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Original Article

Targeting Amide Herbicides by KARI of *Staphylococcus aureus*- an *in silico* Analysis

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ABSTRACT

Background: Herbicides are classified either by toxicity or by mechanism of action, based on the chemical nature of the compound. Herbicides fall into two categories. Contact the herbicide and the transferred herbicide. Herbicides have been selected because they are highly toxic to plants and less toxic to animals and humans, but the main concern is the direct toxic effects of herbicides on animals. Since ketol acid reductoisomerase (KARI) is considered an acceptable target for most amide herbicides, this study conducted an *in-silico* analysis of KARI from *Staphylococcus aureus* against eight amide herbicides, obsessed with investigating by performing virtual molgro molecule docking. **Materials and Methods:** The *ilvC* gene encoding KARI from *Staphylococcus aureus* was amplified and its sequence and chimera were checked using the GenBank project`s CHIMERA CHECK program. It uses the BLAST algorithm and the GenBank database to compare the environment sequence with the GenBank sequence to find evolutionary relatives. Homology modeling of *Staphylococcus aureus* ketol acid reductoisomerase (KARI) was performed because its three-dimensional structure revealed by either X-ray crystallography or NMR studies was not available. The generated model was used to repeat the energy minimization cycle many times using SPDBV software, and the final model was also used to perform docking analysis against the amide herbicide used in the inhibitor study. **Results:** The amplified DNA fragment containing 1005 base sequence BLAST hit, shows an absolute open reading frame encoding the *Staphylococcus aureus* protein KARI. PROSITE method shows active site residues Gln28, Leu79, Leu80, Asp82, Ala106, His107, Pro129, Lys130, Gly131, Pro132, Glu186, Asp190, Glu194, Cys199. The alignment of the protein sequence Ketol acid reductoisomerase (KARI) from *Staphylococcus aureus* and template >1NP3A had a chain length of 327. The three-dimensional structure of *Staphylococcus aureus* ketol acid reductoisomerase (KARI) was predicted using SPDBV. The generated model used SPDBV software to repeatedly repeat the energy minimization cycle, so the final model received a stereochemical evaluation. After energy minimization, the energy of the protein model is 1.10 KJ / mol, which fits the Ramachandran diagram. Docking studies of *Staphylococcus aureus* ketol acid reductoisomerase (KARI) were initiated using the inhibitors reported in the literature. This model was then used to dock to various ligands, amides that act as herbicides. *In silico* models have demonstrated that this enzyme is effective against amide herbicides. **Conclusion:** On the idea of the docking scores, these ligands (amide herbicides) were assigned the results of favorable interactions between the compounds, and therefore the situation of KARI to search out more impregnable candidates out of the screened ligands, optimization of those amides should be extended further.

Keywords: Amide herbicides, KARI, *S. aureus*, Docking.

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INTRODUCTION

It is pretty important to apply agrochemicals globally for reinforcing agricultural produce.[1-2] Their utilization isn`t handiest intended for controlling the pests however additionally to deliver safety towards infections due to microbes[3] in the cutting-edge cultivation of lands, there was an exploitation of numerous sorts of herbicides, insecticides, and pesticides.[4-5] Agrochemicals employed within the control of unwanted plants (weeds) are called herbicides or weedkillers.[6] The herbicides are categorized supported via way of means of chemical shape via way of means of their mode of action, advanced via way of means of the Herbicide Resistance Action Committee then the Weed Science Society of America.[7] The amide herbicides sometimes grouped with the chloroacetamide's possess diverse biological properties. Dimethenamid (Frontier®), registered in 1993, could even be a selective preemergence herbicide that controls the foremost grass weeds, broadleaf's and yellow nuts that come on corn, soybeans, peanuts, dry beans, and sorghum.[8] Amide herbicides like alachlor and metachlor intervene with each protein and supermolecule synthesis.[9] The important poisonous mechanisms of amide herbicides appear to be same in each flora and animals. A completely precise component of plant biochemistry is moreover centered with the aid of using an herbicide; however a unique form of the biochemical receptor ought to also be critical in uncovered animals.[10]

KARI, Ketol-acid reductoisomerase (EC1.1.1.86), additionally known as acetohydroxy acid isomeroreductase is a probable goal for herbicides.[11] Potent and selective inhibitors of KARI display herbicidal activity.[12] KARI is the second enzyme of the parallel chain aminoalkanoic acid pathway[13] which catalyzes an uncommon two-step response performing each as an isomerase and as a reductase concerning an alkyl migration (acetoin rearrangement) and a ketone discount of (S)-2-acetolactate (S2AL) to yield (R)-2,3-dihydroxy-isovalerate. Within the isomerase reaction, S2AL is rearranged via an Mg-dependent methyl migration to produce 3-hydroxy-3-methyl-2-ketobutyrate (HMKB).[14] Within the reductase reaction, this 2-ketoacid undergoes an M^{2+}-dependent (Mg^{2+}, Mn^{2+} or Co^{2+}) discount with the aid of using NADPH to yield 2,3-dihydroxy-3-methyl butyrate whose product is the precursor of every valine and leucine.[15] The third branched-chain chemical compound, isoleucine, is produced during a pathway that parallels that of valine, employing the identical series of enzymes, with KARI catalyzing the conversion of

2-hydroxy-2-ethyl-3-ketobutyrate to 2, 3-dihydroxy-3-ethyl butyrate and pyruvate.[16] These reactions occur at a single site on the enzyme without dissociation of any reaction intermediate. It's an important enzyme, which participates in the synthesis of building block amino acid.[17] KARI is the target of the experimental herbicides Hoe704[18] and IpOHA[19] which are thought to be transition-state intermediates of the alkyl migration step. KARI is encoded by the *ilvC* gene in bacteria.[11] Extensive research on KARI has been performed on the enterobacteria and thus the spinach (spinach) enzymes. KARI was also isolated from *Pseudomonas aeruginosa*,[20] barley,[21] wheat,[22] and rice.[23]

As it is well-known that KARI is additionally a target for the majority of the amide herbicides, therefore this study has been preoccupied to research the *in-silico* analysis of KARI of *Staphylococcus aureus*-herbicide (protein-ligand) interaction on eight selected amide herbicides.

