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Synthesis and biological Evaluation of 1,2,4-triazoles

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Abstract:

The objective of the present research work is to synthesize isoniazid based 1,2,4-triazole derivatives and evaluate for antimicrobial. 1,2,4-triazoles derivatives has been synthesized by reaction of Isoniazid with carbon disulfide in basic medium (KOH) to form Potassium dithiocarbazinate salt and reaction with hydrazine hydrate converted into 4-amino-5-(pyridin-4-yl)-4H-1,2,4- triazole-3-thiol. This compounds was reacted with different benzaldehyde to form 4-[(substituted phenyl)- methylene]-amino-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-thiol (4). The final compounds were synthesized by reaction with 2-choloro-N, N-diethylacetanilide to form 4-[substituted phenyl)-methylene]-amino-3-(Nsubstitutedcarboxamidomethylthio)-5-(pyridine-4-yl)-4H-1,2,4-triazoles derivatives. All these compounds characterized by IR, 1H NMR, 13C NMR and elemental analysis. The antibacterial activity was determined by the cup plate method. : Antibacterial activity of synthesized compounds, compound no. PJ-B4, PJ-B9, and PJ-B13 shows more than 90%, PJ-B2, PJ-B6, PJ-B10 and PJ-B11 shows more than 80% and rest of compounds shows more than 50 and less than 70% of zone of inhibition against both Gram-positive and Gram- negative organisms. Among all these compounds only PJ-B4 and PJ-B13, shows excellent MIC against both Gram- positive and Gram- negative organisms compare to standard drug.

Introduction:

Nitrogen atoms contain heterocyclic ring moieties that are present both in natural products and in synthetic derivatives and exhibited potent anticancer activities against different human cancer cell lines Three nitrogen atoms containing heterocyclic ring such as 1,2,4-triazoles play an important critical role in the structural elucidation of various natural products [33] and are able to form hydrogen bonding with suitable targets leading to improving of pharmacokinetics, pharmacological, and toxicological properties [34, 35]. These 1,2,4-triazole derivatives are associated with different pharmaceutical activities such as anticancer [36], antibacterial [37], antitubercular [38], antifungal [39], antiviral [40], analgesic [41], anti-inflammatory [42], and tubulin inhibitors [43]. In current scenario, microbial resistance is one of the hurdles and needs the development of newer agent to target the diseases. Literature survey indicates that triazole, thiadiazole and triazine derivatives of Indomethacin have been synthesized and tested for anti-inflammatory activity15. The test compounds inhibited the induction of gastric mucosal lesions and their protective effects may be related to inhibition of lipid peroxidation in gastric mucosa16 . Prompted by these findings, it seemed of interest to synthesize new derivatives of 1,2,4-triazole and investigate their anti-inflammatory activity. The object of the current research is to synthesize new 1,2,4-triazoles derivatives of isoniazid as potent antimicrobial and anti-inflammatory agents. In continuation with the above researches we proposed to synthesized some triazole derivatives to design and synthesize new 1,2,4-triazoles derivatives 4-[substituted phenyl)- methylene]-amino-3-(N-substituted-carboxam idmethylthio)-5-(pyridine-4-yl)-4H-1,2,4-triazoles which were expected to show anti-microbial and anti-inflammatory properties. This paper discusses the most common and useful procedure for synthesizing 4-amino-3-mercapto-1,2,4-triazoles.

Material and Methods:

Ligands Preparation:

All Hundred and seventeen compounds selected for this experiment as listed in Table 6.1.1, 6.1.2 and 6.1.3 were sketched in 2D (two dimensional), converted to 3D format and were optimized for docking using VLifeMDS. The Optimization was performed using MMFF force field and Analytical Gradient till a convergence criterion of 0.001 kcal/mol was achieved.

Preparation of Targets for Docking studies

PDB structures for all the selected targets were obtained from Protein Data Bank (PDB). The protein structures were further cleaned for Addition and optimization of Hydrogens, Checking and correcting the incomplete and missing residues in the structure. The Co-crystal ligands for the proteins were extracted and saved separately so as to be used for

docking studies.

Docking Studies:

The Ligands were docked in the selected targets using the GRIP docking method from Vlife MDS. The ligands were docked in the active site for the targets which was identified using the co-crystal ligand from the PDB. The ligands were rotated by 15 degrees and an exhaustive methods was used to screen different poses of ligands using the PLP scoring function. The PLP scoring function provides a good weightage to the hydrogen bonding interactions which are the most desired interactions between the ligand and the target.

