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ANUPDATEDSTATUSOF*ALANINERACEMASE* INHIBITORS:AREVIEW

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ABSTRACT: The predominant function of the enzyme alanine racemase in the formation of mycobacterium cell walls has brought it widespread recognition. Alanine racemase produces D-alanine, a peptidoglycan precursor that is absolutely necessary for the proper development and maintenance of the cell wall. TheThere is great promise in the lipid-rich mycobacterium cell wall as a therapeutic target for the development of novel drugs, as it is common among prokaryotes. Inhibiting Alanine racemase should be the top priority when dealing with different pathogenic infections because of its critical function in mycobacterium - cell wall formation. Renewed attempts to find novel and improved inhibitors with superior therapeutic indices were driven by concerns about cellular toxicity, interference with metabolic processes, and lack of selectivity produced by numerous recognized inhibitors. For the benefit of humanity, this study summarizes the current state of reported Alanine racemase inhibitors based on fragments of evidence in the literature. The goal is to facilitate the rationalization of the drug discovery process as a whole by facilitating the exploration, design, and identification of more precise inhibitors.

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Keywords: **In-situgel,Acyclovir,Anti-viral,HPMCE50 LV,PluronicF-127**

INTRODUCTION: Microorganismsaredefineda s infectious agents of microscopic size, includingbacteria, fungi, protozoan and viruses, responsibleforcausingvarioustypesofinfections.T odealwithinfectiousagents,thereisanurgentneedfo ranantimicrobialagentthatantagonizestheactionof infection-causing microbe [1]. The discovery ofantimicrobialdrugsconferredhugebenefitsonhu manhealthandchangedthefateofmankinddramatic ally.Penicillinwasthefirstantibioticdiscovered by Alexander Fleming, which proved tobeaboon in curinginfectious diseases.As a result, antibiotics were regarded as wonderdrugsandgenerallyusedtomanageinfectio ncaused by pathogens. However, a

overconsumption,whichisakeyissueofconcernfor theresearchers [2].

Nowthegreatestchallengeofthetwenty-firstcenturyisthedevelopmentofdrugresistancere sponsible for causing immense human suffering.Theresistanceproblemurgesiteratedeff orttostriveforantibacterialagentsefficaciousagain stpathogenicbacteriarebellioustocommercialanti biotics [3,4].Thishighlightstheimmediatecallforupgradeda ntibacterial agents with advanced mechanisms forclinical application[5,6].

PotentialTargetSitesfortheSearchofFuturistic Antimicrobials: Thetreatmentofinfectiousdisease sbecomesknottyasmicrobialresistanceshootsupat oddswithantimicrobialagents. Drugs that destroy microbes prevent theirproliferation,andpathogenicactionshavedissi milar structures, inconsistent affinity towardsthe target site, and disparate spectrum of activitywithvariousmechanismsofaction.The advancementinbacterialgenomicshasgreatlyalter ed the antimicrobial therapeutic environment,and manypotential targets standby[7,8].

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moderndrugsacting*via*advancedmechanismsora gainstthelatesttarget sites from natural sources[9]. The probabletargetsforsearchingfornewantimicrobial compoundsmaybefocusedonthefollowingmecha nisms8,whicharedepictedin**Fig.1**asfollows.