MATERIALS AND METHODS

Isolation of DNA, amplification of *ilvC* gene and eluting DNA from agarose gel

Staphylococcus aureus DNA was prepared according to Protocol.[24] The ilvC gene encoding KARI from *Staphylococcus aureus* turned into amplified in a incredibly grasp cycler gradient (Eppendorf, Germany) programmed for five minutes. In the polymerase chain reaction, correct primers (ahead and reverse) (Table 1) had been used to make bigger the genome series of the open studying frame (ORF) of the gene. PCR conditions were extended at 94°C for 2 min, then at 94°C for 1 min, 60°C for 1 min, 72°C for 3 min, and a entire 30 cycles at 72°C for 10 min.[25] The amplified DNA was eluted through agarose gel electrophoresis at 60V.[26] Agarose gels stained with ethidium bromide were visualized underneath a UV transilluminator. A smooth razor blade turned into used to reduce out the portions of interest. Excess liquid turned into eliminated and agarose fragments had been additionally introduced to the spin column. The tube turned into centrifuged at 5500 rpm inside forty-five seconds to elude the DNA. The eluent turned into separated via way of means of migration on an agarose gel and tested for the presence of DNA stained with ethidium bromide the usage of a UV transilluminator. This DNA fraction was sequenced.[27]

Sequencing and Chimera Checking

The eluted PCR products had been subjected to a series reaction allocated with the ABI PRISM Dye Terminator Cycle Sequence Ready Reaction Kit (Applied Biosystems Inc., USA). However, all sequences showing 95% collection similarity to the prevailing GenBank sequences were checked the use of the GenBank project`s CHIMERA CHECK software program with default settings.[28] All consultant sequences include bands of various species.

Phylogenetic Placement

BLAST algorithms[29] and GenBank database[30] had been used to examine environmental sequences with GenBank sequences to look for evolutionary relatives.

Table 1: Primers used for Polymerase Chain Reaction.

Primers	Sequence
Forward	5'- GCCGCTAACTACTTCAATACACT-3'
Reverse	5'- CCACCCGCAACAGCAATACGTTT -3'

In silico Analysis of Ketol-acid Reductoisomerase (KARI) of *Staphylococcus aureus* by Homology Modelling

Staphylococcus aureus ketol acid reductoisomerase (KARI) has no 3D structure elucidated with the aid of using either X-ray crystallography or NMR studies. The DNA collection becomes translated with the aid of using publicity to p-BLAST. The protein series turns into subjected to PSI-BLAST at NCBI from the DNA series obtained with the useful resource of the usage of sequencing. Protein parameters have been analyzed using the Prosite tool (www.expasy.org/prosite). All protein parameters associated with aminoalkanoic acid composition, secondary shape prediction, hydrophobicity, isoelectric point, etc. had been analyzed. The generated version used SPDBV software[31] to copy the electricity minimization cycle many times, and the very last version becomes extensively utilized to carry out docking analysis.

Ligand Drawing

Inhibitors hired withinside the look at have been amides that are having the ability to behave as herbicides. The ligands have been searched towards PubChem and ChemSpider databases for the 2D systems so with the help of open babel [http://openbabel.org/wiki/Main_Page]. These 2D structures had been converted to 3-d structures. ISIS/Draw may be a user-fine drawing package deal that allows the drawing of chemical structures with identical specific signs and signs and symbols employed in paper sketches. ISIS/Draw is generally a 2D drawing software program aleven aleven though it`s prepared with some 3-d rotation capabilities and may interface with Rasmol for 3-d visualization and rendering. The drawn form modified was imported into the TSAR software program software and converted proper right into a 3-d form, minimizing the strength of all molecules.[32] A strength-minimized ligand form modified into used for docking (Figure 1) (Table 2).

Docking of Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* with certain Amide herbicides

The 3D shape of goal Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* turned into generated via way of means of homology modeling the use of SPDBV (Swiss Protein Data Bank Viewer) at 2.6 Å RMSD resolution. The 3-D systems of ligands which have been acquired have been minimized the use of Hyperchem`s MM^+ field. Molegro Virtual Docker V4.2 turned into accustomed hit upon the lively webweb sites and docking turned into executed via way of means of moldock characteristic, that is an implementation of evolutionary algorithms (EAs), targeted on molecular docking simulations. Docking turned into executed with all of the capability lively webweb sites detected on Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* enzyme. During docking, the molecules have been organized and bonds, bond orders, express hydrogens, charges, and bendy torsions, have been assigned in the event that they have been lacking via way of means of the MVD software to each the protein and ligands. From

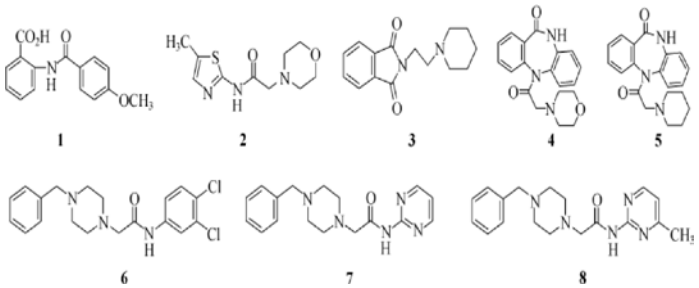

Figure 1: Amide Herbicides used as Ligands.

Table 2: **Amide Herbicides used as Ligands.**

Sl. No	Amide Herbicides
1	2-(4-Methoxy-benzoylamino)-benzoic acid
2	N-(5-Methyl-thiazol-2-yl)-2-morpholin-4-yl-acetamide
3	2-(2-Piperidin-1-yl-ethyl)-isoindole-1,3-dione
4	5-(2-Morpholin-4-yl-acetyl)-5,10-dihydro-dibenzo [1,4] diazepin-11-one
5	5-(2-Piperidin-1-yl-acetyl)-5,10-dihydro-dibenzo [b, e][1,4] diazepin-11-one
6	2-(4-Benzyl-piperazin-1-yl)-N-(3,4-dichloro-phenyl)-acetamide
7	2-(4-Benzyl-piperazin-1-yl)-N-pyrimidin-2-yl-acetamide
8	2-(4-Benzyl-piperazin-1-yl)-N-(4-methyl-pyrimidin-2-yl)-acetamide

the docking, wizard ligands have been decided on and additionally the scoring characteristic used is the Moldock score. The version turned into calibrated using a statistics set from the PDB bind database with recognized binding affinities (expressed in kJ/mol).[33]

Docking evaluation becomes achieved with the use of the default parameters of Molegro. Before docking, the protein shape becomes searched for viable cavities by the use of the hollow space detection set of rules of Molegro. a large sub-floor hollow space of 2266 Å resulted because the first hollow space, and additionally the closing viable 4 cavities are discovered to be very small. Hence, NADPH assured to 1NP3 become extracted and applied as a ligand for scenario identity on version protein. NADPH is permitted to rotate, vibrate and translate all instructed viable ranges of freedom, and additionally, the satisfactory pose becomes evaluated through supported dock rating or binding energies stated as kcal/mol. The test becomes achieved thrice.

