kcal/mol and thereby it shares same platform with the fluorine substitution. Hence, the introduction of chlorine at the C7 position does not have a constructive effect on the binding affinity of 4-amino-DANA. Figure 7 indicates an intramolecular hydrogen bond 2.334Å formation between amino group and carbonyl oxygen and this does not contribute to the higher binding affinity due to the poor charge transfer between C4 and C2 atoms of the pyranose ring[16].

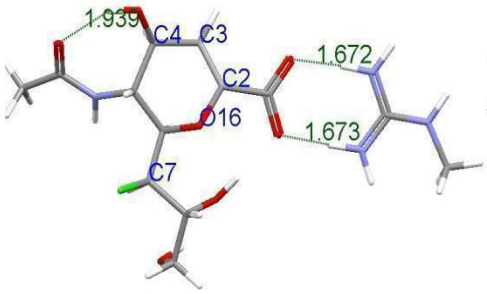

Fig 7: 4-amino-7-chloro-DANA

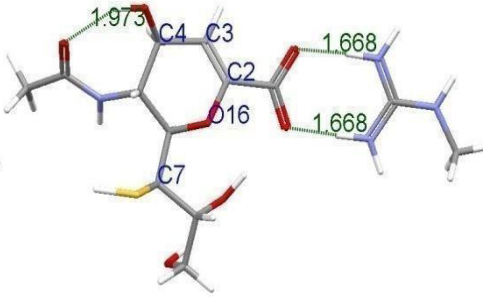

Fig 8: 4-amino-7-thiol-DANA

The introduction of chlorine at the C7 position lengthens the C2=C3 bond distance from 1.319Å to 1.49Å and this lengthening retards the electron delocalization between C2 and C4 atom. Thus it eventually causes low binding affinity of 7–chloro-DANA. Moreover, the lengthening of C2=C3 shown in figure 7 causes structural distortion of the ring and it mitigates the binding affinity of 7-chloro-DANA. Sulfur derivative thiol is introduced at the C7 position of N-DANA (Figure 8) and its interaction with methyl guanidino provides binding energy of 103.40 kcal/mol and it reveals that there is substantial increase in binding affinity of thiol group[17]. Polarization effect of thiol is very less and hence, it cannot strongly exert its electronic effect towards endocyclic oxygen O16 of the pyranose ring. The binding affinity analysis of halogen substituent discloses that it does not have significant effect on the binding energy due to poor delocalization of electrons. Hence, tri-fluoro carbon is introduced at the C7 position of 4-amino-DANA and it is shown in figure 9. Its interaction with methyl guanidino provides binding energy of 104.69 kcal/mol. It is very clear that the effect of tri-fluoro carbon on the C7 position is insignificant. Finally guanidino is introduced at the C7 position to validate its binding affinity.

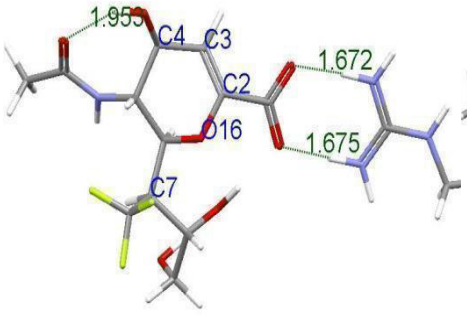

Fig 9: 4-amino-7-CF_3-DANA

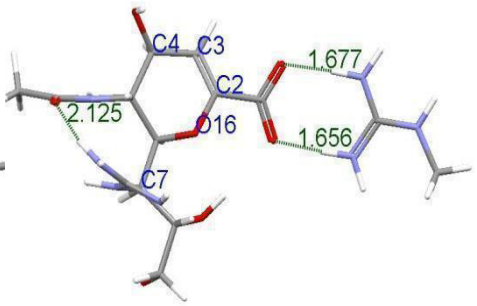

Fig10: 4-amino-7-guandino-DANA

4-amino-7-guandino on interaction with model amino acid (arginine) residue provides binding energy of 109.08 kcal/mol and thereby it shares the same platform with the chloro, fluoro and thiol substituent. The main reason for the higher binding affinity of guanidine derivative of 4-amino-DANA is the formation of intramolecular hydrogen bond between carbonyl oxygen and amino group hydrogen atoms and it has been shown in figure 10. It is clear from Table 1 that the investigation of effect of substituent at the C7 position reveals that guanidino is the only substituent provides higher binding affinity and thus further exploration of dynamics of guanidino derivative will provide a promising opportunity for the design and development of sialidase antiviral inhibitor[18]

