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In silico Docking, ADME and Toxicity studies of Aryl Glyoxamide Derivatives as Anti-Virulence Agents

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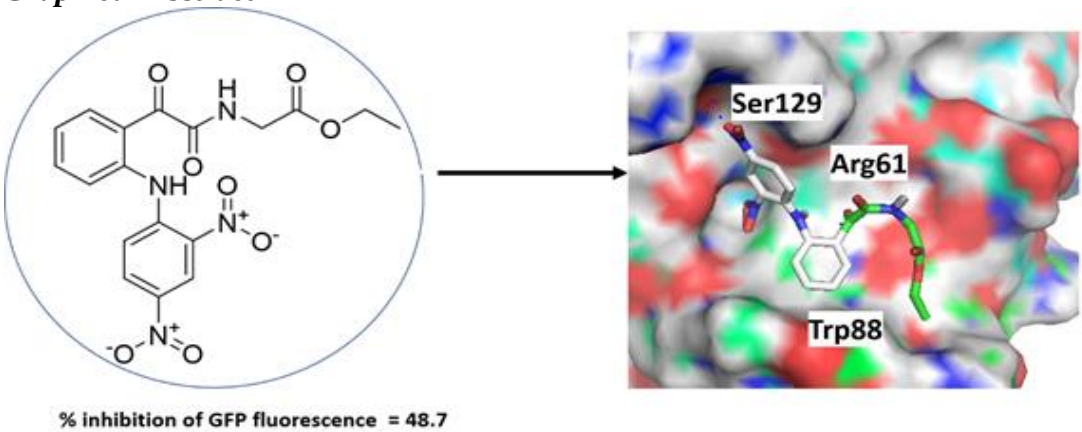
Abstract

The expression of phenotypic characteristics in bacterial species is regulated by the signalling mechanism called quorum sensing (QS). In current scenario, the quorum sensing inhibitors (QSIs) have established themselves as attractive leads which can be exploited to overcome antimicrobial resistance exhibited by various pathogenic bacteria. Aryl glyoxamide derivatives belong to one such class among several chemical classes which are known to inhibit the quorum sensing in P. aeruginosa (MH602) and E. coli (MT102). These derivatives are mostly designed using amino acid esters and found to exhibit fairly good activity and can act as promising leads for QSIs. However, in the field of drug design, the optimization of lead compounds with their activity profile plays a very crucial role. Due to ever growing demand of lead optimization/modification, the use of in silico drug design techniques proves to be most economical and best high throughput screening methods. In present study, QSTR (Quantitative Structure Toxicity Relationship), pharmacokinetic profiling (ADMET) studies and molecular docking studies were carried out on 21 N-Aryl glyoxamide derivatives. The studies implied that these derivatives had less probability to show hepatotoxicity and found to have good oral absorption profile. QSTR (Quantitative Structure Toxicity Relationship) studies performed by using TOPKAT (Toxicity Prediction Komputer Assisted Technology), showed that the compounds devoid of nitro substitution are non-carcinogenic and possess least probability of producing carcinogenicity and mutagenicity among computational models. The molecular docking studies suggest that autodock Vina and gold scores are comparable in determining the biological activity of synthesized compounds. The results indicated that the aryl glyoxamide class of compounds has substantial potential which can be exploited for the development of lead optimization in the field of QSIs.

Graphical Abstract



Keywords: *ADME, Aryl Glyoxamide, Molecular Docking, Quorum sensing, Toxicity studies.*

INTRODUCTION

Bacteria are living organisms. They are unicellular and have unique communication mechanisms. A bacterium was thought to be a self-contained bag of protoplasm that had no sense of community [1]. But this concept was shattered with the discovery of a microbial cell to cell signaling phenomenon known as quorum sensing (QS). *Vibrio fischeri* is a free-living, bioluminescent bacterium which shows initial sign of bacterial quorum sensing (QS). The QS circuit in this species is maintained by *lux* gene which produces bioluminescence [2]. The QS is a biological process developed by many pathogenic bacteria to do variety of functions in response to extracellular signal molecule called as 'Auto-inducers' (AIs) [3]. The bacterial population density is also a dependent parameter which controls by the QS mechanism. This process not only regulates the bacterial communication but also responsible for symbiosis, virulence, bioluminescence, antibiotic production and biofilm formation like processes among bacterial population [4-6].