The observations of each experiment were regularly recorded and documented.

RESULTS

DNA Extraction, Purification and Quantification

The DNA pellet of *S. aureus* become acquired after washing with 70% ethanol gave the impression of white, thick thread like mass. This DNA acquired become in addition quantified through spectrophotometry and agarose gel electrophoresis. It honestly becomes located that *S. aureus* DNA fragments had been located to emit orange fluorescence below UV lamp. The A260/A280 ratio for S. aureus DNA was found to be 1.9 spectrophotometrically. This indicates that the strategy followed for extraction, purification and quantification of DNA had been located to be appropriate for molecular research of *S. aureus*.

Amplification of *ilvC* gene of *S. aureus* by Polymerase Chain Reaction (PCR) and sequencing

The amplified fragment of DNA when analyzed by agarose gel electrophoresis (Figure 2) was of fine quality then the sample was eluted and sequenced.

Wells 1, 2, 3 and 4- Amplified DNA samples, Well M- 2kb DNA ladder

The sequence of the amplified product was as follows:

ATGACAACAGTTTATTATGATCAAGATGTAAAA
ACGGACGCTTTACAAGGCAAA
AAAATTGCAGTAGTAGGTTATGGATCACAAGGT
CACGCGCATGCACAAAAACTTA
AAAGACAATGGATATGATGTAGTCATCGGCATT
CGCCCAGGTCGTTCTTTTGACA
AAGCTAAAGAAGATGGATTTGATGTGTTCCCTG
TTGCAGAAGCAGTTAAGCAAG
CTGATGTAATTATGGTGCTATTACCTGATGAAAT
TCAAGGTGATGTATACAAAAA
CGAAATTGAACCAAATTTAGAAAAACATAATGC
GCTTGCATTTGCTCATGGCTTT
AACATTCATTTTGGTGTTATTCAACCACCAGCTG
ATGTTGATGTATTTTTAGTAGC
TCCTAAAGGACCGGGTCATTTAGTTAGACGTACA
TTTGTTGAAGGTTCTGCTGTA
CCATCACTATTTGGTATTCAACAAGACGCTTCAG
GTCAAGCACGTAATATTGCTT
TAAGTTATGCAAAAGGTATTGGTGCAACTCGTGC
AGGTGTTATTGAAACAACATT
TAAAGAAGAAACTGAGACAGATTTATTTGGTGA
ACAAGCAGTACTTTGCGGTGG
TGTATCGAAATTAATTCAAAGTGGCTTTGAAACA
TTAGTAGAAGCGGTTATCAA
CCAGAATTAGCTTATTTTGAAGTATTACATGAAA
TGAAATTAATCGTTGATTTGA
TGTATGAAGGCGGTATGGAAAATGTACGTTACT
CAATTTCAAATACTGCTGAATT
TGGTGACTATGTTTCAGGACCACGTGTTATCACA
CCAGATGTTAAAGAAAATATG
AAAGCTGTATTAACTGATATCCAAAATGGTAACT
TCAGTAATCGCTTTATCGAAG
ACAATAAAAATGGATTCAAAGAATTTTATAAAT
TACGCGAAGAACAACATGGTC
ATCAAATTGAAAAAGTTGGTCGTGAATTACGCG
AAATGATGCCTTTTATTAAATC TAAAAGCATTGAAAAATAA

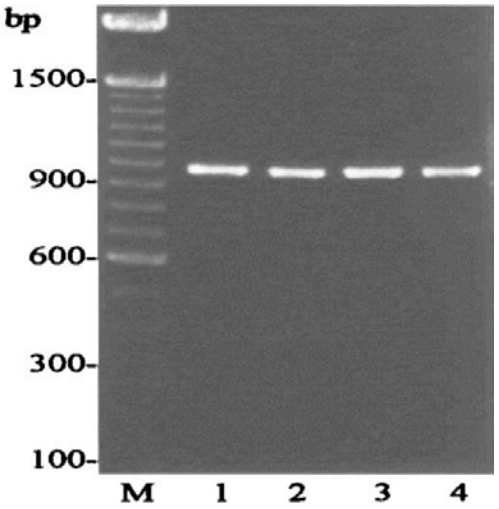

Figure 2: Agarose gel showing amplified ilvC gene of *S. aureus*.

The sequence of the amplified product contains 1005 bases. The sequence when made a BLAST hit, has evinced that it absolutely was having an open reading frame coding for the protein KARI of *S. aureus*.

Phylogenetic Tree of the *ilvC* Gene Constructed by BLAST of NCBI

The sequences received had been made BLAST hit with the sequences of NCBI database. Basing at the alignment occurred, aligned sequences on the maximum diploma had been decided on and consequently the phylogenetic was constructed (Figure 3).

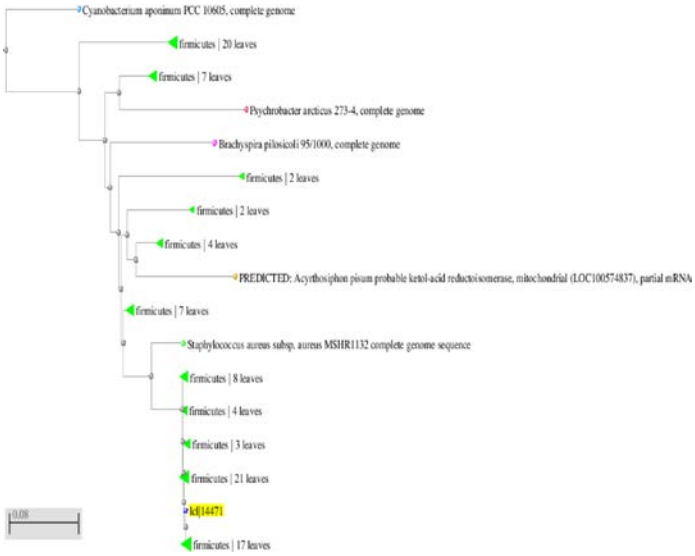

Figure 3: Phylogenetic tree of *S. aureus ilvC* gene.