Experimental

All the chemicals in the synthesis were purchased from S.D. Fine Chemicals LTD., Mumbai. Melting points were determined by open capillary method on Veego (model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using ATR sampling technique.^{1}H NMR spectra was obtained on Bruker AV III 500 MHz spectrometer spectra in $CDCl_3$ and chemical shifts are given in parts per million, downfield from Tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained from Bruker Impact HD 3050 system instrument at the SPPU, Pune. To monitor the reactions, as well as, to establish the identity and purity.

Procedure:

Step I) Synthesis of Chalcones (I):

Proper acetophenone (0.01 mol) in ethanol and aromatic aldehyde (0.01 mol) in ethanol were mixed and 10 ml of 40% sodium hydroxide solution was added with stirring. The subsequent solution was reserved overnight at room temperature. The mixture was then poured over crushed ice and acidified with dil. HCl. The solid gained was filtered, dried and recrystallized from ethanol.

Yield = 60-75%, MP =, Mobile Phase = Chloroform: Methanol (8:2)

Step II) Synthesis of isoxazolines (3, 5-diphenyl-4, 5-dihydroisoxazole) (II):

The isoxazolines were prepared by reacting a mixture of purified chalcones (0.01 mol), hydroxylamine hydrochloride (0.03 mol) and a solution of NaOH (0.01 mol) in dry distilled ethanol by refluxing for 6 h. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol.

Yield = 50-75%, Mobile Phase = Chloroform: Methanol (8:2), Recrystallization solvent: Ethanol (80%)

Step III) General procedure for the synthesis of 3-(3, 5-diphenyl-4, 5- dihydroisoxazol-4-yl) propanehydrazide (III):

To isolation of compound II in ethanol (50 ml, formaldehyde (0.6 ml, 0.02 mol) and ethyl acetate (1.76 ml, 0.02 mol) was added. The reaction mixture was refluxed for 2-6 h. The solvent was distilled off and poured into ice water resulting solids were filtered off dried and recrystallized using appropriate solvent.

Step IV) Synthesis of monopotassium (II) mono (2-(3-(3, 5 -diphenyl-4, 5-dihydroisoxazol-4-yl) propanoyl) hydrazinecarbodithioate) (IV):

Compound III (0.01 mol) and potassium hydroxide (0.067 g, 0.012 mol) in absolute alcohol (20 ml) was refluxed with carbon disulfide (1.14 g, 0.015 mol) for 6- 7 h in RBF. (The mixture used directly for step V)

Step V) Synthesis of 3-(2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl) ethyl)-4H-1, 2, 4-triazol-4-amine (V):

Compound IV potassium dithiacarbazinate (0.01 mol) and substituted hydrazides (0.01 mol) were dissolved in alcohol and refluxed in RBF for 6-10 h. When profuse evolution of hydrogen disulfide was observed. The reaction mixture was cooled and poured in cold water or crushed ice. On acidification with 10 ml HCl, 4-(substituted carboxamide) 3-substituted-5-mercapto-1,2,4-triazole was obtained in good yield as white to brown colour precipitate

which was washed with cold water and recrystallized from aqueous ethanol. Following the same procedure, all the derivatives were synthesized.
Yield = 60-78%, Mobile Phase = Chloroform: ethanol (7:3), Recrystallization solvent: Ethanol (80%) of reactants and products, thin layer chromatography was performed on microscopic slides (2 x 7.5 cm) coated with silica gel G F_{254}, using Benzene: Methanol (7:3) solvent systems and the spots were visualized under ultra-violet light (254nm) or by exposure to iodine vapors.

Result and Discussion:

Present study aim towards development of new therapeutic moieties containing 1,2,4-triazoles and for the treatment of microbial infection.

Antimicrobial: *GlcN-6-P synthase* as described by the reported reference. The pdb enzyme file of receptor was downloaded from the RCSB Protein Data Bank (PDB code 1MOQ) and used as a fixed molecule. The docking study of the potent active isoxazoline, thiazolidine-4-one and 1, 2, 4-triazole.

The docking study of the potent active derivatives toward antimicrobial species inside the active pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase, the active target for antimicrobial agents was explored. As described by the X-ray study, the binding pocket of target enzyme including the following subsequent residues, cysteine 300, glycine 301, threonine 302, serine 303,serine 347, glutamine 348, serine 349, threonine 352, valine 399, serine 401, alanine 602 and lysine 603 as shown in Figure 7.1.5 and 7.1.6

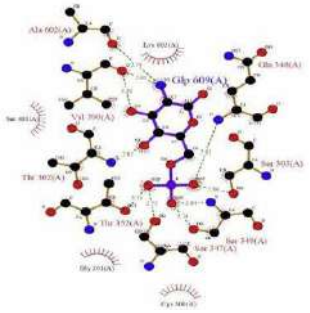

Figure 1: The binding of glucosamine-6-phosphate inside the active site of target enzyme