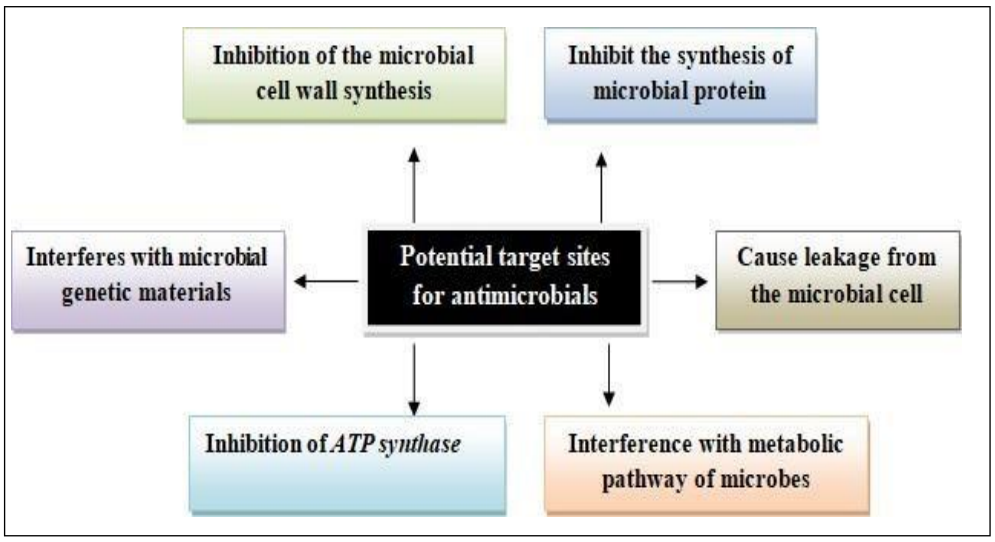

FIG.1:POTENTIALTARGETSITESFORANTIMICROBIALAGENTS

InhibitionofMicrobialCellWallSynthesis: Bacte rialcellsaresurroundedbyacellwallmadeof a peptidoglycan network constitutes an essentialcomponent of the cell wall, serves as a perfect sitefordrugdesignsincecorrespondingbiosynthetic process are lacking in mammalian hosts. Blockageofbacterialcellwallsynthesisisofparamo untimportancefortheactionofantimicrobials.Thepr obable target sites [10-13,8] in cell wall synthesis aresummarizedin**Fig. 2** maybe.

