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CHEMICAL MODIFICATION OF SECNIDAZOLE INTO A NOVEL 5-OXO- IMIDAZOLINE DERIVATIVE: SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING, RP-HPLC METHOD DEVELOPMENT AND VALIDATION, AND BIOLOGICAL EVALUATION

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ABSTRACT

Objective: The present study aimed to synthesize a novel 5-oxo imidazoline derivative of SECNIDAZOLE through chemical modification and to evaluate its physicochemical, characterization, RP-HPLC method development and validation and biological evaluation.

Methods: The synthesized derivative was purified and characterized by TLC, melting point, FT-IR, 1HNMR, Mass spectrometry, and spectral analysis. Molecular docking studies were performed against the selected biological target to predict ligand-receptor interactions and binding affinity. A simple, precise, and accurate RP-HPLC method was developed for quantitative estimation of the synthesized compound and validated according to ICH Q2 R1 guidelines. The biological activity of the synthesized derivative was assessed using suitable invitro, in vivo evaluation methods.

Results: Formic acid: methanol (90:10) made up the ideal mobile phase, for HPLC method development of 5-oxo-imidazoline and the λ max was detected at 254 nm. With a total run time five minutes, the retention time was found to be 3.2 minutes. With a regression coefficient of 0.999, the method showed linearity over a concentration range of 10, 20, 30, 40, 50µg/ml. The 5-oxo-imidazoline respective recognition limits (LOD and LOQ) are 1.350µg/ml and 4.100 µg/ml respectively. %RSD values were found to be <2 for precision studies.

Conclusion: The chemical modification of SECNIDAZOLE successfully yielded a novel 5-oxo imidazoline derivative with confirmed structure, promising docking profile, reliable RP-HPLC analytical method, and biological activity, indicating its potential for further pharmaceutical investigation.

Key words: 5-oxo-imidazoline, RP-HPLC, chemical modification, molecular docking, spectroscopic analysis.

1.INTRODUCTION

5-OXO-IMIDAZOLINE DERIVATIVES these are the compounds containing an imidazole ring with a carbonyl group at the 5- position Oxo-imidazoline derivatives are keto dihydroimidazoles too, known as imidazoline one a five member ring system having 2-nitrogen situated at 1 and 3-positions and C═O at various positions like 2, 4 and 5 of ring. Three possible isomers of imidazoline one observed based on position of C═O substituent at skeleton namely: 1*H*-imidizol-2(5*H*)-one**(2)**, 1*H*-imidizol-5(4*H*)-one**(3)** and 1*H*-imidizol-4(5*H*)-one**(4).** SECNIDAZOLE is Nitro imidazole antimicrobial used for protozoal infections. The chemical modification of nitro group may alters the biological activity and physicochemical properties. CHEMICAL MODIFICATION of nitro group (-NO2) to an amine (-NH2). The amine group is more biologically active than the nitro group in many drug molecules. –NH2 can form hydrogen bonds and ionic interactions with amino acid residues. This may result in better binding affinity, which supports molecular docking results.[1]

2.MATERIAL AND METHODS

2.1 Materials

Chemicals/Reagents: The list of chemicals/reagents used in the study were represented in Table 1

Table No: 1 List of chemicals/reagents

S.NO	CHEMICALS	MANUFACTURE
1	0.1% Formic acid	Merc life science pvt.ltd
2	Methanol	Merc life science pvt.ltd
3	Ethyl acetate	Rankem chemicals
4	Petroleum ether	Rankem chemicals
5	SECNIDAZOLE	Yarrow chemicals
6	HCL	Merc specialties
7	Zinc dust	Sd fine chem pvt ltd
8	Hippuric acid	Merc life science pvt.ltd
9	Benzoyl chloride	Loba chemie pvt.ltd
10	2-Amino acetic acid	Loba chemie pvt.ltd

2.2 Instruments

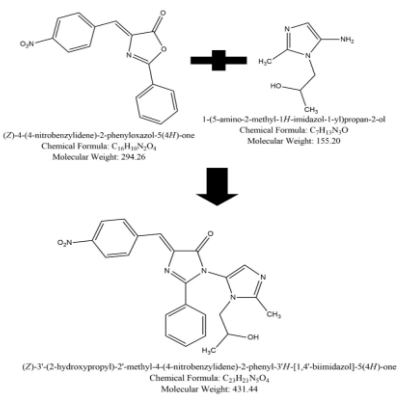
The list of instruments/Software used in study was represented in table 2

Table No:

