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Original Article

Insilico Docking Studies and the Anti-Microbial Potential of Sydnone Derivatives

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Abstract:
The sydnone heterocyclic nucleus founds an important class for new drug development. Molecular Docking study is a key tool in Computer Aided Drug Designing. The main objective of this work is to perform preliminary docking screening using SAR studies, Physicochemical Properties OSIRIS Molecular property Explorer, PASS prediction Activity spectra, and the Lipinski Rule of Five. This study is also an attempt to explore the antimicrobial activity of Sydnone derivative by Molecular docking with *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) via Autodock 4.2. Among all the Sydnone derivatives, compound 5b is the most effective and showed maximum binding energy (-8.8 K/Cal) showed interaction with ASP177, LEU70, GLN196, GLY193, CYS37.
Keywords: Sydnone Heterocyclic compounds, Antimicrobial, Molecular Docking

Introduction

The entire medicine sector is currently up against the challenge of advancing efficiency. The main challenges are the rising costs of inventive work and the correspondingly declining supply of new chemical entities. (NCEs). (NCEs). [1]

The reason for this advancement deficiency is absolutely not science. The unraveling of the human genome has prompted an abundance of medication targets. With in excess of 20,000 human qualities, the supposition will be that somewhere around 1,000 are altogether associated with the rise and course of sickness. Moreover, in light of the fact that every one of these qualities is connected to the capability of somewhere in the range of five and ten proteins, the end is that there may be 5,000 - 10,000 focuses for new medications [2].

Nevertheless, this consistent expansion in Research and Development, the quantity of new chemical entities (NCEs) launching at the market has really diminished emphatically. [3].

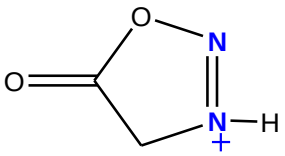
Mesoionic compound are a distinct type of heterocycles (5 or 6 membered) that belong to the class of non-benzenoid aromatic. Mesoionic are compounds having both delocalized negative and positive charges, for which a totally covalent structure cannot be drawn and which can entirely be represented by dipolar canonical formulas. Mesoionic compounds possess 2 or more heteroatoms such as oxygen, nitrogen and sulphur. The most important member of the mesoionic compound is the sydnone ring system. Sydnone is mesoionic compounds having the 1,2,3-oxadiazole skeleton bearing an oxygen atom attached to the fifth position. [4]

Sydnone

The word sydnone was generated from the phrase of 'The University of Sydney'(Sydney + lactone) where this class of compounds was first

discovered by Earl and Mackney in 1935. They proposed the formation of fused 3 and 4 membered ring product (**1**) from the action of acetic anhydride of N-nitroso phenyl glycine. [5].

Sydnones are chemically 1,2,3-oxadizolium– 5-olate (**1**), are exclusively, non-benzenoid heteroaromatic compounds that have bipolar canonical forms as in Figure 1 [6]



Sydnone (1,2,3 oxadiozolium-5-olate (**1**)

Figure 1 Sydnone Nucleus

Sydnone has a unique structure that lets it react with biomolecules like DNA and enzymes. It has both a positive and a negative charge, is aromatic, and has a high lipophilicity. So, Sydnone has a wide range of pharmacological effects, such as being antibacterial, analgesic, anti-arthritic, anti-cancer, cytotoxic, anti-malarial, anti-helminthic, anti-diabetic, anti-hypertensive, etc**.** [17-12]

The present work includes performing Pre - validation studies such as SAR Studies, Physicochemical properties was calculated using OSIRIS Molecular property explorer and Lipinski rule of 5 and PASS prediction, also performed Molecular docking studies against Staphylococcus aureus tyrosyl t-RNA synthetase (PDB ID 1JIJ).

Experimental

Software used

For the purpose of studying in silico docking studies, the following software and databases were used: Chemsketch (https://www.acdlabs.com /resources/freeware/chemsketch/), Drug discovery (BIOVIA), Chem Draw, PASS prediction, Pymol, MGL tools, Autodock 4.2, and Vina.