In silico analysis of Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* by Homology modelling

The translated protein sequence was

M T T V Y Y D Q D V K T D A L Q G K K I A V V G Y G S Q G H A H A Q N L K D N G S Q G H A H A Q N L K D N G Y D V V I G I R P G R S F D K A K E D G F D V F P V A E A V K Q A D V I M V L L P D E I Q G D V Y K N E I E P N L E K H N A L A F A H G F N I H F G V I Q P P A D V D V F L V A P K G P G H L V R R T F V E G S A V P S L F G I Q Q D A S G Q A R N I A L S Y A K G I G A T R A G V I E T T F K E E T E T D L F G E Q A V L C G G V S K L I Q S G F E T L V E A G Y Q P E L A Y F E V L H E M K L I V D L M Y E G G M E N V R Y S I S N T A E F G D Y V S G P R V I T P D V K E N M K A V L T D I Q N G N F S N R F I E D N K N G F K E F Y K L R E E Q H G H Q I E K V G R E L R E M M P F I K S K S I E K

ProtParam

The parameters computed by ProtParam include the mass, theoretical pI, organic compound composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). The relative molecular mass and theoretical pI are calculated by computing pI/MW.[34]

The number of amino acids present within the Ketol acid reductoisomerase (KARI) of *S. aureus* was 325 with a molecular weight: 35597.9 whose theoretical pI was 9.82.

Amino acid composition

Ala (A) 25	7.5%
Arg (R) 12	3.6%
Asn (N) 15	4.5%
Asp (D) 20	6.0%
Cys (C) 1	0.3%
Gln (Q) 16	4.8%
Glu (E) 29	8.7%
Gly (G) 32	9.6%
His (H) 9	2.7%
Ile (I) 21	6.3%
Leu (L) 21	6.3%
Lys (K) 23	6.9%
Met (M) 8	2.4%
Phe (F) 19	5.7%
Pro (P) 13	3.9%
Ser (S) 14	4.2%
Thr (T) 13	3.9%
Trp (W) 0	0.0%
Tyr (Y) 12	3.6%
Val (V) 31	9.3%

The total number of charged residues (Asp + Glu) was 49 and therefore the total number of charged residues (Arg + Lys) was 35. The atomic composition was Carbon-1656, Hydrogen-2576, Nitrogen-442, Oxygen-503, and sulfur-9. The molecular formala is $C_{1656}H_{2576}N_{442}O_{503}S_9$ with total number of atoms of 5186.

Extinction Coefficients

This protein doesn`t incorporate any Trp residues. Experience suggests that this could lead to over 10% mistakes in the computed extinction coefficient. Extinction coefficients are in gadgets of M^{-1} cm^{-1}, at 280 nm measured in water. Extinction coefficient become 17880, Abs 0.1% (=1 g/l) 0.483, assuming all pairs of Cys residues shape cystines.

Estimated Half-life

The N-terminal of the sequence considered is M (Met). The estimated half-life is: 30 hr (mammalian reticulocytes, *in vitro*).

Instability Index

The instability index (II) is computed to be 38.66 which indicate protein stability. The aliphatic index was 83.44 and also the grand average of hydropathicity (GRAVY) was -0.304.

SOPMA

10 20 30 40 50 60 70
| | | | | | |

MTTVYYDQDVKTDALQGKKIAVVGYGSQGHAHAQNLKDN
GYDVVIGIRPGRSFDKAKEDGFDVFPVAEAV

hheeeccccchhhhttceeeeeeccccchhhhhhhhttceeeeeeccccchhhhhhttcee
ehhhhhh

KQADVIMVLLPDEIQGDVYKNEIEPNLEKHNALAFAHGFNIHFG
VIQPPADVDVFLVAPKGPGHLVRRTF

hhhheeeeeccccchhhhhhhhcccccttceeeeettceeeeeeccccccceeeeecttccccc
ehhhh

VEGSAVPSLFGIQQDASGQARNIALSYAKGIGATRAGVIETTFKEET
ETDLFGEQAVLCGGVSKLIQSGF

htttccceeeeeeccccchhhhhhhhhhhttcccceeeehhhhhhhhhhhhhhhheehtc
hhhhhhhhh

ETLVEAGYQPELAYFEVLHEMKLIVDLMYEGGMENVRYSISNTAE
FGDYVSGPRVITPDVKENMKAVLTD

hhhhhttcchhhhhhhhhhhhhhhhhhhhttchhhhhhhcchhhccccccccchhhh
hhhhhhhhhhhh

IQNGNFSNRFIEDNKNGFKEFYKLREEQHGHQIEKVGRELREMM
PFIKSKSIEK

hcttchhhhhhhhhttccchhhhhhhhhhhcchhhhhhhhhhhhhhhhttchhc

The Sequence duration became 334 whose Alpha helix (Hh) money owed for one hundred seventy amino acids of approximately 50.90%. The prolonged strand (Ee) had fifty-two amino acids accounting for

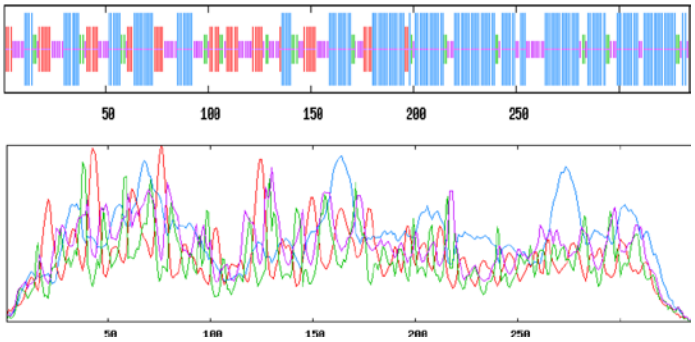

Figure 4: Significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments (SOPMA).