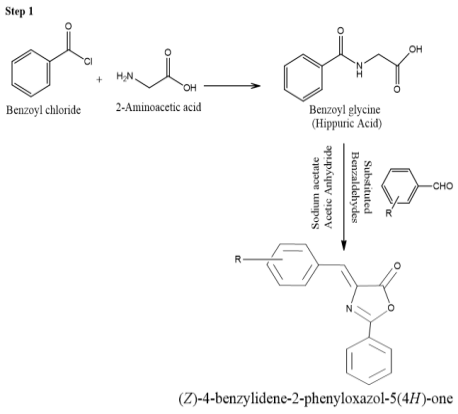
S.NO	INSTRUMENT/SOFTWARE	MARKER	MODELNO	PURPOSE
1	HPLC	SHIMADZU	LC-20AD	Method development &validation
2	UV Visible spectroscopy	SHIMADZU	UV-1800 NG240AV, software: UV robe2.0	Absorbance measurement
3	Electronic balance	SHIMADZU	ATY224	Accurate weighing of chemicals and samples
4	Hot air oven	VISION TECH	ISO 9001-2015	Drying of glassware and synthesized compound
5	Vacuum filtration pump	BOROSIL	-	Rapid filtration
6	UV cabinet	MONOQUARTZ	-	Visualization of TLC spots and monitoring reaction progress
7	FTIR	BRUKER	Alpha	Functional group analysis
8	Magnetic stirrer with hot plate	-	-	Heating and stirring
9	Reflux condenser	-	-	Reflux during synthesis
10	TLC chamber	-	-	Reaction monitoring
11	Melting point apparatus	-	-	Purity check
12	Auto Dock 4.2.6.1(Docking software)	-	-	Molecular docking studies
13	Discovery studio visualizer	-	-	Visualization of ligand-protein interactions

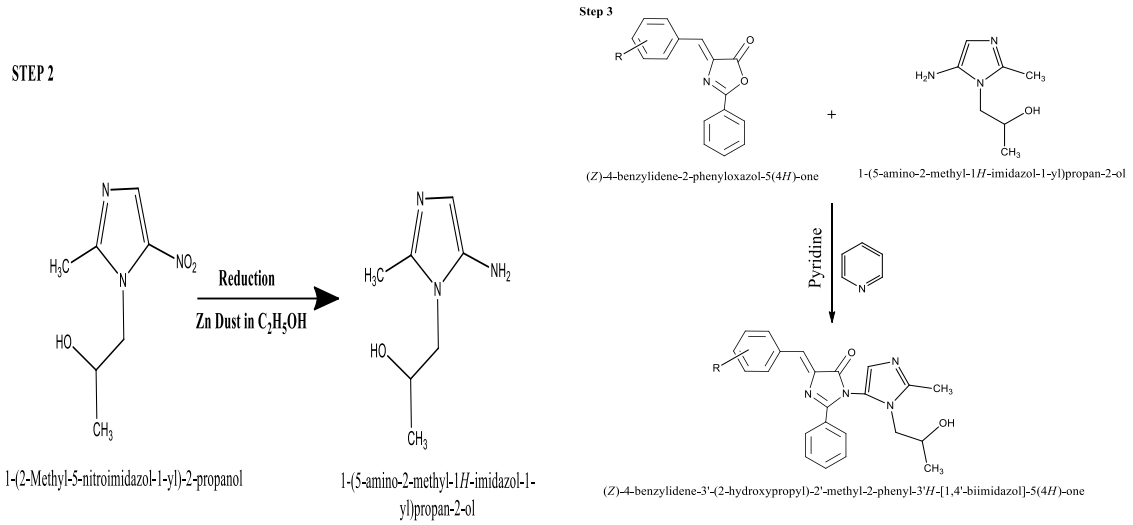
2.3 synthesis of novel 5-oxo imidazoline derivative

SYNTHESIS PROCEDURE



SCHEME OF WORK





STEP WISE SYNTHESIS

Step I: Preparation of 4-Benzalylidine-2-phenyl oxazolone-5-one:

A mixture of Hippuric acid 0.0239mol (4.3gm), Benzaldehyde 0.0239mol (2.5ml), Acetic anhydride 0.739mol (7ml), and anhydrous sodium acetate 0.0239(1.95gm) heated with constant stirring. A liquefied mixture was transferred for reflections for 2hours. Then add ethanol (2.5ml) slowly to the contents of the flask and the mixture was allowed to stand for overnight. The resultant crystalline precipitate was filtered with suction and wash the precipitate with 2 portions of ice-cold alcohol (3ml) and finally with 2 portions of boiling water. The solid mass thus separated out was filtered, dried, and purified by recrystallization from ethanol. (Yield-80%)[10],[23]

Step II: Preparation of 1-(5-amino-2-methyl-1H-imidazol-1-yl)-propan-2-ol:

The mixture of 1-(2-Methyl-5-nitroimidazol-1-yl)-2-propanol (0.0lmol), Zinc dust (0.01mol), and Conc. HCl (10ml) in anhydrous ethanol (40ml) was refluxed for 8 hrs. Concentrate the solution and allow it to cool. Neutralize the reaction mixture with 10% Sodium hydroxide. The resultant crystalline precipitate was filtered with suction and washed the residue with ice-cold water. The solid mass thus separated out was filtered, dried, and purified by recrystallization from ethanol.[10],[23]