Table 1 Effect of C7 Substituent on the binding affinity of 4-Amino-DANA

Substituent's	Binding Energy HF\6-31g (kcal/mol)	Binding Energy B3LYP\6-31G(d) (kcal/mol)	Charges at the carbon C2 atom	Charges at the O16 oxygen atom
Hydroxyl	107.95	112.62	0.313	-0.789
Amino	108.03	110.44	0.336	-0.789
Methoxy	105.93	109.25	0.313	-0.784
Fluorine	105.92	109.35	0.316	-0.780
Chlorine	104.99	108.15	0312	-0.766
Methyl	111.02	115.33	0.327	-0.782
Thiol	107.04	109.67	0.334	-0.772
GuanidinoGuandino	106.11	109.08	0.349	-0.803
CF3	104.39	108.08	0.31	-0.769

3.1 Binding Affinity Of C7 Substituent In Solvent Phase

Most of the biological process in human physiology occurs in solvent phase and hence effect of substituent in solvent phase is inevitable and it provides a reliable platform to design the effective sialidase inhibitors[19]. The parent 4-amino-DANA on interaction with methyl guanidino provides binding energy of 18.03 kcal/mol. However, the introduction of substituent at the C7 drastically decreases the binding affinity between substrate and receptor. Introduction of an amino group at the C7 position drastically decreases the binding affinity with the binding energy of 12.84 kcal/mol. Substitution of methoxy group at the C7 position provides binding affinity of 13.38 kcal/mol. The presence of polarized oxygen atom in the methoxy group polarizes in the solvent phase and thus attains better binding affinity than the amino group at the C7 position[20]. Introduction of methyl group at the C7 position in solvent phase provides solvated binding energy of 13.43 kcal.mol. Methyl group is hydrophobic in nature and thus it inherits less electrostatic interactions. So it provides a fair binding energy equivalent to the methoxy group. Introduction of chlorine at C7 position forms 7-chloro-4-amino-DANA; which on interaction with methyl guanidino provides solvated binding energy of 13.28 kcal/mol; which indicates that the chlorine derivative does not have significant effect on the binding energy[21]. Thiol group at the C7 position of 4-amino-DANA provides binding energy of 13.42 kcal/mol. It appears that thiol also fail to exert their high binding affinity with the receptor due to their hydrogen bonding interactions with the solvent water molecules[22]. Introduction of the guanidino group at the C7 position of 4-amino-DANA has a stellar effect on the binding affinity. The guanidino derivative yields binding energy of 18.93 kcal/mol with its amino acid receptor (Methyl guandiano). It is clear from table 2 that guanidino is the only substituent that attains higher binding affinity among all the seven substituents taken into account for the analysis. Guanidino substituent contains two polarizable amino groups in its structure and this polarization accounts for higher binding affinity[23]. The electronic charge transfer between substrate and receptor also favors high binding affinity. Hence, the great asset of substituent analysis of 4-amino-DANA at C7 position is guanidine group. Therefore it can be attested that the 4-amino-7-guandino-DANA is the potential candidate for the design and development of futuristic sialidase antiviral inhibitors.