The *in silico* drug design methods are vital tools used in the computational chemistry for designing potent scaffolds for the target protein. Among several approaches rational drug design methodology is highly fruitful as it increases the chances for lead discovery at low cost. This approach utilizes molecular docking technique which plays an important role in the judiciously designing compounds of interest [7]. Prediction of the correct binding modes of different ligands into a wide variety of receptor sites has remained the principal objective of the many ligand docking methods available today [8].

The present computational study uses Autodock Vina 1.1.2,(Trott and Olson 2010) [9] Argus lab(Thompson 2004) [10] and TOPKAT (Toxicity Prediction Komputer Assisted Technology) software for performing various computational analyses [11,12]. The 3D structure of molecules inherent varied physicochemical information which can be utilized in terms of Quantitative structure activity/toxicity relationship (QSAR/QSTR) studies. The present study utilizes the QSTR for accurately and rapidly assessing the toxicity of chemical compounds solely from their 2D molecular structure. It uses a range of robust, cross validated quantitative structure-toxicity relationship (QSTR) models for assessing specific toxicological endpoints. TOPKAT currently supports assessment of developmental toxicity potential (DTP), mutagenicity (Ames test), NTP carcinogenicity, skin sensitization (GPMT), Rat Oral LD50, Inhalational LC50, Aerobic biodegradability and Ocular irritation. It also easily predicts pharmacokinetic profiling of drugs. In the present study, ADME and toxicity profiles of all the novel derivatives was studied thoroughly [13].

MATERIALS AND METHOD

Drawing and Cleaning of Chemical Structures

Chem Bio Draw Ultra version 12.0 (2010) (Cambridge Soft, Chem Bio Office Ultra, 2010) [14] was used for the drawing and geometry optimization of the two dimensional (2D) structures of the aryl glyoxamide derivatives.(Ultra 2001)

Energy Minimization and Geometric Optimization

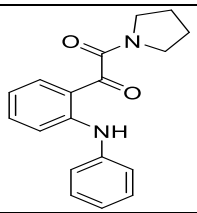
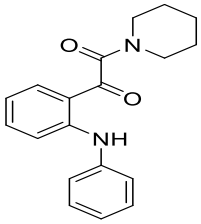
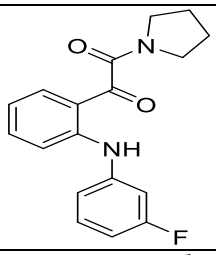
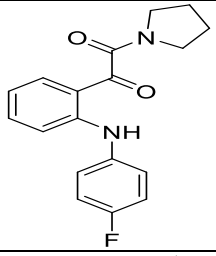
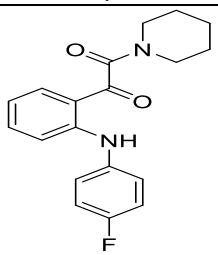
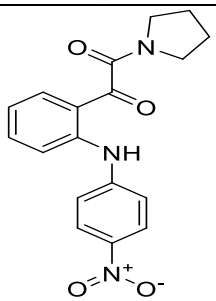
Energy minimization and geometric optimization involves a systematic and repeated modification and evaluation of the various atomic coordinates of a molecule to find a stable conformation of the molecule. This molecular modelling technique has application in predicting the binding site and key amino acid residue which are involved in forming the drug receptor interaction.