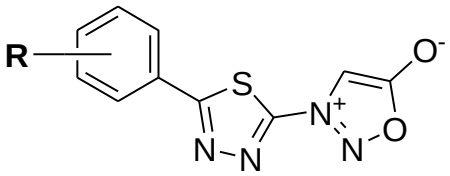
Methodology

In silico screening - In silico screening is currently widely utilized to track important variables that could help in assessing a compound's chemical and physical qualities.

Evaluation of Pre validation parameter:

1. **Structure-Activity Relationship [SAR] Studies [7-8]**

   It was done on the basis of a literature review, the most striking sites for substitution on the Sydnone nucleus are Nitrogen -1, Carbon -2, 5, and oxygen is present (Figure 2). [49-52]



5(a-c)

Figure 2 N-Substituted-1,3,4-thiadiazole – 2 - yl) - 2, 3-dihydro – sydnone.

2. OSIRIS Molecular Property Explorer–Using an automated Java-based programme, one can sketch the chemical structure and calculate attributes that are important to drugs. Table 1 and Figure 3 displays the predicted outcomes for all suggested compounds. The outcomes are shown in terms of drug score, c Log P, drug likeness, polar surface area (PSA), and risk factors. Table 1 shows properties in red that are more likely to have undesirable outcomes, such as mutagenicity or poor intestine absorption. A green color, on the other hand, denotes drug-consistent behavior. Using OSIRIS online, severe toxicity screening including tumorigenicity, mutagenicity, reproductive effect, and skin irritating effect was examined.[9]

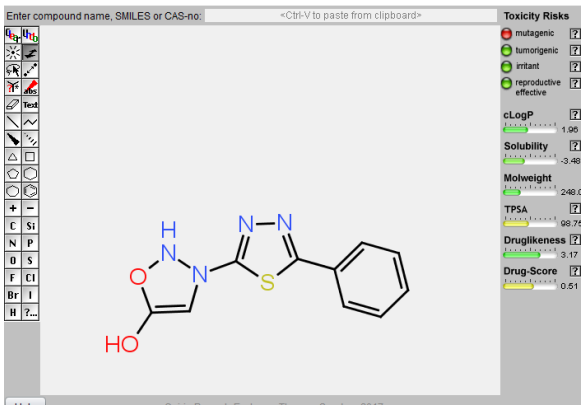
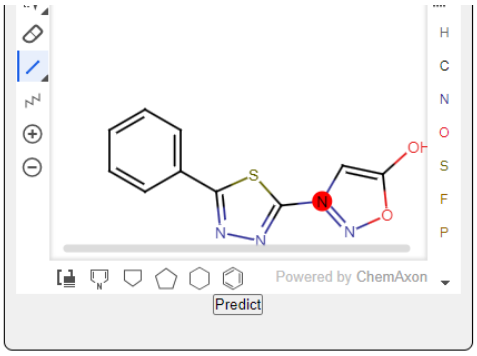


Figure 3 OSISRIS Molecular Property Explorer Properties

3. PASS Prediction – A built-in online platform called PASS (Prediction of Activity Spectra for Substances) is used to assess the general biological activity of any organic or synthetic drug-like compound [10]. In terms of *Pa* (*probability "to be active"*) and *Pi* (*probability "to be inactive*"), it also enables the structure to be sketched and the results to be calculated. All potential sydnone compounds' *Pa* and Pi values were displayed in Table 2 and Figure 4



0,193	0,080	Magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase inhibitor
0,211	0,097	TRPA1 agonist
0,186	0,074	Metallocarboxypeptidase D inhibitor
0,310	0,197	Thromboxane B2 antagonist
0,168	0,055	Phenylalanine decarboxylase inhibitor
0,216	0,104	Antibacterial
0,234	0,122	Nitrite reductase (NO-forming) inhibitor
0,187	0,076	Glucuronate isomerase inhibitor
0,212	0,100	L-threonine 3-dehydrogenase inhibitor
0,220	0,109	X-methyl-His dipeptidase inhibitor
0,184	0,073	Rhamnulose-1-phosphate aldolase inhibitor
0,182	0,071	4-Hydroxyglutamate transaminase inhibitor

Figure 4 PASS prediction proposed activity

3. Lipinski Rule of Five - "*Lipinski rule*" or "*rule of five*" was established by Lipinski [55] for in-silico studies. It was created in 1995 and published in 1997. This rule specifies the molecular characteristics necessary for a drug's pharmacokinetic qualities, including log P (Partition Coefficient), Molecular Weight, Hydrogen Bond Acceptor and Donor (HBD, HBA), and Total Polar Surface Area (PSA). The rule does not, however, foretell any of the molecule's pharmacological characteristics. In-silico investigations must follow the criterion when determining whether a compound has drug-like physiochemical characteristics and protein selectivity. [11].