Table 2 Effect of Binding affinity of 4-Amino-DANA in Solvent Phase at C7 position

Substituent's	Binding Energy HF\6-31g (kcal/mol)	Binding Energy B3LYP\6-31G(d) (kcal/mol)	r(C2=C3) (Å)	r(C2–O16) (Å)
Hydroxyl	6.33	18.03	1.320	1.382
Amino	6.52	12.84	1.322	1.373
Methoxy	6.42	13.38	1.320	1.381
Fluorine	6.63	13.58	1.320	1.379
Chlorine	6.41	13.28	1.320	1.380
Methyl	3.91	13.16	1.321	1.375
Thiol	6.62	13.42	1.320	1.372
Guanidino	3.42	18.93	1.320	1.374
CF3	6.34	14.47	1.32	1.38

4. CONCLUSION

Ab initio and DFT investigation of substituent effect at the C7 position of 4-amino-DANA in gas phase reveals that the methyl group attains higher binding affinity than all other substituent's. Methyl group exerts their positive inductive effect along the glycerol side chain and thus favors the electronic transfer between cyclic oxygen atom and side chain, thus it increases the binding affinity. It yields binding energy of 111.02 kcal/mol. Next to the methyl group, the amino functional group attains higher binding affinity at the C7 position. Hence the study concludes that 7-methyl-4-amino-DANA is the promising candidate for the futuristic design and development of sialidase antiviral inhibitors. However the solvent phase analysis of binding affinity at the C7 position reveals that the guanidino group attains higher

binding affinity of 18.93 kcal/mol and rest of the substituent do not have a significant binding affinity.Therefore the quantum chemical electronic investigation concludes that the methyl derivative of 4-amino-DANA and guanidino derivative of 4-amino-DANA yields higher binding affinity and thus these two potential candidates can be further recommended for the design and development of sialidase antiviral drugs.