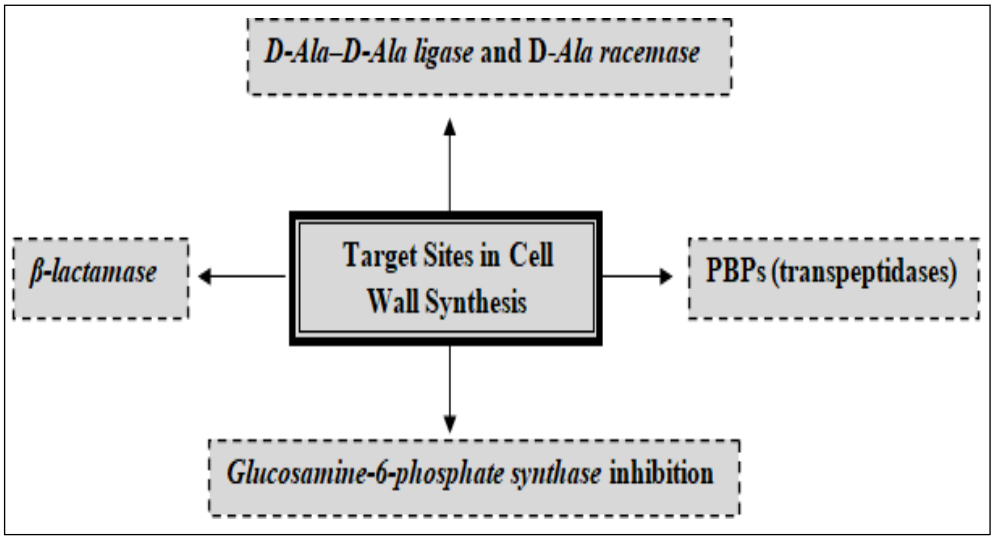

FIG.2:TARGETSITESINCELLWALLSYNTHESIS

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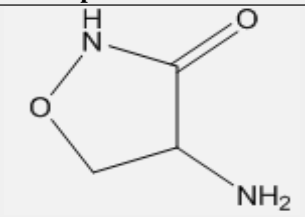
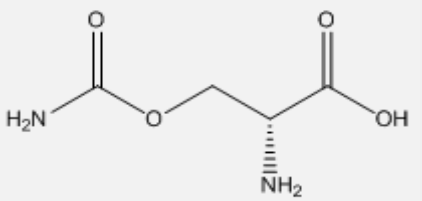
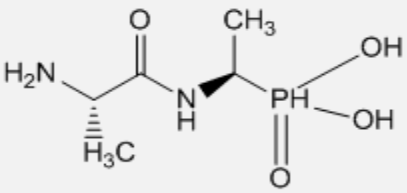
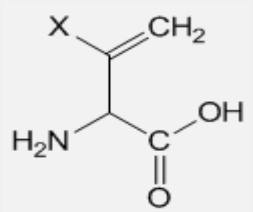
***Alanineracemase*PromisingTargetforAntimicrobial agents:** *Alanine racemase*(Alr, EC5.1.1.1)isapyridoxal-5-phosphate(PLP)dependent homodimeric enzyme that brings aboutreversibleracemizationofL-alanineandD-alanine.Thisbacterialenzymeexecutepredominating role in cell wall synthesis of bacteria[14,15]byproviding D-alanine(D-ala) whichserveasakeymoleculeforthebiosynthesisofpeptidoglycan network of mycobacterial cell wall;hence its inhibition has been reported to be fatal topathogen viability in the deprivation of D-alaninesupplementation [16,17]. The lipid-rich mycobacterialcell wall is common amidst prokaryotes, making*Alanine racemase*a putative target for the designand development of pharmacologically active drug[18-21].D-alanineprovidedby*Alanineracemase*is vital for maintaining cell wall growth and integrity.D-alanineactsasapivotalprecursorforpeptidoglycan biosynthesis in bacterialcell walls*via* D-ala-D-ala formed by the enzyme D-ala-D-alaligase[22].Thismanifestshowtheinhibitionof*alaninera cemase*isimportunate.Thispaperprovidesanoverviewoftheupdatedstatusofreported Alanineracemaseinhibitorsbasedonshredsofliterature.Theproductsderivedfromnaturalsourceshavebeenrecognizedtoplayasignificant role by being the lead molecules to beselectedaspotentialcandidatesfordrugdevelopment. Various researchers have synthesizedderivativesofdifferentscaffoldsandevaluatedthem for *Alanine racemase*properties, summarizedin **Table 1.**

TABLE1:REPORTEDINHIBITORSOFENZYME *ALANINERACEMASE*

Sr.no.	ReportedInhibitors	ResearchFindings
		Sources:*Streptomycesgaryphalus*or*S.orchidaceus*.Dissociation
		
		
		

1.D-cycloserine(DCS)
2.*O*-carbamyl-D-serine
3.Alafosfalin
followed by subsequent rearrangement of DCS with substitutedoximeunriddle*alanine racemase*reactivation in cellular pool.DCS, earlier proved to be an effective competitive inhibitor ofenzyme, unfit for *S. aureus*Alr due to the absence ofconformationessentialforthe moleculetobound withsubstrateregion[23-25].

Enzymekinetics-
Km=4.6*10^{-4}M(D-alanine)
Km= 9.7 * 10^{-4} M (L-alanine)Sources:*Streptococcusfaecalis*
Determination of primary site of action of *O*-carbamyl-D-serineon Alr on the basis of UDP-NAC muramyl-L-ala-D-glu-L-lysaccumulationandintheabsenceofD-ala-O-carbamyl-D-serine
[26, 27]

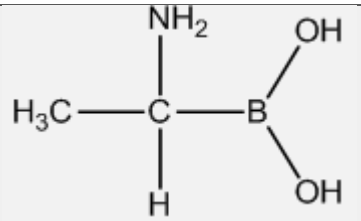
Enzymekinetics-Km=4.8*10^{-4}M(D-alanine),Km=6.8*10^{-3}M(L-alanine)

Sources:Synthetic L-alanineanalog
Containstwoparts-AlaRinhibitorfosfalinandcarrieralaninemoiety
Baseduponalafosfalinformationofexternalaldimine withPLPcofactor, phosphonate group rendered catalytic residuesinaccessiblefor catalysis.
Variableactivityagainstgrampositiveandnegativebacterialstrains.
Phosphonodipeptidewithantibacterialproperties[27-29]

4. Sources:synthesizedfrom*N*-(benzyloxycarbonyl)-vinylglycinemethyl ester which in turn obtained from methionine.Irreversibleinhibitor ofAlr obtainedfrom*E.coli.*[27,30]

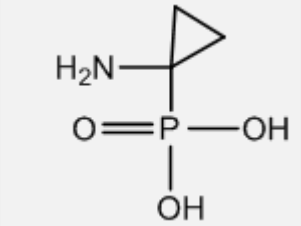
D-chlorovinylglycine:
MIC value- 32 µg/mL (*S.aureus*)64 µg/mL(*S.faecalis*)

X=Cl,FHalovinylglycine

5. 