Step III: Preparation of (Z)-4-benzylidene-3'-(2-hydroxypropyl)-2'-methyl-2-phenyl-3'H-[1,4'-biimidazol]-5(4H)-one:

To a mixture of 1-(5-amino-2-methyl-1H-imidazol-1-yl)propan-2-ol (0.01mol) and different substituted 4-benzylidene-2-phenyl Oxazol-5-one (0.01mol) in a pyridine (15ml) was refluxed for 4hrs in a sand bath. The excess of solvent was removed under reduced pressure and the reaction mixture was poured into crushed ice, filtered, dried, and recrystallized from ethanol. Thin Layer Chromatography monitored the completion of the reaction. The various (Z)-4-benzylidene-3'-(2-hydroxypropyl)-2'-methyl-2-phenyl-3'H-[1,4'-biimidazol]-5(4H)-onewere prepared in a similar manner. The physicochemical data of these compounds (3a - j).[10],[23]

2.4 Monitoring of reaction by TLC

Thin layer chromatography was employed to monitor the progress of the reaction and to assess the purity of the synthesized compound. Pre-coated silica gel plates were used as stationary phase. The mobile phase consisting of (petroleum ether: ethyl acetate in specific ratio) was optimized to achieve proper separation.The developed plates were visualized under ultraviolet light at 254 nm. The retention factor (Rf) value of the synthesized compound was calculated using the following formula:

$$RF = \text{Distance travelled by compound} / \text{Distance travelled by solvent front}$$

The presence of a single spot on the TLC plate indicated satisfactory purity of the isolated compound.

2.5 Purification by column chromatography

The purification of synthesized drug was purified by recrystallization using ethanol. Further purification was performed by column chromatography.

2.6 characterization of synthesized compound[36]

2.6.1 Melting point

The melting point of the purified compound was determined using a digital melting point apparatus. A small quantity of the dried sample was filled into a capillary tube and placed in the apparatus. The temperature at which the compound changed from solid to liquid was recorded. A sharp melting point range indicated good purity of the synthesized compound.

2.6.2 FT-IR spectral Analysis

Using an FT-IR spectrophotometer and the KBr pellet method, the synthetic compound's infrared spectrum was captured. The 4000–400 cm^{-1} range was used to scan the spectrum. To determine whether distinctive functional groups were present in the molecule, the detected absorption bands were examined. We looked closely at certain peaks that corresponded to functional groups like N–H, C=O, C=N, C–N, and aromatic C–H stretching. The parent compound's structural change was validated by the existence of expected functional group peaks.

2.6.3 ^{1}H NMR Spectral Analysis

NMR spectrometer was used to record the produced compound's ^{1}H NMR spectra. An established analytical laboratory conducted the analysis. Tetramethyl silane (TMS) was utilized as the internal standard, and chemical shifts were reported in parts per million (ppm) using a deuterated solvent, such as DMSO-d6/ $CDCl_3$.The structural characteristics of the synthesized molecule were verified by analysing the obtained spectrum data.

2.6.4 ^{13}CNMR Spectral Analysis

To determine the various carbon habitats contained in the produced molecule, the ^{13}C NMR spectra was captured. Signals from carbonyl carbons, aromatic carbons, and other aliphatic carbons were seen in the spectrum. To validate the synthetic derivative's structural framework, the chemical shift values were examined.

2.6.5 Mass spectral Analysis

The molecular weight of the produced molecule was ascertained by mass spectral analysis. The spectrum's molecular ion peak (M^{+}) matched the predicted molecular mass of the suggested structure. The compound's structural assignment was further validated by the fragmentation pattern.

2.7 Molecular docking studies

COMPUTER AIDED DRUG DESIGN

A binding interaction between a small molecule ligand and an enzyme protein results in activation or inhibition of the enzyme, which results in agonism or antagonism. Identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using docking programs. This method is known as virtual screening.

Selection of target protein : Molecular docking study was carried out to evaluate the interaction between the synthesized compound and the selected biological target. The three- dimensional crystal structure of the target protein was retrieved from the protein data bank (PDB). Table 3 [2]

Table 3 details of selected protein

PARAMETER	DESCRIPTION
Protein name	Probable decaprenyl phosphoryl-β-D-ribose oxidase(DprE1)
PDB ID	4P8L
Resolution	2.02*A*°
source	RCSB Protein data bank

Docking software

Molecular Docking by AUTODOCK 4.2.6.1 is an automated procedure for predicting the interaction of ligands with bio macromolecular targets. Progress in biomolecular x-ray crystallography continues to provide important protein and nucleic acid structures. These structures could be targets for bioactive agents in the control of animal and plant diseases, or simply key to the understanding of fundamental aspects of biology. The precise interaction of such agents or candidate molecules with their targets is important in the drug discovery process.