According to the 'Rule of five' by Lipinski, a chemical must adhere to the following conditions in order to be orally bioavailable: -

S. No	Lipinski Parameters	Limits
1	Molecular Weight	180 – 500 Dalton
2	Log P [Partition Coefficient]	0.4 - 5.6
3	Hydrogen Bond Donor [HBA]	<5
4	Hydrogen Bond Acceptor [HBA]	<10
5	Polar Surface Area	<140 A^2
6	Molar Refractivity [MR]	40-130 m^3/mol

All these parameters were calculated by using Marvin Sketch 5.0 and chem Draw Software. All proposed compounds showed limits under the standard values of Lipinski 'Rule of Five' which are shown in Table 5.

Docking Analysis

Molecular docking is a widely used method in computer-assisted structure-based rational drug design. The Staphylococcus aureus tyrosyl t-RNA synthetase is the target macromolecule, and this study evaluates the interactions between the ligands (proposed sydnone derivatives). (PDB: 1JIJ) are connected. [12].

A set of automated docking tools called Auto Dock Tools (ADT) is intended to predict how tiny molecules would bind to a protein with a known 3D structure. The position of the ligand in the enzyme binding site can be seen in these docking studies in addition to producing binding energies [69]. It can be utilized to create possible ligands and understand how binding works [69–70]. In order to determine how well sydnone derivatives bind to Staphylococcus aureus tyrosyl t-RNA synthetase, docking studies were carried out. (PDB: 1JIJ) [13].

The Protein Data Bank (http://www.pdb.org/pdb/home/home.do) was used to retrieve the crystal structure of Staphylococcus aureus tyrosyl t-RNA synthetase (PDB: 1JIJ) (Figure 5). The resolutions were 2.92 and 2.70, respectively. The improved ligand molecules were docked into an improved Staphylococcus aureus tyrosyl t-RNA synthetase enzyme model using "Ligand Fit" in Autodock 4.2. [14]. **Figure 5 B** shows the Ligplot/2D Interaction of a common ligand with enzyme (1JIJ) from the PDB sum, which shows every amino acid residue in the active **pocket.**