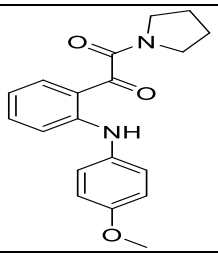
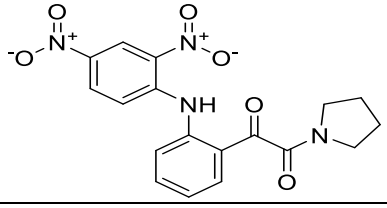
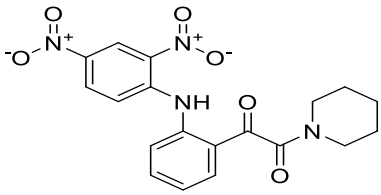
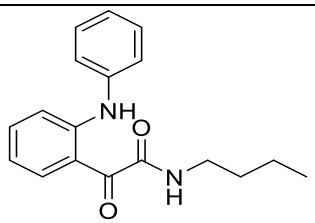
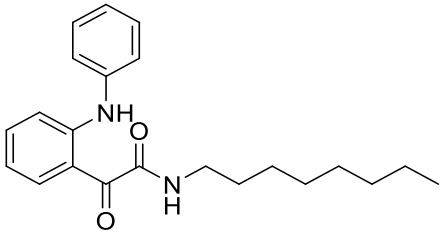
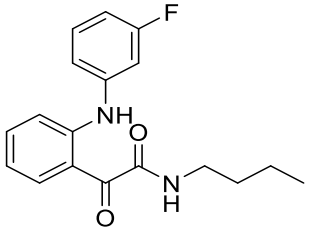
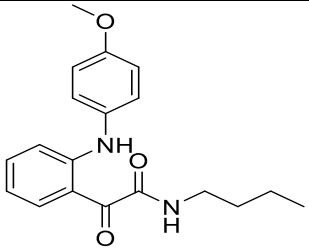
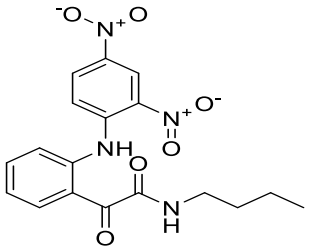
The 2D-structures were first transformed into three dimensional (3D) structures

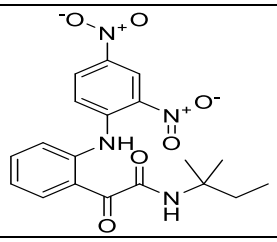
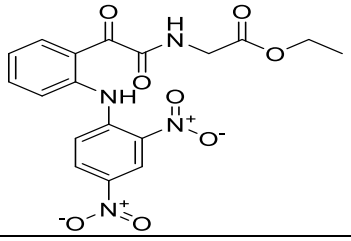
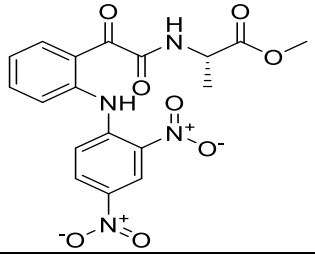
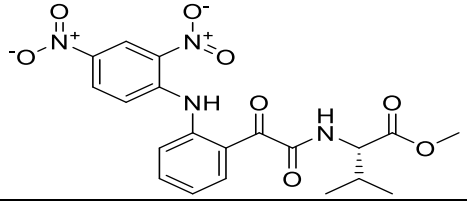
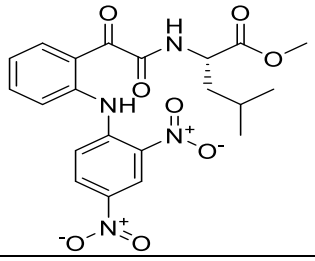
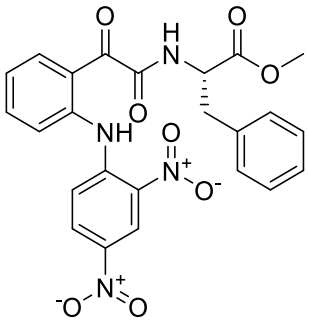
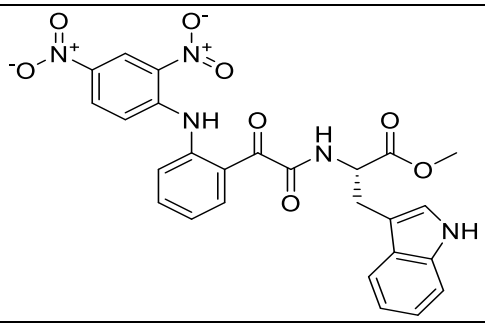
using the converter module of ChemBio3D Ultra 12.0 [14]. After the conversion, energy minimization of the structures was carried out using molecular mechanics 2 (MM2) force field which is in-built in ChemBio3D Ultra version 12.0 (2010) software to obtain stable conformer with minimum energy of each molecule. These structures were further minimized by Argus lab software using Parameterization3 (PM3) method.