15.57%, Beta turn (Tt) manufactured from 28 amino acids making up eight.38%, and random coil (Cc) manufactured from eighty-four amino acids accounting for 25.15%. There became no 310 helix (Gg), Pi helix (Ii), Beta bridge (Bb), Bend region (Ss), Ambiguous states, and different states. The parameters had been window width of 17 with a similarity threshold of eight and consequently the quantity of states is 4[35] (Figure 4).

Thre-Dimensional Structure determination of KARI of *S. aureus*

PROSITE method (with gear and information) protected through this documentation for the lively web website online residues Gln28, Leu79, Leu80, Asp82, Ala106, His107, Pro129, Lys130, Gly131, Pro132, Glu186, Asp190, Glu194, Cys199. This become showed through acting Pfam-Protein Family analysis (http://pfam.sanger.ac.uk/family?acc=PF00704).[36] Alignment of Protein Sequence Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* and template >1NP3A with a sequence duration of 327 is as follows.

Score = 504 bits (1299), Expect = e-144, Method: Composition-based stats.

Identities = 185/327 (56%), Positives = 236/327 (72%), Gaps = 1/327 (0%)

Query: 2 TTVYYDQDVKTDALQGKKIAVVGYGSQGHAHAQNLKD NGYDVVIGIRPGRS-FDKAKEDG 60

V+YD+D +QGKK+A++GYGSQGHAHA NLKD+G DV +G+R G + KA+ G

Sbjct: 1 MRVFYDKDCDLSIIQGKKVAIIGYGSQGHAHACNLKD SG VDVTVGLRSGSATVAKAEAHG 60

Query: 61 FDVFPVAEAVKQADVIMVLLPDEIQGDVYKNEIEPN LEK HNALA FAHGFNIHFGVIQPPA 120

V V AV ADV+M+L PDE QG +YK EIEPNL+K LAFAHGF+IH+ + P A

Sbjct: 61 LKVADVKTAVAAADVVMILTPDEFQGRLYKEEIEPNLKK GATLAFAHGFSIHYNQVVPRA 120

Query: 121 DVDVFLVAPKGPGHLVRRTFVEGSAVPSLFGIQQDASG QARNIALSYAKGIGATRAGVIE 180

D+DV ++APK PGH VR FV+G +P L I QDASG A+N+ALSYA G+G R G+IE

Sbjct: 121 DLDVIMIAPKAPGHTVRSEFVKGGGIPDLIAIYQDASGN AKNVALSYACGVGGGRTGIIE 180

Query: 181 TTFKEETETDLFGEQAVLCGGVSKLIQSGFETLVEAGY QPELAYFEVLHEMKLIVDLMYE 240

TTFK+ETETDLFGEQAVLCGG +L+++GFETLVEAGY PE+AYFE LHE+KLIVDLMYE

Sbjct: 181 TTFKDETETDLFGEQAVLCGGCVELVKAGFETLVEAGYA PEMAYFECLHELKLIVDLMYE 240

Query: 241 GGMENVRYSISNTAEFGDYVSGPRVITPDVKENMKAV LTDIQNGN FSNRFIEDNKNGFKE 300

GG+ N+ YSISN AE+G+YV+GP VI + + M+ L IQ+G ++ FI + +

Sbjct: 241 GGIANMNYSISNNAEYGEYVTGPEVINAESRAAMRNAL KRIQDGEYAK MFITEGAANYPS 300

Query: 301 FYKLREEQHGHQIEKVGRELREMMPFI 327

R H IE++G +LR MMP+I

Sbjct: 301 MTAYRRNNAAHPIEQIGEKLRAMMPWI 327

An alternative method for locating the homologous protein i.e., the fold prediction method was used. There's an automatic server for protein modeling which searches the homologous protein by fold prediction and sequences are modelled with a high degree of accuracy. The generated model was subjected to many repeated cycles of energy minimization using SPDBV software and therefore the final model was subjected to stereochemical evaluation. The fold prediction method discovered Template 1NP3 from a protein data bank of the Crystal structure of sophistication I acetohydroxy acid isomer reductase from *Pseudomonas aeruginosa* was found to be the simplest homolog and modeling was distributed. The generated model was subjected to many repeated cycles of energy minimization using modeler software that's performed by the satisfaction of spatial restraints and also the final model was subjected to stereochemical evaluation. After Energy minimization, the energy of the protein model is found to be -1.10 KJ/mol which fits Ramachandran Plot (Figure 5).

Docking of ligands onto Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* with certain Inhibitors (Amides)

Docking studies of Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* had been initiated using inhibitors advised withinside the literature, precise as ligands 1 to 8 respectively because of the presence of a massive hole area withinside the protein, template docking end up completed through manner of way of considering NADPH as a template (Table 3, Figure 6). t were evinced that ligands 2, 3, 4 and 7 displayed susceptibility to not unusual place hobby with model protein even as ligands 1, 5, 6, and 8 represented moderate hobby even as located next to NADPH (dock score: -155.482 kcal/mol). It has been observed that ligands 2, 3, and 4 are destitute of any H-bond interactions, and the

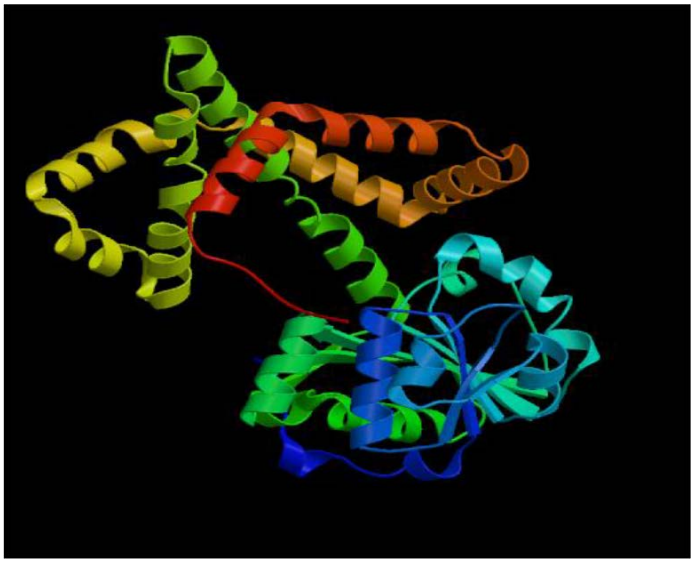

Figure 5: *In silico* 3-Dimensional Structure of KARI of *Staphylococcus aureus*.