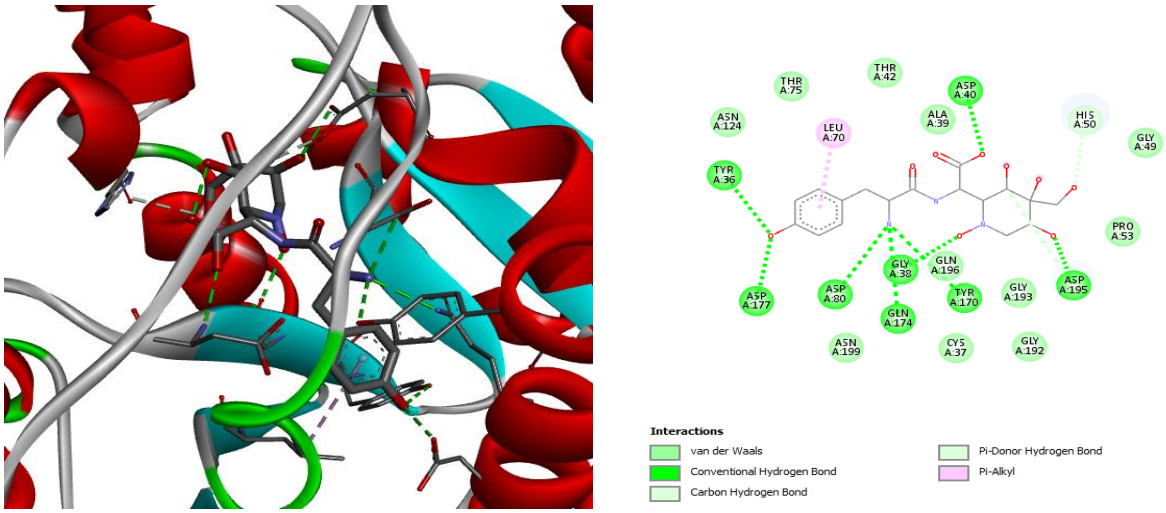


Figure 5 A Crystal structure of *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB: 1JIJ) [Downloaded from https://www.rcsb.org/structure/1JIJ retrieved on 12/02/2023] and B. 2D Interaction of standard Ligand results for 1JIJ showing all amino acid residues

All proposed sydnone derivatives were produced in Chem Draw 3D. The 3D structures of the ligands were converted to PDBQT using Autodock 4.2, and energy minimization was applied to each molecule. Using Autodock 4.2, a grid box comprising all amino acid residues (Ser 165, Val 236, Thr 237, Met 233, and Glu 108) was created for the Staphylococcus aureus tyrosyl

t-RNA synthetase in association with SB-239629 (Standard Ligand). Figure 6 illustrates the center grid unit's measurements, which were -1.111 x 0.566 x 0.639. [15 -16] In order to conduct docking studies, Autodock Vina and the Lamarckian genetic algorithm were employed. The interface was examined using Discovery Studio and Autodock 4.2. Every conceivable interaction was shown in Table 5 and Figure 12.

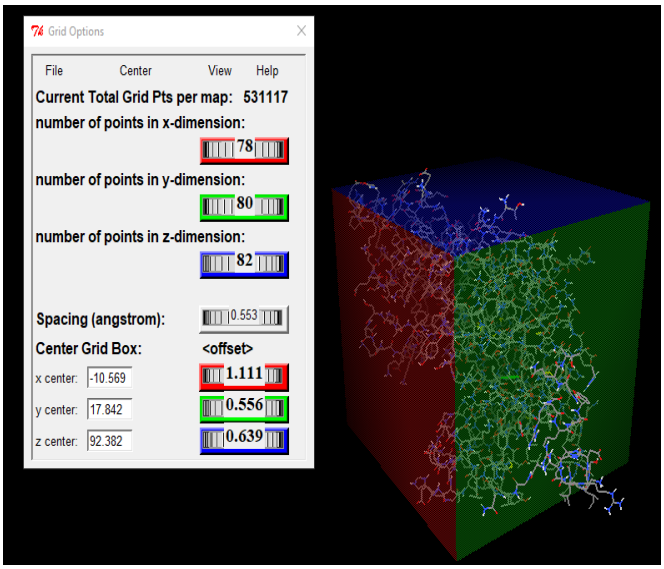


Figure 6. Grid box for the *Staphylococcus aureus* tyrosyl t-RNA synthetase (PDB ID: 1JIJ) with the standard ligand.

Result & Discussion

The present work consists of Molecular Docking Studies and the synthesis of Some Newer sydnone derivatives, as well as an investigation into their antimicrobial activity. Sydnone is a versatile pharmacophore that exhibits a wide range of biological activities. The activity spectrum includes antimicrobial, analgesic, anti-inflammatory, ulcerogenic, anticonvulsant, anticancer, antitumor, and anthelmintic properties. [17]

In Silico Docking Studies

Computed drug-like properties were used to optimize the lead of the chosen compounds. SAR studies, Molecular property prediction (OSIRIS property explorer), Prediction of Activity Spectra for Substances (PASS), and Lipinski's rule were utilized to evaluate the pre-validation of all compounds.

Pre validation Studies

1. SAR Studies – An appropriate substituent facilitates the development of molecules with prospective activity. As depicted in Figure 7. The Sydnone Nucleus SAR was used to develop new derivatives. [11,13]

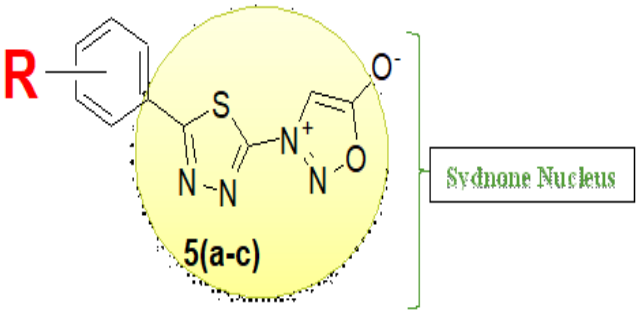


Figure 7 Pharmacophore basis of Sydnone Derivatives

- The aromatic ring is responsible for binding to the receptor.[13]
- Side chains are intended for further replacements.