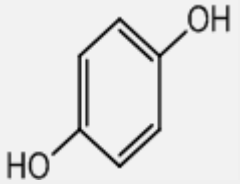
(1-aminoethyl)boronicacid

Structuralanalogofalanine
Showedtime-dependentinhibitoryactivitytowards Alrisolatedfrom*B. stearothermophillus*
CompetitiveinhibitorofD-alanine at D-alaninebindingsites[27,31]

6. 

1-aminocyclopropanephosphonate

Slow but potent inhibitor of Alr obtained from *B.stearothermophillus*strain.
Second order rate constant: <150$M^{-1}S^{-1}$
K_m/K_iratio:2000[27,32]

7. 

Hydroquinone

Sources:Blueberry,pears,broccoli,onions,tea,coffee,beer,redwine, wheatandcerealsetc.
Alr-2inhibitorsfoundtobeactiveagainst*Aeromonashydrophila*
IC_{50} value= 11.39 µMMICvalue=25µg/mL
Potent inhibitor of Alr isolated from *Streptococcus iniae*HNM-1[18,33]

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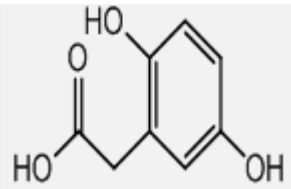
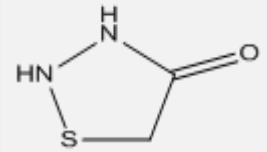
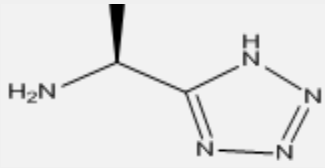
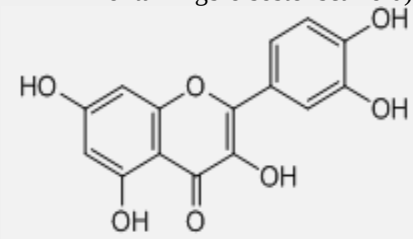
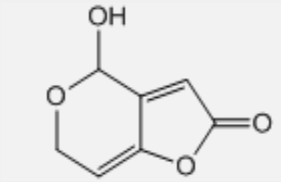
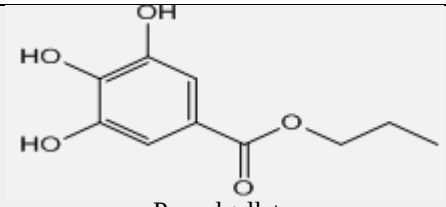
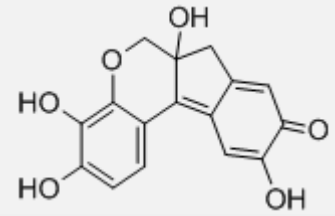
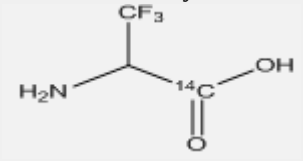
8.	 Homogentisicacid	Sources: Aebutusunedo (strawberry-tree), honey, *Xanthomonascampestris*pv. Phaseoll,Yarrownialipolytica Alr-2inhibitorsfoundtobeactiveagainst*Aeromonashydrophila* IC_{50}value=0.2µMMICv alue=1.73µg/mL Potent inhibitor of Alr isolated from *Streptococcus iniae*HNM-1[18,33,34]
9.	 Thiadiazolidionone	Inhibitory activity against *M. tuberclosis*and *M. smegmatis*AlrwithIC$_{50}$valueranging from<0.03 to28µMand23 to>150µMrespectively[35].
10.	 1-aminoethyltetrazole(Tetrazolec ontainingbioisoster scaffold)	C-terminal1-aminoethyltetrazolecontainingdiandoligopeptidessynthesized by solid phase peptide coupling technique wereidentifiedasnovelandpotential *alanineracemase*inhibitors[29].
11.	 Quercetin	Sources:Apples,berries,broccoli,grapes,citrusfruits,cherries,greentea,coffee,redwine,onionsandcapersetc. M.W=302.24 IC_{50} value= 0.33 µMDockingscore=-102.489kcal/mol PotentinhibitorofAlaR Effectivedruginthetreatmentofpulmonarytuberculosis[18,36]
12.	 Patulin	Sources: Apple, peach, grapes, fruit juices, several species ofAspergillus,Pencilliumand Byssochlamys[18,37-39] M.W=154.12 IC_{50}value=14.7(0.27)µM MICvalue=20(0.89)µg/mL
13.	 Propylgallate	M.W=212.2 IC_{50}value=8.6(0.5)µM[18]
14.	 Haematoxylin	Sources:HeartwoodofthelogwoodtreeHaematoxylumcampechianum[40] M.W=302.29 IC_{50}value=15.6(0.23)µM
15.	 β,β,β-triflouroalanine	Exhibitedtime-dependentinhibitionofagainst AlaRobtainedfrom*S.typhimurium*and*B.stearothermophillus*[27] $K_i$values=65±10and>100mMK_{ina} $_{ct}$=0.08±0.02and>2.0min^{-1}