Table 3: H-bond interactions and dock score (kcal/mol) of NADPH against KARI of *Staphylococcus aureus*.

Ligand	Dock score (kcal/mol)	H-bond interactions
NADPH	-155.482	Gln28, Leu79, Leu80, Asp82, Ala106, His107, Pro129, Lys130, Gly131, Pro132, Glu186, Asp190, Glu194, Cys199
Ligand 1	-72.979	Gln28, Lys130
Ligand 2	-57.076	Nil
Ligand 3	-57.636	Nil
Ligand 4	-64.524	Nil
Ligand 5	-70.126	Gln28
Ligand 6	-74.799	Asp82
Ligand 7	-62.147	Asp82
Ligand 8	-85.161	His107, Asp190, Glu194

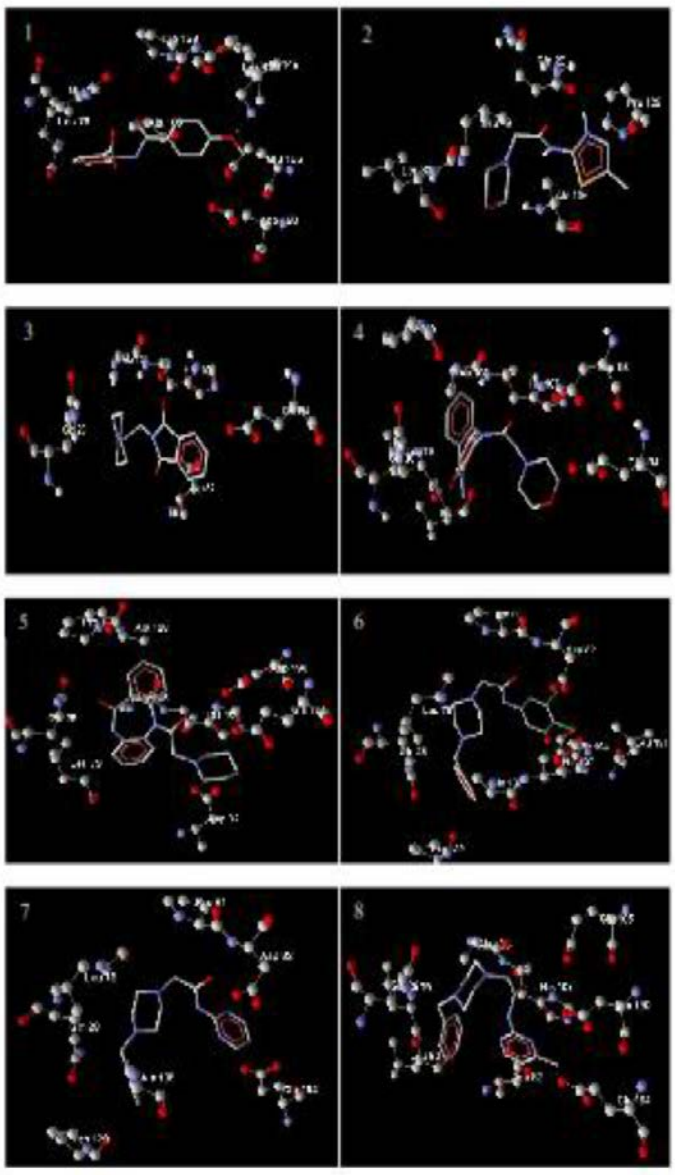

Figure 6: Docking of Amide Herbicides onto KARI of *Staphylococcus aureus*.

ultimate all showed one h-bond except ligand 8. The excessive score (-85. 161 kcal/mol) of ligand eight can be attributed to the mode of h-bond interplay with His107, Asp190, and Glu194 residues, respectively.

The three-dimensional shape Ketol-acid reductoisomerase (KARI) of *Staphylococcus aureus* become expected via way of means of the usage of SPDBV. Later via way of means of the usage of this version it have been docked via way of means of exceptional ligands which can be amides performing as herbicides. The *in-silico* version proved that this enzyme is green in opposition to amide herbicides.

DISCUSSION

The mechanism of action of herbicides plays a pivotal role in understanding the classification, organization, practices, and hierarchy of the herbicides. It's quite essential in understanding herbicide resistance, which happens to be a controversy in sustainable agricultural management. The overuse of herbicides, just like one of a kind of pesticides, has delivered approximately extra fantastic development of resistance among weeds, causing harm and destruction of useful flowers in agriculture, land management, and one of a kind of related industries. Supported the mode of action, it should target an enzyme or a metabolic pathway of a plant that the herbicide may work, either injuring or disrupting the plant growth and development and eventually leading to the death of the plant.[37-39] Efficacy of herbicides is related to an imbalance in carbon-nitrogen after comparing the physiological consequences of inhibitors of consecutive enzymes in the biosynthesis of branched-chain amino acids, acetolactate synthase (ALS), and ketol-acid reductoisomerase (KARI).[40] Free natural compound accumulation brought on via way of means of herbicides in wild-kind vegetation turned into alleviated in the mutants, indicating that vegetation that lack ADH1 or AOX1a are much less liable to the herbicides` phytotoxicity.

The indiscriminate use of herbicides with the equal mechanism of movement brought about the upward push of the choice pressure resulting withinside the producing of ever more resistant weeds in present day years. Therefore, novel herbicides with new biochemical purpose web sites are important to alleviate the choice pressure with the modified mechanism of movement withinside the plant`s metabolism.[41] The crystal systems of rice KARI – Mg2+ in complicated with 5 fragments from Zenobia library, 3-aminopyridine (ZT038), 3-hydroxypyridine (ZT042), 3-aminobenzonitrile (ZT389), 2-amino-four-methylphenol (ZT398) and 3, four-diaminotoluene (ZT393) had been solved to 1. five - 2. five Å resolution. The outcomes displays that ZT398 is sure close to the energetic web website online and the opposite fragments are sure at the floor of the protein. The binding web website online of ZT038 and ZT042 is close to the Lys105, part of the protein that can be essential for area movement at the same time as ZT393 binds close to the doorway of the energetic web website online. In addition, via the screening of a Maybridge fragment library, new rice KARI inhibitors, 3-[(2-thienylthio) methyl]benzoic acid (KM02425) and 2-hydroxy-2-phenylacetic acid (JFD3933) had been recognized with Ki values of 212 ± 132.four μM and 311.five ± 122.five μM, respectively. To higher apprehend the mode of binding of KM02425, the crystal shape of KM02425 in complicated with rice KARI – Mg2+ enzyme turned into decided to be 2. forty five Å. The outcomes display that this molecule binds inside the enzyme`s energetic web website online. Docking research had been additionally undertaken and recommend an opportunity mode of binding close to the energetic web website online for this molecule is possible.[42] KARI catalyzes the second common location step in branched-chain amino acid biosynthesis. The catalytic procedure includes steps, step one being the alkyl switch from one carbon atom to an adjoining atom. Since the capacity transition country is a cyclopropane by-product, the layout, and synthesis of a today's series of cyclopropane-carbonyl thiourea