- Two nitrogen presents in the thiadiazole ring contributes significantly to its antimicrobial properties.
- The fourth and third carbons of the sydnone ring are the primary substitution sites.
- The N-C-S linkage in 1,3,4 thiadiazole and the N-C-O linkage in the skeleton of Sydnone are responsible for the antimicrobial activity's broad spectrum.

2. OSIRIS Property Explorer (Molecular property Prediction) [14]

This is an online platform for drawing structures and calculating drug-relevant properties to determine whether or not a structure is valid. It describes the drug store, the drug-likeness, the solubility, and the toxicity studies of compounds. All results are displayed in Table 1, which indicates that the Drug Score of all compounds ranges from 0.51 to 0.55, Drug-likeness ranges from 3.17 to 3.29, and Solubility ranges from -3.48 to -3.51. Except for compound **5a,** which exhibited minimal mutagenicity, all compounds exhibited No Mutagenic Risk.

Table 1. OSIRIS Molecular Property Explorer Data

S. No.	Compound Code	OSIRIS (Molecular Property Explorer)					
		c log P	Drug Score	Drug Likeness	TPSA (A^2)	Solubility	Toxicity Risk
1.	5a	1.96	0.51	3.17	98.76	-3.48	Mutagenic
2.	5b	1.66	0.53	3.23	98.09	-3.39	No Risk
3.	5c	1.99	0.55	3.29	96.41	-3.51	No Risk
4.	Ciprofloxacin (Standard)	2.96	0.26	-2.08	92.31	-4.11	No Risk

3. PASS (Prediction of Substance Activity Spectra) [15]

It is a piece of software designed as an instrument for assessing the general biological potential of an organic molecule resembling a drug. Based on the structure of organic compounds, PASS generates predictions for multiple categories of biological activity. The calculated values of all compounds were interpreted in Table 2 as antimicrobial activity in terms of Pa (probability "to be active") greater than Pi (probability "to be inactive"). Compounds 5b have higher Pa values of 0,265 and 0,108.

Table:2. PASS Prediction data

S. No.	Compound Code	PASS Prediction	
		Antimicrobial	
		Pa	Pi
1.	5a	0,216	0,104
2.	5b	0,265	0,108
3.	5c	0,216	0,114
4.	Ciprofloxacin (Standard)	0,847	0,002

Pa = probability "to be active, Pi= probability to be inactive,

4. Lipinski 'rule of five' [16]

These parameters, including log P, Molecular weight, Hydrogen Bond donor (HBA), Hydrogen Bond acceptor (HBA), and Polar Surface Area (PSA), were predicted using Chemdraw and Marvin Sketch software. Log P ranges from 2.73 to 3.20, indicating that compounds have excellent cell membrane permeability. All compounds were found to have a Polar surface area of less than 140 A^o, indicating that they readily bound to the receptor or enzyme. All compounds have a molecular weight less than 500, and the number of

hydrogen donors is less than five (the sum of OHs and NHs) and the number of hydrogen bond acceptors is less than ten (the sum of Os and Ns) [55]. These parameters are summarized in Table 3, and all compounds adhere to the Lipinski Rule of Five.