Ligand Data Set

Table 1: *A list of data set of 21 aryl glyoxamide derivatives used in this study [16].*

S. No.	Compound	% inhibition of GFP fluorescence in *P. aeruginosa* MH602
1		20.3
2		16.6
3		22.7
4		24.0
5		20.8
6		30.1

7		32.1
8		22.7
9		23.1
10		17.9
11		13.8
12		24.9
13		28.5
14		30.4

15		25.7
16		48.7
17		42.7
18		33.4
19		37.3
20		31.3
21		35.2

21 compounds having aryl glyoxamide framework, as listed in Table 1, were used as the ligand data set. These compounds were taken from the report of Nizalapur *et al.* of 2016 [15] where they have reported the synthesis of these compounds and tested their biological activity on QS circuit of *P. aeruginosa* and *E. Coli* pathogens. (Nizalapur, Kimyon et al. 2016) [16] Table 1 contains the 2D structure of various aryl glyoxamides derivatives (**1-21**) along with their % inhibition values of GFP fluorescence in *P. aeruginosa* MH602 at 250 µM concentration [16].

Molecular Docking

Molecular docking studies aimed at understanding the binding profile of the aryl glyoxamide derivatives in the binding site pocket of QS receptor protein (LasR). The crystal structure of protein (PDB ID: 2UV0) taken from RCSB-PDB (Research Collaborators for Structural Bioinformatics-Protein Data Bank) (http://www.rcsb.org) was used as the receptor for performing docking studies [17]. Molecular docking was carried out using AutoDock Vina 1.1.2 software (Trott and Olson 2010) [9] (Scripps Research Institute La Jolla, CA, USA) under the following three important steps as per reported in its manual.

Receptor Preparation

The 3D structure of target protein generally retrieves from the Protein Data Bank [17].Then it is selected as a template for docking. There are some steps need to be done before docking like removal of water molecule as they may mask the protein surface from the ligand. If protein molecule contains more than one chain and binding site so other chains need to be deleted. The polar hydrogen were added up and partial atomic charges (Kollman Charges) were assigned. This is followed by adding AD4 atom types to all the atoms present in the molecule.

Ligand Preparation

In ligand preparation, module of Autodock the hydrogen's and Gasteiger charges were added up and later on the all the atoms of ligand were assigned AD4 atom type [18]. This is followed by the generation of different conformations of the ligand by setting of various torsions across the rotatable bonds in the structure of the ligand.

ADME Studies

Computer aided ADME studies have been done by using the software Accord for Excel (Accelrys Discovery studio software). These studies are solely based on the chemical structure of the molecule. Some of the parameters that are calculated using Accord for Excel includes Atom based Log P98 (A LogP 98), Absorption level (Abs level), Aqueous solubility (AQ sol.), Blood brain barrier level (BBB level), Cytochrome P4502D6 (CYP2D6), Hepatotoxicity level (Hepatox level), and Plasma protein binding level (PPB LEV). The structures provided in Table 1 are used for performing ADME studies [19].

Virtual Toxicity Studies

The molecular structure of the desired/query compound (1-21) is given in sdf format and a desired TOPKAT predictor for its analysis was selected. If the structure is a member of the training set, the database information for the compound is displayed. The virtual toxicity studies which are done in the present study using TOPKAT are NTP carcinogenicity call (male mouse), NTP carcinogenicity call (female mouse), ames mutagenicity and developmental toxicity potential studies.