derivatives that encompass a one-pot segment transfer catalyst reaction. The KARI inhibitory interest of those compounds turned into evaluated and 5-butyl substituted (3e) and 3-pyridinyl substituted (3n) compounds reached a hundred% at a hundred mg / ml. The structure-interest dating suggests that the long-chain by-product confirmed better KARI inhibitory interest. On the opposite hand, the substitution on the 4-role of the benzene ring confirmed better KARI inhibitory interest than the substitution on the 2-role and the 3-role. This turned into completed with the aid of using reading the interplay of compound 3n with the energetic webweb page of to be had spinach KARI. This turned into regular with the effects analyzed with the aid of using the frontier molecular orbital theory.[43]

CONCLUSION

KARI has a large cavity and template docking was also performed using NADPH as a template. Ligands 1, 5, 6, and 8 showed moderate activity when placed next to NADPH (dock score: 155.482 kcal / mol). It was observed that ligands 2, 3, and 4 had no hydrogen bond interactions and all but ligand 8 showed hydrogen bonds. The excessive rating of ligand 8 (85.161 kcal/mol) can be because of the character of hydrogen bond interactions with His107, Asp190, and Glu194 residues, respectively. However, efficacy studies designed to demonstrate the efficacy of these ligands for KARI *in vitro* and *in vivo* should be avoided and the conclusions should be postponed.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Kumar V, Kaur S, Singh S, Upadhyay N. Unexpected formation of N′-phenyl-thiophosphorohydrazidic acid O,S-dimethyl ester from acephate: Chemical, biotechnical and computational study. 3 Biotech. 2016;6(1):1. doi: 10.1007/s13205-015-0313-6, PMID 28330071.
2. Singh S, Kumar V, Chauhan A, Datta S, Wani AB, Singh N, *et al.* Toxicity, degradation and analysis of the herbicide atrazine. Environ Chem Lett. 2018;16(1):211-37.doi: 10.1007/s10311-017-0665-8.
3. Kumar V, Singh S, Singh R, Upadhyay N, Singh J. Design, synthesis, and characterization of 2,2-bis(2,4-dinitrophenyl)-2-(phosphonatomethylamino)acetate as a herbicidal and biological active agent. J Chem Biol. 2017;10(4):179-90. doi: 10.1007/s12154-017-0174-z, PMID 29075355.
4. Mishra V, Gupta A, Kaur P, Singh S, Singh N, Gehlot P, *et al.* Synergistic effects of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in bioremediation of iron contaminated soils. Int J Phytoremediation. 2016;18(7):697-703. doi: 10.1080/15226514.2015.1131231, PMID 26682583.
5. Benbrook CM. Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur. 2016;28(1):3. doi: 10.1186/s12302-016-0070-0, PMID 27752438.
6. USEPA. Pesticides industry, sales and usage 2006 and 2007: Market estimates archived. Vol. 2011; 2015.
7. Wood A. Compendium of pesticide common names; 2021. Available from: http://www.alanwood.net/pesticides/class_herbicides.html [cited 6/29/2022].
8. George WW, David MW. An introduction to herbicides. 2nd ed, Extracted from The Pesticide. 6th ed. Willoughby, OH: Published by MeisterPro Information Resources, A division of Meister Media Worldwide; 2004.
9. Au AM. Academic press. Pestic Herbicides|Types, Uses, and Determination of Herbicides, Encyclopedia of Food Sciences and Nutrition. 2nd ed. 2003;4483-4487. doi: 10.1016/B0-12-227055-X/00910-X.
10. Beasley VR. Direct and indirect effects of environmental contaminants on amphibians, reference module in earth systems and environmental sciences. Elsevier; 2020. doi: 10.1016/B978-0-12-409548-9.11274-6.
11. Dumas R, Biou V, Halgand F, Douce R, Duggleby RG. Enzymology, structure, and dynamics of acetohydroxy acid isomeroreductase. Acc Chem Res. 2001;34(5):399-408. doi: 10.1021/ar000082w, PMID 11352718.
12. Zabalza A, Zulet A, Gil-Monreal M, Igal M, Royuela M. Branched-chain amino acid biosynthesis inhibitors: Herbicide efficacy is associated with an induced carbon-nitrogen imbalance. J Plant Physiol. 2013;170(9):814-21. doi: 10.1016/j.jplph.2013.01.003, PMID 23394788.
13. Tyagi R, Lee YT, Guddat LW, Duggleby RG. Probing the mechanism of the bifunctional enzyme ketol-acid reductoisomerase by site-directed mutagenesis of the active site. FEBS Journal. 2005;272(2):593-602. doi: 10.1111/j.1742-4658.2004.04506.x, PMID 15654896.
14. Chunduru SK, Mrachko GT, Calvo KC. Mechanism of ketol acid reductoisomerase-steady-state analysis and metal ion requirement. Biochemistry. 1989;28(2):486-93. doi: 10.1021/bi00428a012, PMID 2653423.
15. Rane MJ, Calvo KC. Reversal of the nucleotide specificity of ketol acid reductoisomerase by site-directed mutagenesis identifies the NADPH binding site. Arch Biochem Biophys. 1997;338(1):83-9. doi: 10.1006/abbi.1996.9802, PMID 9015391.
16. Bastian S, Liu X, Meyerowitz JT, Snow CD, Chen MM, Arnold FH. Engineered ketol-acid reductoisomerase and alcohol dehydrogenase enable anaerobic 2-methylpropan-1-ol production at theoretical yield in Escherichia coli. Metab Eng. 2011;13(3):345-52. doi: 10.1016/j.ymben.2011.02.004, PMID 21515217.