Table 3 Data showing Lipinski Rule limits

S. No.	Compound Code	Lipinski Parameters						Lipinski Rule followed
		Mol. Weight	HBA	HBD	PSA(A^2)	log P	MR (m^3/ mol)	
1.	5a	246.25	2	0	30.18	2.73	69.48	Yes
2.	5b	262.22	2	0	30.18	3.33	74.26	Yes
3.	5c	280.60	3	0	39.41	2.57	75.94	Yes
4.	Ciprofloxacin (Standard)	265.01	4	2	92.31	3.20	73.97	Yes

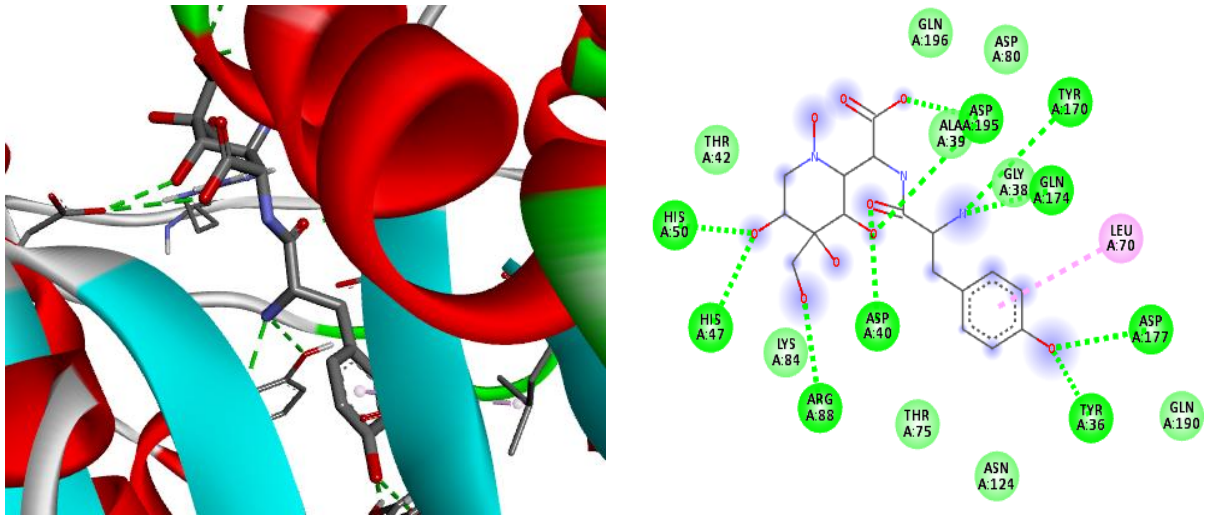
HBD =Hydrogen Bond Donor, HBA = Hydrogen Bond Acceptor, TPSA = Total Polar Surface Area, MR = Molar Refractivity.

Docking Analysis

Molecular docking is a frequently employed instrument in computer-aided structure-based drug design and can be characterized by the "Lock and key" mechanism. It can be defined as an **optimization** problem that identifies the optimal ligand-protein orientation. [15]

The optimal conformation was determined by minimizing the docking energy. Table 4 demonstrates the average binding affinity. As shown in Figures 8 A & B, discovery studio analyzed the interactions of a complex Staphylococcus aureus tyrosyl t-RNA synthetase enzyme (PDB ID: 1JIJ) protein and standard confirmation, including hydrogen bond and bond length.

Molecular docking predicts the binding relationship between macromolecules and a minor ligand. It is the examination of the prospective pharmacological effects of a compound prior to its actual synthesis. Understanding binding affinity is necessary to comprehend intermolecular interaction [89]. The binding affinity quantifies the intensity of the binding interaction. Therefore, binding affinity (kcal/mol) is used to classify virtual screening results [16]. The 1JIJ PDB was chosen for the investigation. Tyrosyl-tRNA synthetase catalyses the covalent bonding of amino acids to their respective tRNA and is essential for protein synthesis 17]. For comparing the binding affinities depicted in Figures 8, the ligand present in the protein molecule was used as a standard.



A **B**

Figure 8: 2D & 3D interactions of a ligand and Staphylococcus aureus tyrosyl t-RNA synthetase enzyme (PDB ID: 1JIJ)

Table 4: Docking Score and interaction of synthesized derivatives with 1JIJ.