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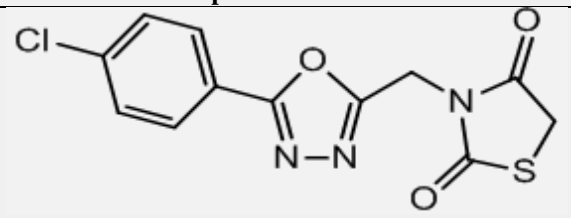
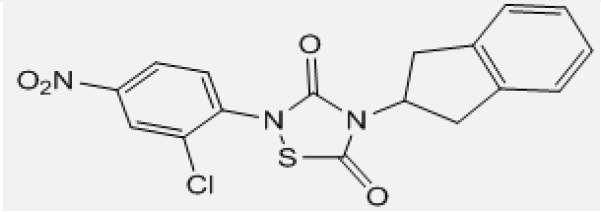
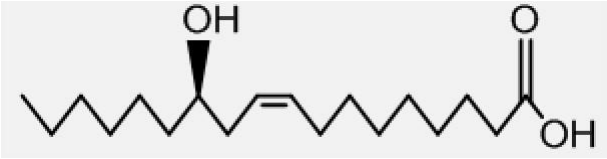
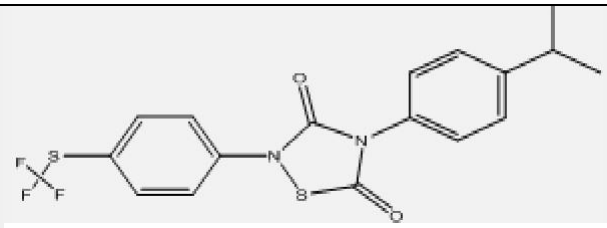
Indrugdiscovery,computer-aideddrugdesign(CADD)offerseffectiveandreliablemethodologiesforleadoptimization,virtualscreening,anddesigningnewdrugcandidates.Molecular docking is a computational drug designapproachthatprovidesinsightsintomolecularrecognition[41].Inanattempttoconductwetlaboratoryexperimentssmoothlyandeffectively,

this method is useful for predicting the compoundarchitecture,preferredorientationandcon-formation (binding pose), interaction, and bindinggeometry of small ligands into the catalytic pocketsofbiomoleculartargetsbasedondockingscorefunction [42]. Based on literature evidence, moleculardocking studies of some reported *Alanine racemase*Inhibitorshavebeen presented in**Table2.**

TABLE2:REPORTEDMOLECULARDOCKING-BASEDSTUDIESOF *ALANINERACEMASE* INHIBITORS

Sr.no.	ReportedInhibitors	ResearchFindings
1	 3-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione	PDB code: 1XFCDockingsoftware-Schrodinger[43] IC_{50} value =13.1µMNumberofH-bonds-6 Saltbridgeinteraction-Arg228
2	 2-(2-chloro-4-nitrophenyl)-4-(2,3-di hydro-1H-inden-2-yl)-1,2,4-thiadiazo-lidine-3,5-dione	PDBcode:1XFCIC_{50} value=0.05µMNumberofH-bonds-3 π-πstacking-His172,Tyr175[43]
3	 Ricinoleicacid	PDB code: 3E5PDockingsoftware-AutoDock Docking score: -7.81, Number of H-bonds-5Amino acid residues – Ser207, Val225, Tyr356,Tyr267. Bioavailabilityscore:0.56 with highGIabsorption[44]
4		PDB code: 1XFC, Resolution: 1.9 ÅDocking software-SchrodingerInduced fitdocking-Desmond Induced fit docking results revealed that Lys42,Tyr46,Tyr175andTyr364residuesthatwererresponsibleforthestabilizationofinhibitor-proteincomplexes[45].