In any docking scheme, two conflicting requirements must be balanced: the desire for a robust and accurate procedure, and the desire to keep the computational demands at a reasonable level. The ideal procedure would find the global minimum in the interaction energy between the substrate and the target protein and exploring all available degrees of freedom (DOF) for the system. Auto Dock combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom.

The current version of Auto Dock using the Lamarckian Genetic Algorithm and empirical free energy scoring function typically will provide reproducible docking results for ligands with approximately 10 flexible bonds.

The quality of any docking results depends on the starting structure of both the protein and the potential ligand. The protein and ligand structure need to be prepared to achieve the best docking results.[3]

- **Protein preparation**
- **Ligand preparation**
- **Receptor grid generation**
- **Ligand docking (screening)**

DOCKING PROCEDURE

Preparation of protein:

- Read molecule from the file (allows reading of PDB coordinate files.)
- Edit -Charges – Compute Gasteiger (for arbitrary molecules)
- Edit – Hydrogen –Merge non polar
- Save as .**PDB** in Auto Dock folder

Preparation of Ligand:

- Ligand –Input from file
- Ligand – Torsion –choose torsion: Rotatable bonds are shown in green, and non-rotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user are shown in magenta.
- Ligand – Torsion –set number of torsion: sets the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable.
- Ligand – Output – save as**. PDBQT** in Auto Dock folder

Grid preparation:

- Grid – Macromolecule -open (open the PDB file that has been saved and then save it in **PDBQT** extension in Auto Dock folder)
- Grid – Set map types –open ligand: tools to define the atom types for the grids that will be calculated
- Grid – Grid box – launches interactive commands for setting the grid dimensions and center (Set dimension of 60 **x** 60 **x** 60 – Center: center
- on macromolecule)
- File – Close saving current
- Grid – Output – save as**. gpf** (grid parameter file)
- Open command prompt **[cd Auto Dockcd 4.2.5.1 autogrid4.exe –p a.gpf –l a.glg]**

Preparation of Docking Parameters:

- Docking –Open the macromolecules – set rigid file name.
- Docking – ligand – open the ligand.
- Docking –search parameters – genetic algorithm parameters: this command opens a panel for setting the

parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.

- Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and output options.
- Docking- output –Lamarkian GA –save as **.dpf (**docking parameter file**)**
- Open command prompt **[autodock4.exe –p a.dpf –l a.dlg]**

Visualization / Interpretation of Docking

- Analysis –Docking – open. dlg (docking log file) file
- Analysis – open the macromolecule
- Analysis – Confirmation –Play and Play ranked by energy: Play- will use the order of conformations as they were found in the docking calculations, and Play Ranked By Energy will order the conformations from lowest energy to highest energy.
- Analysis – Load: Information on the predicted interaction energy is shown at the top and the individual conformations
- Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.[4]

2.8 RP-HPLC method development

Instrumentation

Chromatographic analysis was performed using RP-HPLC system equipped with UV detector and data acquisition software. Separation was achieved using reverse phase c18 column. (Table 4)[34],[35]

Table 4 HPLC Instrument details

PARAMETER	SPECIFICATION
Instrument	R-P HPLC
Column	C18
Detector	UV Detector
Software	Lab solutions

Chromatographic conditions

Various mobile phase compositions were evaluated to achieve optimal peak resolution and symmetry. The optimized mobile phase consisted of 0.1% Formic acid and Methanol in the ratio of 90:10 the flow rate was maintained at 1.2 ml/min and detection wavelength was set at 254nm

Table 5 optimized chromatographic conditions

PARAMETER	OPTIMIZED CONDITION
Column	C18
Mobile phase	0.1%Formic acid: methanol
Flow rate	1.2 ml/min
Detection wavelength	254nm
Injection volume	10
Run time	5.5 min

2.9 preparation of standard solution

Accurately weighed 10 mg of synthesized compound was dissolved in suitable solvent methanol To prepare stock solution. further dilutions were made to obtain working standard solution of required concentration.

2.10 method validation as per ICH Q2(R1)

2.10.1 system suitability: It is a test to determine the stability and effectiveness of a chromatographic system prior to use.

Table 6 Parameters recommendations

SNO	PARAMETERS	RECOMMENDATIONS
1.	Theoretical plates	> 2000
2.	Tailing factor	≤2
3.	Resolution (Rs)	>2 between the peak of interest and the closest eluting potential interference
4.	Repeatability	RSD≤1% and N>2000is desirable
5.	Capacity factor	>2.0

six replicates of the prepared concentrations, or 10 µg/ml (from the above working standard) were taken in order to assess the system appropriateness. Fallowing the injection of the six repetitions and the establishment of optimal conditions, the chromatograms were examined to determine the peak heights, retention times, tailing factors, theoretical plates and peak areas.