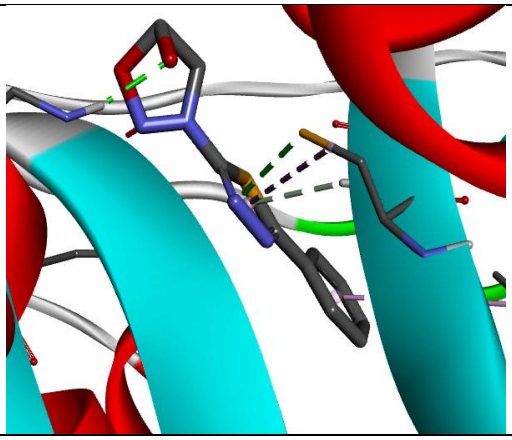
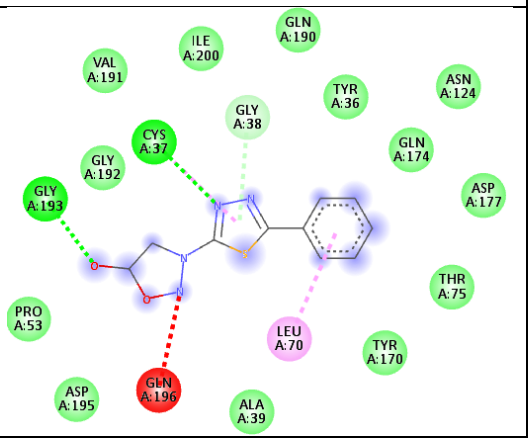
Compound ID	Binding affinity (kcal/mol)	Root Mean Square Deviation [RMSD]	Interacting Amino Acid
5a	-8.2	3.644	GLN196, CYS37, LEU70, GLY193, GLY38
5b	-8.8	3.489	ASP177, LEU70, GLN196, GLY193, CYS37
5c	-7.3	5.236	THR75, ASP177, ASP195, PRO53, ALA39, ASP177
Standard Ligand	-8.3	0.000	THR36, ALA39, ASP88, LEU70, THR75, LYS84, ARG88, ASN124, TYR170, GLN174, LEU70, ASP80

Figure 6 depicts the preparation of the binding domain as a grid box in Autodock, following the visualization of the binding interaction with the reference ligand in the discovery studio.

Figure 9 depicts the primary bonding interactions of all of the compounds in the binding pocket, and Table 5 depicts the binding affinity of sydnone derivatives to the target protein with amino acid interaction.

All Sydnone derivatives bind to the Staphylococcus aureus tyrosyl-tRNA synthetase (PDB: 1JIJ) with a higher affinity than the standard ligand SB-219383. The 2D and 3D interactions of N-substituted Sydnone derivatives with the active domain amino acid are depicted in Figure 12. The binding affinity of all compounds ranged from –8.2 to 8.8 kcal/mol for Staphylococcus aureus tyrosyl-tRNA synthetase, compared to –8.3 kcal/mol for the reference ligand SB-219383. This docking result indicates that Sydnone derivatives possess a higher affinity for the target protein. Among these three synthesize derivatives compound 5b showed better binding affinity with less RMSD as compared to a standard ligand.

Compound 3-(5-(4-hydroxy phenyl)-1,3,4-thiadiazol-2-yl)-2,3-dihydro-sydnone **5(b)** binds powerfully to the active compartment of tyrosyl-tRNA synthetase. The amino acids responsible for ligand binding and present in the binding domain were ASP177, LEU70, GLN196, GLY193, and CYS37. This amino acid and the ligand interact via carbon-hydrogen, Pi-cation, Pi-anion, alkyl Pi-alkyl, Van der Waals, and conventional hydrogen bonds. All of these interactions and binding affinity analyses indicate that the derivatives inhibit t-RNA synthetase protein more effectively than the reference ligand.