RESULTS AND DISCUSSION

Binding Affinity Studies against LasR QS Receptor Target Protein

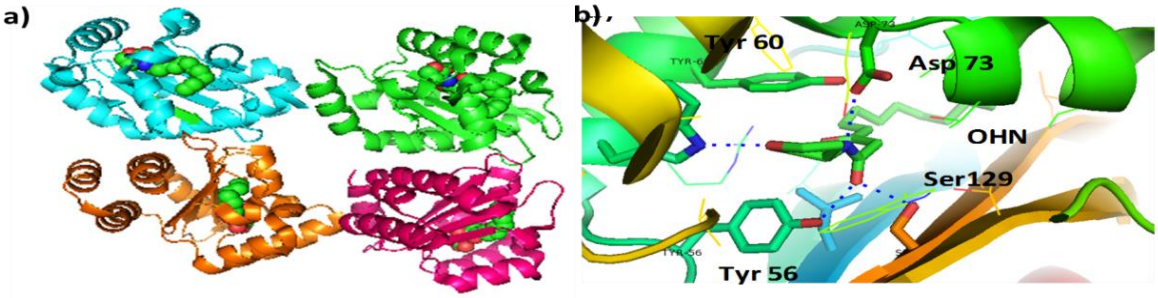
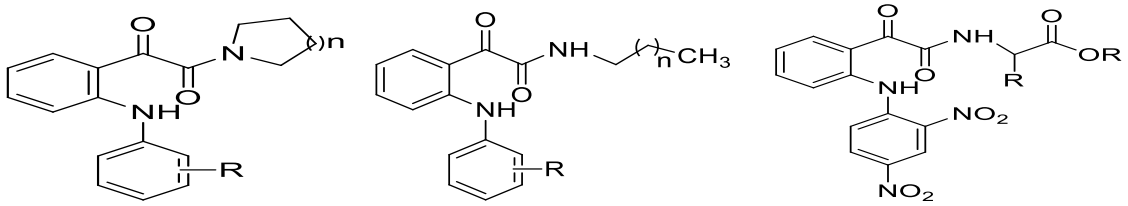


Figure 1: *The 3D-ribbon representation of LasR (PDB Id: 2UV0) protein and b) docked pose of co-crystallized ligand N-3-oxo-dodecanoyl-L-homoserine lactone (OHN).*

The aim of the molecular docking study was to elucidate the binding affinity and docking pose of aryl glyoxamide derivatives against QS target protein LasR of *P. aeruginosa*. The native ligand re-docking study indicates that the software predicts the reliable results as shown in Fig.1. Thereafter, the predicted active derivatives were subjected to molecular docking studies.The docking results provided pertinent information about the binding affinity, binding energy and orientation of ligand-receptor interactions. The docking results are summarized in Table 2.

Table 2: *List of Gold Score and Autodock Vina docking score of the 21 aryl glyoxamide derivatives.*



(1 – 9) **(10 – 15)** **(16 – 21)**

S.no	R	n/OR	Gold Score[a]	AutodockVina docking Score (kcal/mol)
1	H	1	49.9	-7.8
2	H	2	50.1	-6.5
3	3-F	1	49.4	-6.4
4	4-F	1	50.0	-9.2
5	4-F	2	47.7	-6.7
6	4-NO_2	1	48.6	-9.8
7	4-OCH_3	1	51.2	-6.1
8	2,4-dinitro	1	44.3	-6.8
9	2,4-dinitro	2	43.1	-6.6
10	H	2	68.1	-5.8
11	H	6	67.6	-6.2
12	3-F	2	66.8	-6.0
13	4-OCH_3	2	70.5	-8.2
14	2,4-dinitro	2	51.4	-6.1
15	2,4-dinitro	3	55.4	-6.0
16	Glycine	OEt	59.8	-9.0
17	L – Alanine	OMe	55.1	-8.6
18	L – Valine	OMe	49.6	-7.9
19	L – Leucine	OMe	53.6	-8.2
20	L – Phenylanine	OMe	58.0	-7.5
21	L – Tryptophan	OMe	65.8	-8.1

[a] *Data obtained from ref. 15*

It is concluded from the results that molecules showing percentage GFP inhibition in the range of 13−20% have shown docking scores ranging from -5.8−7.8, however another set of compounds which are showing inhibitory activity from 20−30% are reflecting docking scores of -6.0−8.6 kcal/mol. However, compounds showing inhibition above 30% is binding with the affinity of -6.1−9.8. The compound no. 16 is displaying maximum activity due to the presence of electron withdrawing nitro groups and the keto amide.