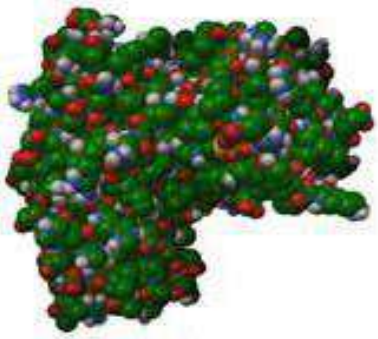

Figure .2: 3D structure of glucoseamine-6-phosphatesynthase (GlcN-6-p)

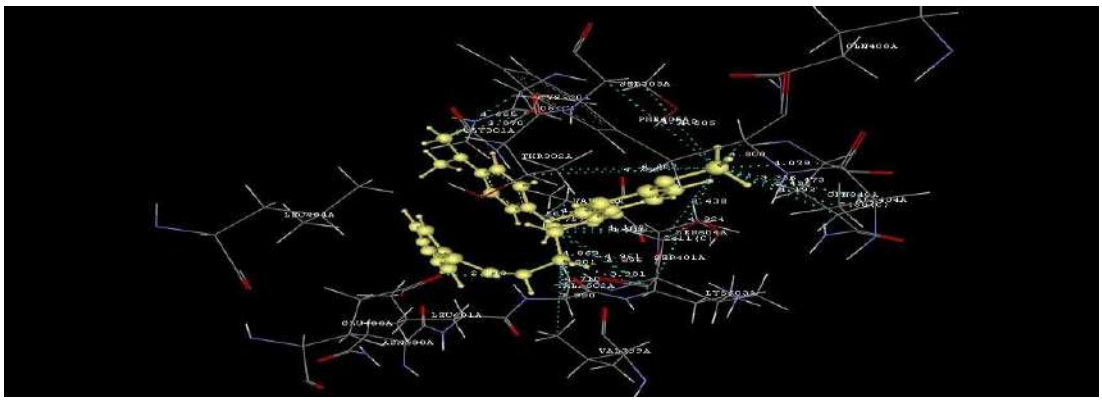

Figure 3: The Docking studies carried out on the Co-crystal ligand to SA 21 shows hydrogen bonding with CYS300A, GLY301A, SER349A, THR302A and shows 8 hydrophobic bonding with GLY301A ALA602 AVAL605 AGLN348A THR302A SER349A LEU601A LYS603 (docking score= -33.15)

derivatives toward antimicrobial species inside the active pocket of L- Glutamine: D-fructose-6-phosphate amidotransferase, the active target for antimicrobial agents was explored.

Figure 4: Compounds SG2, SG16, SG19 and SG20 have shown moderate antifungal activity as compared with Fluconazole, used as standard drug.

Initial chalcones were synthesized by using differently substituted acetophenone and aromatic aldehyde following Claisen- Schmidt‟s condensation reaction. Further the chalcones were treated with nucleophilic reagent hydroxylamine hydrochloride under reflux to get isoxazoline compounds. The target compounds, 3- (2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl) ethyl)-4H-1, 2, 4-triazol-4-amine (SS6, SS7, SS11, SS12, SS16, SS26, SS41 & SS42) are synthesized from monopotassium

(II) mono (2-(3-(3, 5 -diphenyl-4, 5-dihydroisoxazol-4-yl) propanoyl) hydrazinecarbodithioate) (IV) by using methanol as solvent and using isonicotinic acid hydrazide, pyrazinoic acid hydrazide and sulphanilamide . Structures of all derivatives have been elucidated by ^{1}H-NMR, HR-MS and IR spectral measurements. The results obtained from this study confirmed that the product has formed. The solid state IR (ATR,cm^{-1}) spectra of these compounds reveal a characteristic N-H Stretch secondary amine 3500-3100 of hydrazide and aromatic Stretch between 3150-3050 cm^{-1}. The C-H stretch alkane group present in the at 3000-2850 cm^{-1} .The C-H bend (aliphatic -CH_2 bend) group reveal peaks at 1375-1465 cm^{-1}. The C=C group of Aromatic ring showed stretching vibrations at around 1600-1475 cm^{-1}.The ^{1}H NMR spectra of all target derivatives (SS6, SS7, SS11, SS12, SS16, SS26, SS41 & SS42) were recorded in $CDCl_3$. ^{1}H NMR has revealed signal around at δ 3.32-3.70 accounting for isoxazoline nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all the protons were accounted for the respective structures. Mass spectra were also in accordance with the proposed structures.

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