Compound ID	3d Interaction of Docked Pose	2D interaction of Docked Pose
5a		

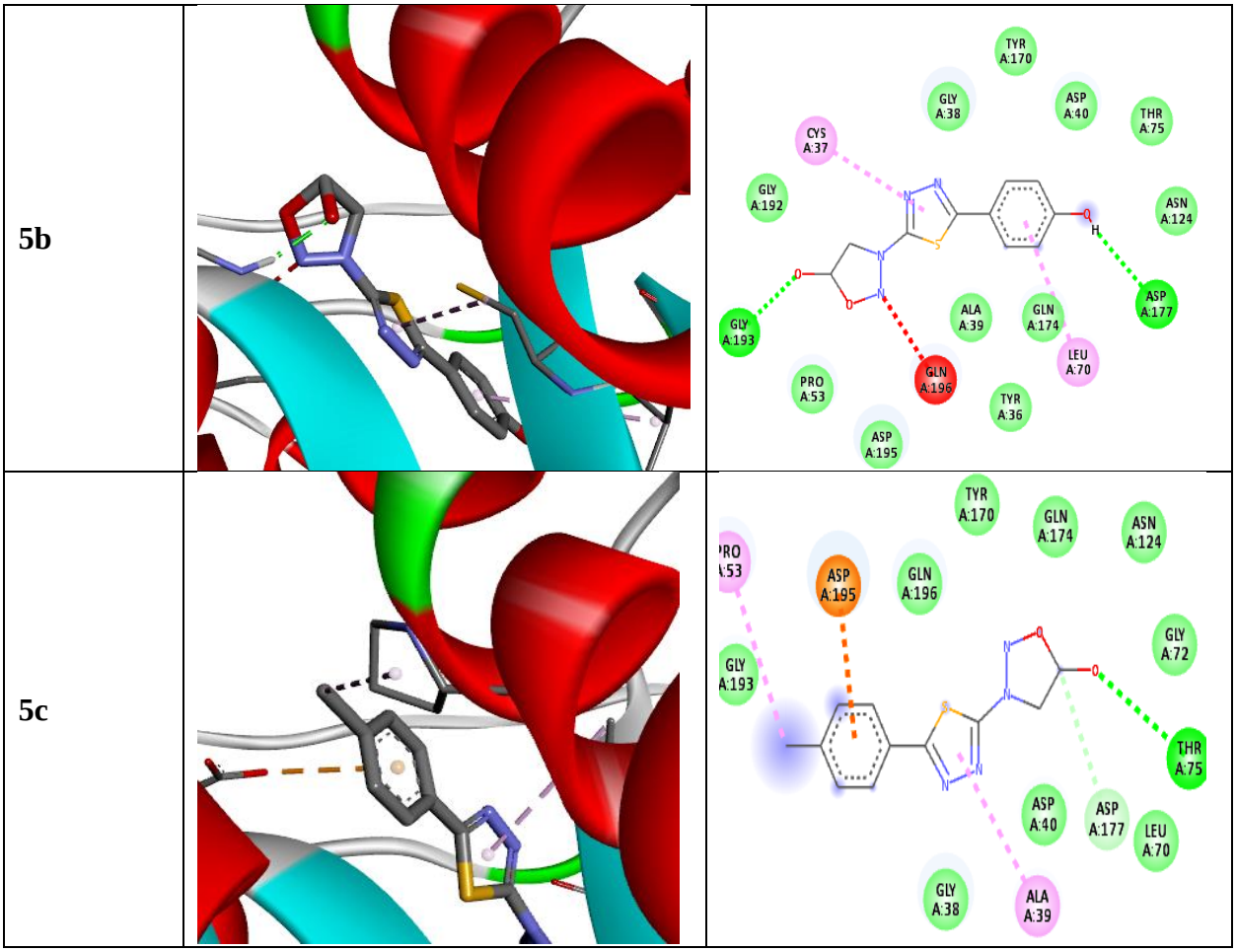


Figure 9 2D & 3D interactions of a Compound 5a, 5b, and 5c with Staphylococcus aureus tyrosyl t-RNA synthetase enzyme (PDB ID: 1JIJ).

Conclusion

Sydnones are very adaptable and robust heteroaromatic chemicals in the mesoionic class. They have a wide range of fascinating chemical and physical properties as well as biological functions.[21] The present work, which has been comprised of Molecular docking studies, synthesis and characterization of Sydnone derivatives. In this view, have made an attempt in reviewing the literature on substituted sydnone nucleus for their medicinal significance with help of chemical abstracts, journals, and various search engines. Finally, it was concluded that compound (3-(5-(4-hydroxy phenyl)-1,3,4-thiadiazol-2-yl)-2,3-dihydro-sydnone) (**5b**) is the most effective and showed maximum binding energy (-8.8 K/Cal) as compared to standard (-8.3 K/Cal)

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