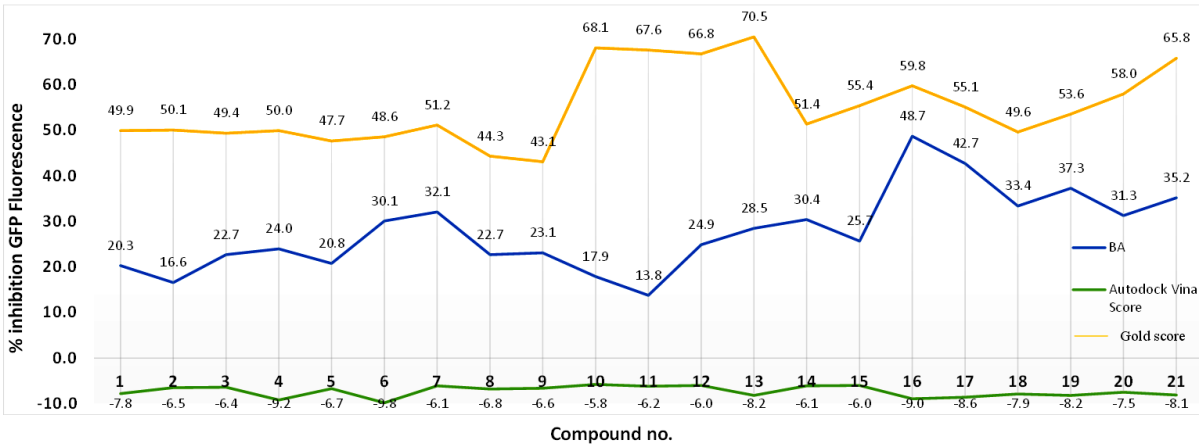


Figure 2: *A comparative analysis of Gold score, Autodock Vina docking score and the percentage inhibition of GFP fluorescence s against the P. aeruginosa MH602.*

It is found that the Gold scores reported by Nizalpaur *et al.*(Nizalapur, Ho et al. 2016) [15] Is comparative to the docking score which we get by using Autodock Vina (Fig. 2). The key amino acid residues found to interact with the ligand site are Ser129, Trp88, Trp60, Tyr56. The hydrogen bonding thus plays a major role in governing the stability of ligand-receptor complex in the active site pocket of LasR QS protein. It is in line with the results reported by Stacy *et al.*[20] where they have proven that hydrogen bonding is the key factor in the recognition of ligand at the receptor site.

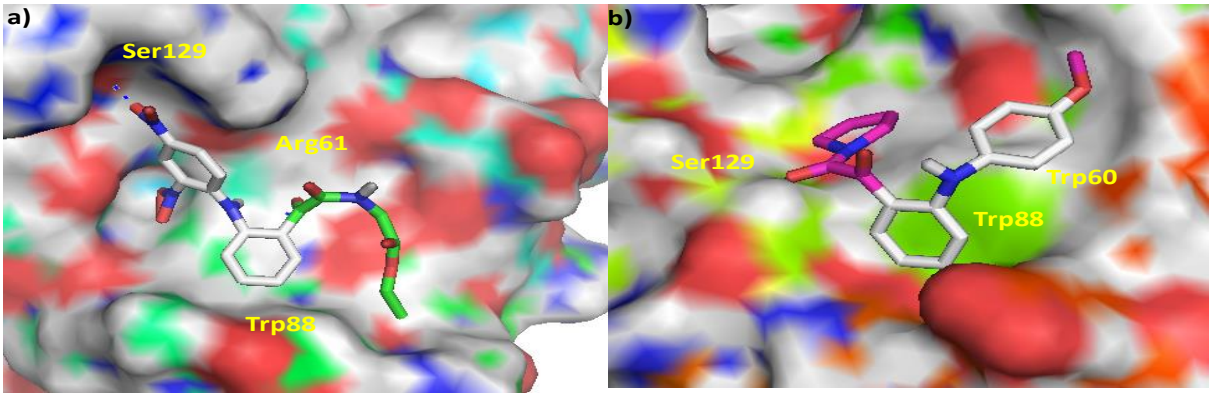


Figure 3: *A docked pose of a) compound **16** and b) compound **7** inside the active site pocket of 2UV0.*

The docked pose of active compounds (16 and 7) was shown in Fig. 3. The hydrogen bonding plays a crucial role in stabilizing the docked pose of compound. The compounds are also stabilized by hydrophobic amino acid residues Trp88 and Trp60.