2.10.2 Preparation of linearity concentrations: The linearity of this method was established. Accurately weigh 10mg of 5-oxo imidazoline derivative transferred to 10ml volumetric flasks, and makeup with diluents and labelled as standard stock solution (1000µg/ml of 5-oxo imidazoline) from this solution prepare following concentrations of 10-50µg/ml. These solutions are injected and the corresponding peak areas are found and calibration curve is produced.

2.10.3 Precision: The Intraday and Inter-day Precision was carried out by injecting 15µg/ml solution was injected in six replicates for 3 trails on a same day for Intra precision and 1 trail for alternate three days for inter-day precision and the peak response factor, %RSD were calculated.

2.10.4 Limit of Detection (LOD): Detection limit is the parameter which is determined by analysing the samples with known concentration and by establishing the minimum level at which the analyte can consistently quantified.

2.10.5 Limit of Quantification (LOQ): Quantitation limit is the parameter which is determined by analysing the samples with known concentration and by establishing the minimum level at which the analyte can consistently quantified.

2.10.6 Robustness: Small deliberate changes in method like Flow rate, Temperature and Wavelength are made but there were no recognized change in the result and are within range as per ICH Guidelines. Robustness conditions like flow minus (1.0ml/min), Flow plus (1.4ml/min), Temperature minus (25ºc), and Temperature plus (35ºc) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limits.

2.10.7 Degradation Studies or stability studies:

Acid Degradation Studies: To 0.5ml of stock solution, 0.1ml of 0.1N HCL was added and refluxed for 30min at 600ºc. The resultant solution was diluted to obtained 100µg/ml solution and 10µl solution was injected into the system. And the chromatogram was recorded to asses the stability of sample.

Alkali Degradation Studies: To 0.5ml of stock solution , 0.1ml of 0.1 NAOH was added and refluxed for 30min at 600ºc. The resultant solution was diluted to obtained 100µg/ml solution and 10µl solution was injected into the system. And the chromatogram was recorded to assess the stability of sample.

Oxidation: To 0.5ml of stock solution, 0.1ml of 3% Hydrogen Peroxide (H_2O_2) was added separately. The solution was kept for 30min at 600ºc. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solution and 10µl solution was injected into the system. And the chromatogram was recorded to assess the stability of sample.

Peroxide Degradation Studies: 0.3ml of the sample solution was taken from the prepared dummy solution and placed in a 10ml volumetric flask. 0.3ml of 3% H2O2 was added to the flask and make up to the volumetric flask mark using mobile phase. The solution was then allowed to sit at room temperature for 12hrs before being injected into an HPLC, where the peak responses were noted and the chromatogram is displayed

Thermal Degradation: From the Prepared stock solution, 0.3ml of the sample solution was transferred to a 10ml volumetric flask along with 1ml of mobile phase. The mixture was then heated to 60ºc for 10min and allowed to cool, makeup the difference with mobile phase, which was then injected into HPLC.

2.11 Biological evaluation

Experimental Protocol for anti-TB activity using Alamar Blue Dye

Procedure:

- The anti-mycobacterial activity of synthesized compounds were evaluated against M. tuberculosis using micro plate Alamar Blue assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
- Briefly 200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
- The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate.
- The final drug concentrations tested were 100 to 0.2 µg/ml.
- Plates were covered and sealed with Para film and incubated at 37ºC for five days.
- After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
- A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
- The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.[6],[7],[8],[9]

3.RESULTS AND DISCUSSION

3.1 synthesis and reaction outcome

3.1.1 Monitoring of reaction by TLC

The progress of reaction was monitored by thin layer chromatography. Initially, the starting material showed a single spot with Rf value 0.0.20 up on completion of reaction, a new spot appeared at Rf value 0.50 with disappearance of the starting material spot, indicating formation of the desired product. The appearance of a single spot in the purified compound confirms reaction completion and purity.

3.1.2 purification by column chromatography

The reaction mixture was subjected to column chromatography. Elution with optimized solvent system (petroleum ether, ethyl acetate) resulted in separation of impurities. The purified compound exhibit a single spot on TLC, indicating successful purification.

3.1.3 predicting molecular properties of compounds

Table 7: Predicted molecular properties of the compounds

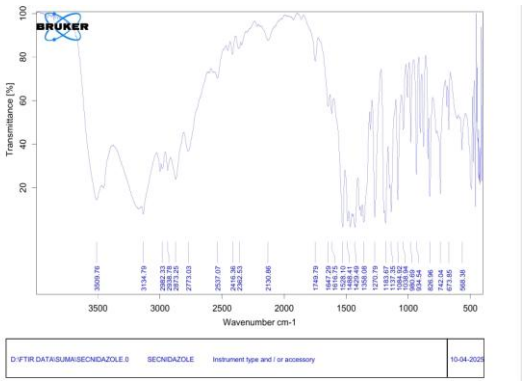
Compound Code	Formula	Molecular Weight (gm/mol)	No. of H-B acceptors	No. of H-B Donors	I LOGP	TPSA
3a	$C_{23}H_{22}N_4O_2$	386.45	4	1	3.03	70.72
3b	$C_{23}H_{21}N_5O_4$	431.44	6	1	2.8	116.54
3c	$C_{26}H_{28}N_4O_2$	428.53	4	1	3.77	70.72
3d	$C_{25}H_{27}N_5O_2$	429.51	4	1	3.31	73.96
3e	$C_{23}H_{21}N_4O_4$	431.44	6	1	3.02	116.54
3f	$C_{23}H_{21}FN_4O_2$	404.44	5	1	3.42	70.72
3g	$C_{23}H_{20}Cl_2N_4O_2$	455.34	4	1	3.63	70.72
3h	$C_{25}H_{21}ClN_4O_2$	420.89	4	1	3.53	70.72