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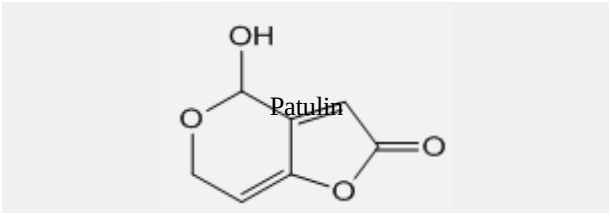
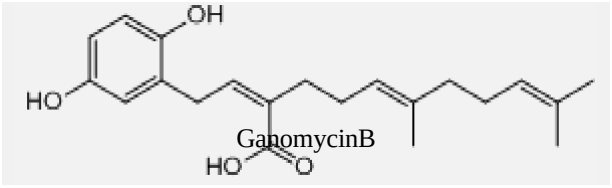
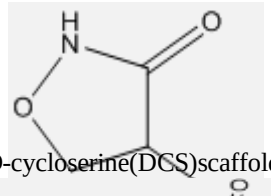
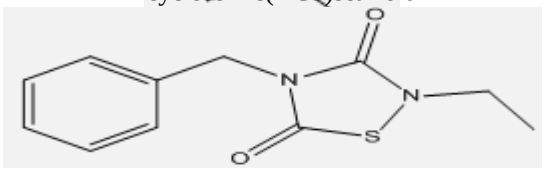
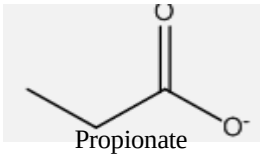
5	4-[4-(propan-2-yl)phenyl]-2-{4-[(trifluoromethyl) 38.88kcal/molsulfanyl]phenyl}-1,2,4-thiadiazolidine-3,5-dione	Bindingenergyvalue=- IC_{50}value=0.17 µM PDBcode:2rjh.1.A Docking software: AutoDockCompoundpossessedinhibitoryactivit yagainst *Aeromonashydrophilla*[18]
6	 Patulin	IC_{50}value=0.62µMagainstCaco-2cells Alsoexhibitsstrongcytotoxiceffectsandreducetheviab ilityofHeLacells upto99%at6.25µg/ml.
7	 GanomycinB	PDB code:2RJG Docking software: AutoDock4PredictedXscoreKi =0.15µM CompoundformsH-bondswithresiduesArg280,Tyr274 and prosthetic group PLP. Compoundhadnarrowaccess totheactivesite[46]
	 D-cycloserine(DCS)scaffold	
8	 4-benzyl-2-ethyl-1,2,4-thiadiazolidine-3,5-dione	PDBcode:1XFC-A(Mtb-Alr) Software:MODELLER(Homologymodelling)D ocking software: AutoDockVina[15]Molecular Volume: 205.03
	 Propionate	PDBcode:1SFT Program: LigBuilder (to generate phrmacophoremodel)Non-covalentinhibitorfitsonlytwo featuresofthedynamicpharmacophoremodel with no excluded volumes, made the compound insufficientinordertobeorientedontothe modelwithexcluded volumes[47]

CONCLUSION: The imperative role of*alanineracemase*inmycobacteriumcellwallsynthesisimplies that its inhibition is of utmost priority indealingwithvariouspathogenicinfections.Thispaperprovidesanoverviewofstructuralinformationonvariouslyreportedinhibitorsof*alanine racemase*based on convincing shreds ofevidencefromliteraturesothatmorepreciseinhibitorscouldbeexplored,designedandidentified to rationalize the overall drug discoveryprocesswhichwillbetrueserendipityforthemankind.

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