Virtual Toxicity Studies

The toxicity profiles calculated for all the compounds are tabulated in Table 3 and 4.

Table 3: *Virtual toxicity data of investigated compounds.*

S. No.	Models			
	NTP Carcinogenicity call (Male mouse)		NTP Carcinogenicity call (Female mouse)	
	Discriminant score	Computed probability	Discriminant Score	Computed Probability
1	-8.473	0.000	-7.879	0.000
2	-8.010	0.000	-8.144	0.000
3	2.996	0.952	0.943	0.720
4	3.169	0.960	0.712	0.671
5	3.694	0.976	0.460	0.613
6	2.519	0.925	-5.201	0.005
7	-2.705	0.063	-2.650	0.066
8	-7.662	0.000	-7.396	0.001
9	-6.971	0.001	-7.637	0.000
10	-11.210	0.000	-8.637	0.000
11	-7.081	0.001	-9.324	0.000
12	-1.316	0.211	0.101	0.525
13	-5.535	0.004	-3.470	0.030
14	3.083	0.956	-6.931	0.001
15	1.906	0.871	-6.657	0.001
16	3.920	0.981	-10.119	0.000
17	-7.631	0.000	-1.065	0.256
18	14.419	1.000	-2.162	0.103
19	-2.183	0.101	-3.609	0.026
20	-46.077	0.000	14.795	1.000
21	-66.820	0.000	23.834	1.000

Values in red indicate the compounds with maximum toxic potential, blue indicate minimal chances of exhibiting toxicity while, values in black show zero probability of producing toxic effects in experimental models

Table 4: *Virtual toxicity data of investigated compounds.*

S.no	Models			
	Ames mutagenicity		Developmental toxicity potential	
	Discriminant score	Computed probability	Discriminant score	Computed probability
1	-24.074	0.000	-24.350	0.000
2	-24.814	0.000	-21.282	0.000
3	-15.807	0.000	-17.049	0.000
4	-16.253	0.000	-17.871	0.000
5	-16.983	0.000	-14.875	0.000
6	-17.576	0.000	-20.925	0.000
7	-19.015	0.000	-20.599	0.000
8	-10.635	0.000	-15.254	0.000
9	-11.446	0.000	-12.487	0.000
10	-20.523	0.000	-19.643	0.000
11	-23.281	0.000	-13.230	0.000
12	-12.478	0.000	-12.806	0.000
13	-15.503	0.000	-15.821	0.000
14	3.935	0.981	-7.547	0.001
15	5.091	0.994	-11.730	0.000
16	3.422	0.968	-6.294	0.002
17	9.608	1.00	-10.328	0.000
18	15.594	1.000	-21.613	0.000
19	-4.611	0.010	-15.148	0.000
20	14.046	1.000	22.730	1.000
21	12.002	1.000	13.6222	1.000

Values in red indicate the compounds with maximum toxic potential while, values in black show zero probability of producing toxic effects in experimental models

TOPKAT carcinogenicity predictor experimentation studies estimate that the compounds carrying nitro groups are more liable for probable toxicity especially in NTP carcinogenicity call (Male mouse) and Ames mutagenicity test models as shown by their positive detrimental scores (+1.91−+14.05) and computed probability values of above 0.87. However, the compounds which are devoid of nitro groups and have H or methoxy (OCH_3) substitution (compounds ranging from 1−9) are showing negative discriminant score with almost zero probability of exhibiting toxicity. In this category, also the compounds having fluorine substitution (3−5) have shown probability of producing toxicity in NTP carcinogenicity call (Male mouse) model. Most of the compounds are found to be safe against developmental toxicity potential model which predicts the safety profile of compound for consumption by pregnant women. TOPKAT studies concluded that although dinitro groups have maximum biological activity but they are more liable to act as a carcinogen and a mutagen. So, the bioisosteric replacement of nitro groups can be tried so as to avoid the toxic liabilities, these groups can be oxadiazole, furanone and a nitrile motifs which can produce electronically similar environment inside the active site of the receptor.