3i	$C_{23}H_{22}N_4O_3$	402.45	5	2	3.18	90.95
3j	$C_{24}H_{24}N_4O_4$	432.47	6	2	3.45	100.18

3.2 spectral characterization

3.2.1 FT-IR interpretation

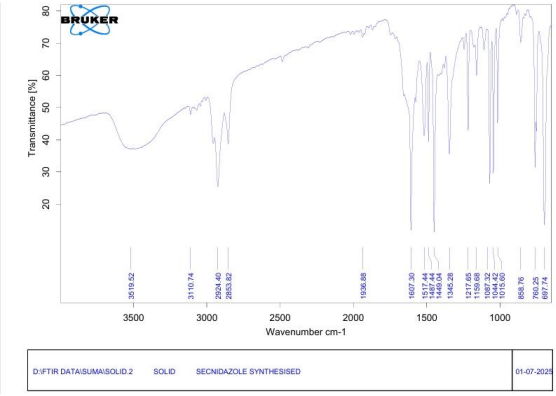
SECNIDAZOLE: FTIR spectrum of secnidazole showing characteristic nitro group (1528 &1349), carbonyl and imidazole ring absorption bands(1749cm1)



FT-IR (cm-[1]): 1749 (c=o5-oxo-imidazole ring), 1270 (C-N Imidazole ring), 3500-3300(O-H), 1528(N-O Nitro group (No2), 2982(C-H Aliphatic CH_3/CH_2)

Figure:1 FTIR Spectra interpretation of SECNIDAZOLE

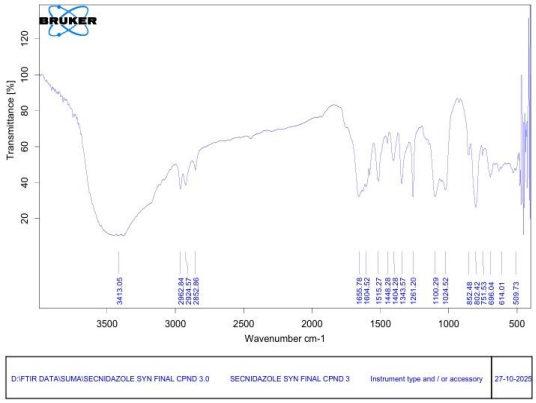
Reduction of nitro group interpretation of amine group: FTIR spectrum of the reduced product showed distinct spectral changes compared to parent secnidazole, the complete disappearance of nitro group indicating successful chemical reduction. Here the carbonyl stretching bands observed near 1600-1700 cm-1 confirm the presence of 5-oxo-imidazoline moiety. the absence of nitro group(NO2) characteristic bands at ~1520 and ~1350 cm-1 confirmed complete reduction of nitro group (NO2) to Amine group(NH3).



FT-IR (cm-[1]): 3519(N-H Primary amine), 3110(C-H imidazole ring), 2924(C-H Aliphatic CH_3/CH_2), 1607(c=o5-oxo-imidazole ring), 1150-1200(C-N Amine linkage)

Figure:2 FTIR Spectra interpretation of chemical modification of SECNIDAZOLE

FINAL SYNTHESIZED COMPOUND 5-OXO-IMIDAZOLINE DERIVATIVE: This FTIR spectra fully supports the final compound compound structure. All the functional groups present in 5-oxo-imidazoline derivative are clearly observed.



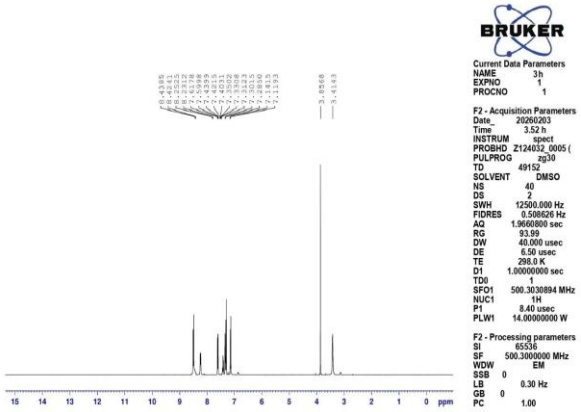
FT-IR (cm-[1]): 3413(N-H secondary Amine), 2962,2924, (C-H Aliphatic CH_3/CH_2), 1655(c=o5-oxo group), 1604(C=N imidazoline), 1515(C=C Aromatic phenyl ring), 1343,1261,1100,1024(C-N Amine).