17. Bayaraa T, Kurz JL, Patel KM, Hussein WM, Bilyj JK, West NP, *et al.* Discovery, synthesis and evaluation of a ketol-acid reductoisomerase inhibitor. Chemistry. 2020;26(41):8958-68. doi: 10.1002/chem.202000899, PMID 32198779.
18. Schulz A, Spönemann P, Köcher H, Wengenmayer F. The herbicidally active experimental compound Hoe 704 is a potent inhibitor of the enzyme acetolactate reductoisomerase. FEBS Lett. 1988;238(2):375-8. doi: 10.1016/0014-5793(88)80515-5, PMID 3049163.
19. Aulabaugh A, Schloss JV. Oxalyl hydroxamates as reaction-intermediate analogues for ketol-acid reductoisomerase. Biochemistry. 1990;29(11):2824-30. doi: 10.1021/bi00463a027, PMID 2189496.
20. Eom SJ, Ahn HJ, Yoon HJ, Lee BI, Bae SH, Baek SH, *et al.* Crystallization and preliminary X-ray crystallographic analysis of acetohydroxy acid isomer reductase from Pseudomonas aeruginosa. Acta Crystallogr D Biol Crystallogr. 2002;58(12):2145-6. doi: 10.1107/s0907444902015573, PMID 12454481.
21. Durner J, Knorzer OC, Boger P. Ketol-acid reductoisomerase from barley (Hordeum vulgare) (purification, properties, and specific inhibition). Plant Physiol. 1993;103(3):903-10. doi: 10.1104/pp.103.3.903, PMID 12231988.
22. Donadini R, Copeland L. Acetohydroxy acid reductoisomerase of wheat. Functional Plant Biol. 2000;27(5):417-23. doi: 10.1071/PP99181.
23. Lee YT, Ta HT, Duggleby RG. Cyclopropane-1,1-dicarboxylate is a slow-, tight-binding inhibitor of rice ketol-acid reductoisomerase. Plant Sci. 2005;168(4):1035-40. doi: 10.1016/j.plantsci.2004.11.020.
24. Tsai YL, Olson BH. Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Appl Environ Microbiol. 1992;58(7):2292-5. doi: 10.1128/aem.58.7.2292-2295.1992, PMID 1386212.
25. Volossiouk T, Robb EJ, Nazar RN. Direct DNA extraction for PCR-mediated assays of soil organisms. Appl Environ Microbiol. 1995;61(11):3972-6. doi: 10.1128/aem.61.11.3972-3976.1995, PMID 8526511.
26. Sambrook J, Fritsch ER, Maniatis T. Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1989.
27. Downey N. Extraction of DNA from agarose gels. Methods Mol Biol. 2003;235:137-9. doi: 10.1385/1-59259-409-3:137, PMID 12904656.
28. Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A, *et al.* Determinants of *Staphylococcus aureus* nasal carriage. Clin Diagn Lab Immunol. 2001;8(6):1064-9. Doi: 10.1128/CDLI.8.6.1064-1069.2001, PMID 11687441.
29. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, *et al.* Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997;25(17):3389-402. doi: 10.1093/nar/25.17.3389, PMID 9254694.
30. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, *et al.* GenBank. Nucleic Acids Res. 2013;41((Database issue)):D36-42. Doi: 10.1093/nar/gks1195.
31. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis. 1997;18(15):2714-23. Doi: 10.1002/elps.1150181505, PMID 9504803.
32. Shoichet BK, Kuntz ID, Bodian DL. Molecular docking using shape descriptors. J Comput Chem. 1992;13(3):380-97. doi: 10.1002/jcc.540130311.
33. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. J Med Chem. 2006;49(11):3315-21. Doi: 10.1021/jm051197e, PMID 16722650.
34. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, *et al.* (In). In: Walker JM, editor: The proteomics protocols handbook. Humana Press; 2005. p. 571-607.
35. Geourjon C, Deléage G. SOPMA: Significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Comput Appl Biosci. 1995;11(6):681-4. doi: 10.1093/bioinformatics/11.6.681, PMID 8808585.
36. Sigrist CJ, Cerutti L, Hulo N, Gattiker A, Falquet L, Pagni M, *et al.* PROSITE: A documented database using patterns and profiles as motif descriptors. Brief Bioinform. 2002;3(3):265-74. doi: 10.1093/bib/3.3.265, PMID 12230035.
37. Duke SO. Overview of herbicide mechanisms of action. Environ Health Perspect. 1990;87:263-71. doi: 10.1289/ehp.9087263, PMID 1980104.
38. Torrens F, Castellano G. Molecular classification of pesticides including persistent organic pollutants, phenylurea and sulphonylurea herbicides. Molecules. 2014;19(6):7388-414. doi: 10.3390/molecules19067388, PMID 24905607.

39. Varshney S, Hayat S, Alyemeni MN, Ahmad A. Effects of herbicide applications in wheat fields: is phytohormones application a remedy? Plant Signal Behav. 2012;7(5):570-5. doi: 10.4161/psb.19689. PMID 22516826.
40. Wang B, Ma Y, Li Y, Wang S, Li Z. The design, synthesis of amide KARI inhibitors and their biological activities. Front Chem China. 2009;4(2):186-90. doi: 10.1007/s11458-009-0021-z.
41. Duke SO. Why have no new herbicide modes of action appeared in recent years? Pest Manag Sci. 2012;68(4):505-12. doi: 10.1002/ps.2333, PMID 22190296.
42. Bhumkar AV. Functional studies of rice KARI and inhibitor discovery [MPhil thesis]. School of Chemistry and Molecular Biosciences, The University of Queensland; 2013.
43. Liu XH, Zhang CY, Guo WC, Li YH, Chen PQ, Wang T, *et al*. Synthesis, bioactivity and SAR study of N′-(5-substituted-1,3,4-thiadiazol-2-yl)-N-cyclopropylformyl-thioureas as ketol-acid reductoisomerase inhibitors. J Enzyme Inhib Med Chem. 2009;24(2):545-52. doi: 10.1080/14756360802234943, PMID 18763167.

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