5. ACKNOWLEDGEMENT

Author is grateful to Sri Krishna College of Engineering and Technology

8 REFERENCES

1. Sangma C, Hannongbua S. Structural information and computational methods used in design of neuraminidase inhibitors. Current Computer-Aided Drug Design. 2007 Jun 1;3(2):113-32.
2. Gerdon AE, Wright DW, Cliffel DE. Hemagglutinin linear epitope presentation on monolayer-protected clusters elicits strong antibody binding. Biomacromolecules. 2005 Nov 14;6(6):3419-24.
3. Jarreau PH, Harf A, Levame M, Lambré CR, Lorino H, Macquin-Mavier I. Effects of Neuraminidase on Airway Reactivity in the Guinea Pig1, 2. Am Rev Respir Dis. 1992;145:906-10.
4. Holzer CT, Von Itzstein M, Jin B, Pegg MS, Stewart WP, Wu WY. Inhibition of sialidases from viral, bacterial and mammalian sources by analogues of 2-deoxy-2, 3-didehydro-N-acetylneuraminic acid modified at the C-4 position. Glycoconjugate journal. 1993 Feb;10(1):40-4.
5. Chandrasekaran K. Abintio and DFT investigation of C4 and C7 position of sialidase anti-viral inhibitor, Journal of Chemical and Pharmaceutical Research, 2015 Aug; 7(4): 425-430.
6. Karplus PA, Faerman C. Ordered water in macromolecular structure. Current opinion in structural biology. 1994 Oct 1;4(5):770-6.
7. Goodford PJ. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. Journal of medicinal chemistry. 1985 Jul;28(7):849-57.
8. Kim CU, Lew W, Williams MA, Liu H, Zhang L, Swaminathan S, Bischofberger N, Chen MS, Mendel DB, Tai CY, Laver WG. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. Journal of the American Chemical Society. 1997 Jan 29;119(4):681-90.
9. Chong AK, Pegg MS, Taylor NR, Von ItzsteiN M. Evidence for a sialosylcation transition-state complex in the reaction of sialidase from influenza virus. European journal of biochemistry. 1992 Jul;207(1):335-43.
10. Chandrasekaran, K. Quantum chemical investigation of C12 and C6 position of Oseltamivirsialidase antiviral inhibitor, Journal of Applied Pharmaceutical Science, 2015; 5(09): 120-123.
11. Stoll V, Stewart KD, Maring CJ, Muchmore S, Giranda V, Gu YG, Wang G, Chen Y, Sun M, Zhao C, Kennedy AL. Influenza neuraminidase inhibitors: structure-based design of a novel inhibitor series. Biochemistry. 2003 Jan 28;42(3):718-27.
12. AndzelmJ, Kolmel C, Klamt A, et al. Incorporation of solvent effects into density functional calculations of molecular energies and geometries. Journal of Chemical. Physics, 1995; 103: 9312-9315.
13. M.J.Frisch GW, Tucks, HB, Schlegel GE, Scuseria MA, Robb JR, Cheeseman VG. Zakrzewski JA, Montgometry RE. Stratmann, JC, Burant S. Dapprich JM. MillamAD, Daniels KN, .Kudin MC. Strain O. Farkas J, Tomasi V. Barone M. Cossi R, Cammi B,. Mennucci C, Pomelli C, Adamo S, Clifford J .Ochterski GA, Peterson PY, Ayala Q, Cui K, Morokuma DK. .Malick AD, Rabuck K,.Raghavachari JB. Foresman J, Cioslowski, JV, Ortiz BB. Stefanov G, Liu A. Liashenko P. Piskorz I, .Komaromi R,.Gomperts RL,. Martin DJ. Fox T. Keith MA. Al-LahamCY, Peng A,. NanayakkaraC, Gonzalez M, Challacombe PM, Gill BG,. Johnson W. Chen MW, Wong JL, Andres M, Head-Gordon ES, Replogle, JA. Pople, Gaussian 03,Gaussian,Inc., Pittsburgh, PA, 2003.
14. Hemalatha K, Chakkaravarthi V, Murthy KG, Kayatri R, Girija K. Molecular Properties and Docking Studies of Benzimidazole Derivatives as Potential Peptide Deformylase Inhibitors. Asian Journal of Research in Chemistry. 2014;7(7):644-8.
15. Sarika V. Khandbahale, Kanchan R. Pagar, Rupali. V. Khankari. Introduction to Enzymes. Asian J. Res. Pharm. Sci. 2019; 9(2):123-130.
16. Faust R, Garratt PJ, Pérez MA, Piccio VJ, Madsen C, Stenstrøm A, Frølund B, Davidson K, Teh MT, Sugden D. 7-Substituted-melatonin and 7-substituted-1-methylmelatonin analogues: Effect of substituents on potency and binding affinity. Bioorganic & medicinal chemistry. 2007 Jul 1;15(13):4543-51.
17. Smith BJ, Colman PM, Von Itzstein M, Danylec B, Varghese JN. Analysis of inhibitor binding in influenza virus neuraminidase. Protein Science. 2001 Apr;10(4):689-96.
18. Kairys V, Baranauskiene L, Kazlauskiene M, Matulis D, Kazlauskas E. Binding affinity in drug design: experimental and computational techniques. Expert opinion on drug discovery. 2019 Aug 3;14(8):755-68.
19. Khade S, Pallavi MP. A Computational Study Of Molecular Docking For Antiviral Compounds Against Sars-Cov-2. Int J Pharm Bio Sci. 2022 Jan;13(1):37-51.
20. Alyar S, Şen T, Özmen ÜÖ, Alyar H, Adem Ş, Şen C. Synthesis, spectroscopic characterizations, enzyme inhibition, molecular docking study and DFT

6. AUTHOR CONTRIBUTION STATEMENT

Corresponding author of this article prepared and compiled the manuscript with data interpretation and no other co-authors have been involved in this work. Quantum chemical calculation and level of theory of current article were performed by corresponding author. The corresponding author discussed extensively the methodology and computational methods of this research article.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

calculations of new Schiff bases of sulfa drugs. Journal of Molecular Structure. 2019 Jun 5;1185:416-24.
21. Nadzirin IA, Chor AL, Salleh AB, Rahman MB, Tejo BA. Discovery of new inhibitor for the protein arginine deiminase type 4 (PAD4) by rational design of α-enolase-derived peptides. Computational Biology and Chemistry. 2021 Jun 1;92:107487.
22. Chandrasekaran K. Investigation of solvation dynamics and Hydration pattern of sialic acid by ab initio and DF method. Int J. Pharm Sci Res. 2020 May;11(5):2482-2488.
23. Magee CA, Peterson LW, Cafiero M, Selner EF. The effects of ligand charge, orientation and size on the binding of potential inhibitors for aldehyde dehydrogenase. Computational and Theoretical Chemistry. 2020 Sep 1;1185:112868.