Figure:3 FTIR Spectra of 5-oxo imidazoline derivative

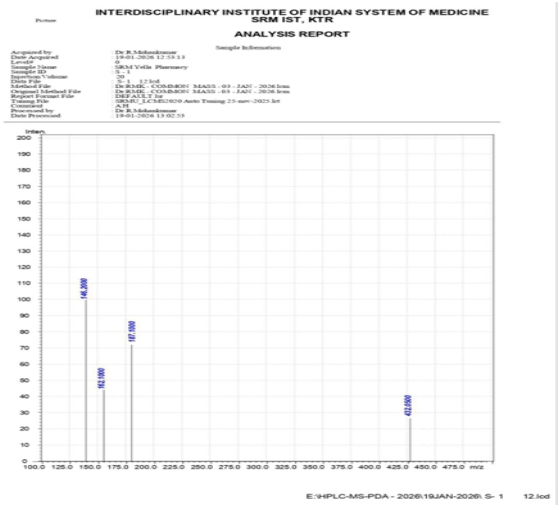
3.2.2 NMR Interpretation

1HNMR(500MHZ,DMSO-d6)ppm:

3.07-3.58(2H,m,-ch2),7.27-7.39(1H,m,-CH-),7.40-7.55(2H,m,Ar-H),7.73-8.63(2H,m,Ar-H),7.83-8.93(3H,m,Ar-H), 8.93-9.07(1H, s, -SH-).



3.2.3 Mass spectral analysis



ESI-MS (m/z): 432.44(M+H)+, major fragment ions at m/z 187.20, 162.10 and 146.20.

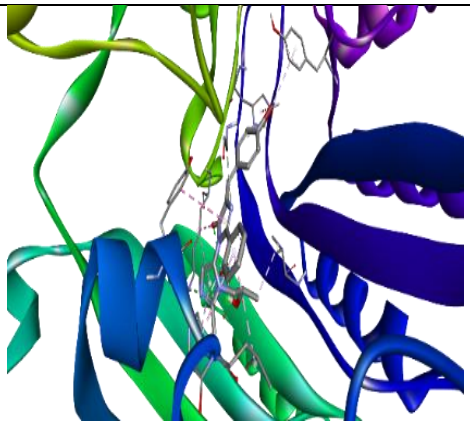
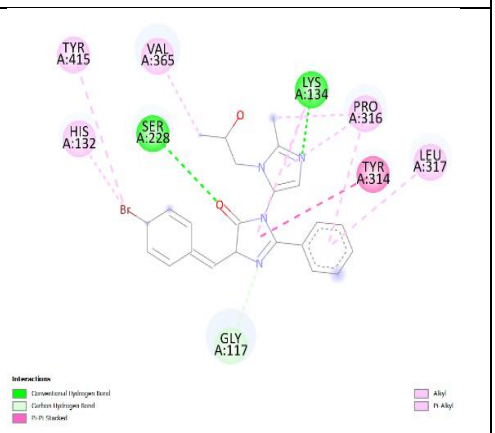
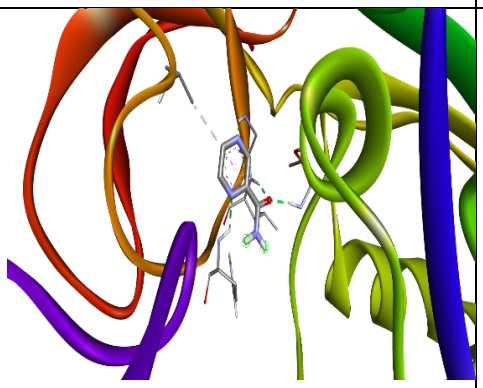
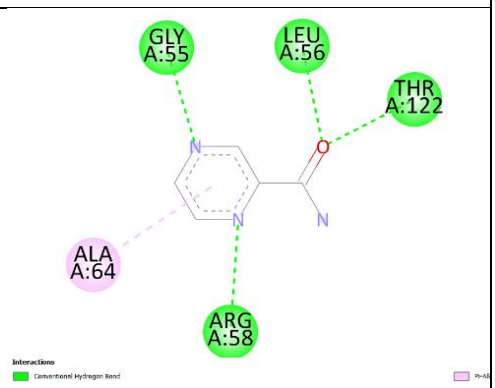
3.3 molecular docking results

Molecular docking studies were performed for the synthesized 5-oxo imidazoline derivative against the 4PSL protein using Auto dock 4.2.6 the docking scores of the synthesized compound along with the standard drug pyrazinamide are summarized in Table 1The binding energies for the synthesized derivatives are ranging from -9.2 to -9.0 kcal/mol, which were significantly better than the standard drug pyrazinamide-5.4 Among the synthesized series, compound 3h exhibits the highest binding score. (Table:8) & (Table:9).