ADME studies/Pharmacokinetics profiling studies

Pharmacokinetic profiling (ADMET) studies were carried out on all the 21 aryl glyoxamide derivatives. The first parameter in this study was hepatotoxicity score which predicts the hepatotoxic nature of the chemical compounds. The data shown in Table 4 implied that these derivatives had less probability to show hepatotoxicity as indicated by low scores (0 and 1) and found to have good oral absorption profile (scores between -1 to +6) as the scores which is depicted in Table 5 is under the 95% absorption ellipse. Most of the compounds in this series is having score of 4 for the blood brain barrier (BBB) parameter. This parameter gives the idea that which compound is more liable to cross the BBB and can cause CNS effects. So, if the score is high the compound is having least probability to cross BBB, however the compound having score 1 is having 5:1 ratio of its concentration in brain: blood. The aqueous solubility (AQ sol) level of most of the compounds was found to be 2 and 3 which indicates very little aqueous solubility.

Table 5 *Computer aided ADME data of the investigated aryl glyoxamide derivatives.*

S.no	Descriptor	Compounds																				
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
1	Hepatox level	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
2	BBB level	1	1	1	1	1	3	2	4	4	4	1	1	1	2	4	4	4	4	4	4	4
3	Abs level	0	0	0	0	0	0	0	2	2	2	0	0	0	0	3	3	3	3	3	2	3
4	AQ sol	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	2	3
5	CYP2D6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0
6	PPB level	2	2	1	1	1	1	2	0	0	0	2	2	2	2	1	0	0	0	0	0	0
7	A logp98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2

The cytochrome P450 2D6 model predicts CYP2D6 enzyme inhibition using 2D chemical query structure as input. This model was developed from the diverse set of 100 compounds which are known to interfere with CYP2D6 inhibition. The model classifies compounds as either 0 or 1 for non-inhibitor or inhibitor and provides an average-class-value estimate of confidence. Most of compounds are having score 1 in this category so it can be said that they can act as inhibitors of

CYP2D6 enzyme. The highly active compound 16, is having score 0 and thus it is believed that it may not interfere with CYP2D6.

Plasma protein binding model (PPB) is a parameter which can measure the binding of drug with the plasma proteins. This parameter is also dependent on the scores of atom based A logp98. If the score is 0 as shown by compounds 8−10 and 16−21, it indicates that these compounds have low probability of binding with the plasma protein (< 90 %). Score 1 indicates that the compounds are binding with > 90 % value. However, beyond this if score is 2, it acts as a measure of high protein binding compounds (> 95 %). The plasma protein drug complex is the measure of how much drug is available for undergoing metabolism and excreted from the body. The bound drug can remain as a reservoir in the body which can be slowly released and thus alter the biological half-life.

CONCLUSION

In current scenario, the quorum sensing inhibitors have established themselves as attractive leads which can be exploited to overcome antimicrobial resistance exhibited by various pathogenic bacteria. Aryl glyoxamide derivatives belongs to one such class among several chemical classes which are known to inhibit the quorum sensing in *P. aeruginosa*. These derivatives are mostly designed by using amino acid esters and found to exhibits fairly good activity and can act as promising leads for quorum sensing inhibitors. However, in the field of drug design the optimization of lead compounds with their activity profile plays a very crucial role due to ever growing need of lead optimization.

The thorough investigation of result of docking studies showed that the compounds having two nitro substitution are binding with the greater affinity with the LasR receptor. These compounds have shown greater biological activity however TOPKAT studies have shown that these molecules have liability to show mutagenic effects in Ames mutagenicity models. The ADME analysis done using various parameters mainly predicts that the compounds are having low hepatoxicity scores and fairly good oral absorption. The aqueous solubility of the investigated compounds is found to be low and some of them may interfere with CYP2D6 enzyme and can cause toxic effects. However, the most active compound 16, is falling in safer zone as ADMET parameters are concerned. With, these encouraging results, the compounds belonging to this scaffold can be further explored for structural modification and detailed investigations can be made to arrive at possibly newer potent agents with better therapeutic activity.

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COMPETING INTERESTS

Authors declare that no competing interest exists.

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