Table:8 Docking score in Auto dock 4.2.6

S. No.	Compound code	Receptor	Binding score
1.	3a	4P8L	-9.2
2.	3b	4P8L	-9.29
3.	3c	4P8L	-9.1
4.	3d	4P8L	-9.47
5.	3e	4P8L	-8.66
6.	3f	4P8L	-9.39
7.	3g	4P8L	-9.4
8.	3h	4P8L	-9.87
9.	3i	4P8L	-9.18
10.	3j	4P8L	-9.0
11.	STD (PYRAZINAMIDE)	4P8L	-5.4

Table no 9: Docking pose and 2D Diagrams of all compounds on **4P8L** Docking

Compound code	Docking pose	2D interaction diagram
3h		
STD PYRAZINAMIDE		

4 RP-HPLC method development

Table 10:Optimized conditions

S.NO	Parameters	Conclusion
1.	Mobile phase	methanol and formic acid in the ratio of 90:10
2.	Column	C18
3.	Flow rate	1.0ml/min
4.	Detection of wave length	254nm
5.	Column oven temperature	40°C
6.	Run time	5.2min
7.	Retention time	3.2min
8.	Injection volume	20µl
9	Concentration	10µl

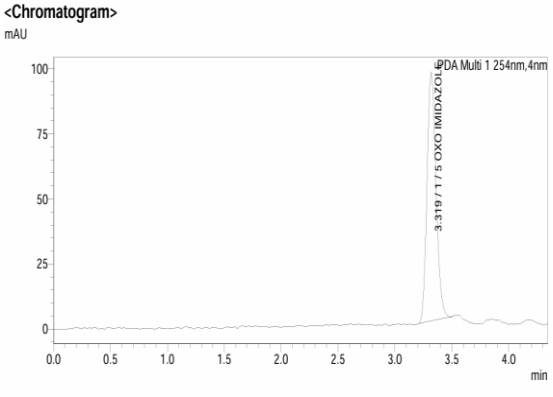


Figure:4 optimized chromatogram of synthesized derivative

3.5 Method validation results

Analytical method validation and their acceptance criteria'

Table 11: parameters and their acceptance criteria

Parameters	Acceptance criteria
Linearity	R^2= 0.999-1.0
Precision	RSD<2%
Detection limit	S/N>2 or 3
Quantification limit	S/N>10
Robustness	% Assay 99-102%

System suitability parameters

Table 12: system suitability parameters

SYSTEM SUITABILITY					
S.NO	PEAK AREA	RET TIME	PLATE COUNT	PEAK HEIGHT	TAILING
1	868555	3.246	4731	130057	1.334
2	861118	3.246	4775	129767	1.336
3	861513	3.246	4803	129724	1.344
4	869708	3.248	4720	129653	1.339
5	866796	3.246	4778	130431	1.334
6	865502	3.246	4813	130385	1.341
Average	865532.00	3.25	4770.00	130002.83	1.26
STDEV	3572.45	0.00	37.54	342.95	0.00
% RSD	0.41	0.03	0.79	0.26	0.32
Limits			>2000		<2.0
% RSD	<2.0	<2.0	<2.0	<2.0	<2.0

LINEARITY

Table 13: results of linearity

LINEARITY			
S.No	Linearity level	Conc. (µg/ml)	Peak area
1	50%	10	538940
2	75%	20	720257
3	100%	30	863422
4	125%	40	1035851
5	150%	50	1205950

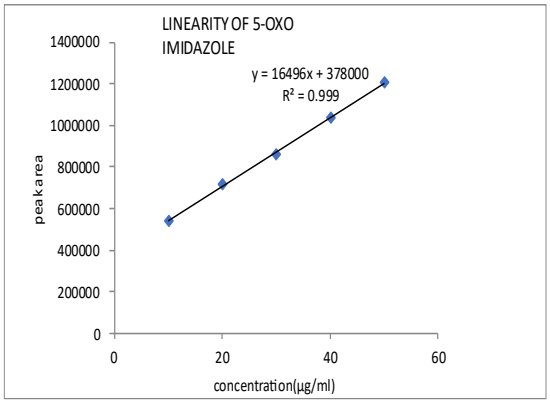


Figure 5: linearity of 5-oxo imidazoline

Inter-day precision

Table 14: inter-day precision

	peak area		
S.No	Day 1	Day 2	Day 3
1	866754	867472	869720
2	860934	854533	856720
3	866368	863771	868758
4	869668	850897	864585
5	863932	851062	861880
6	861576	864642	865764
Average	864872.00	858729.50	864571.17
STDEV	3347.73	7410.19	4782.18
% RSD	0.39	0.86	0.55
Limits	% RSD: <2.0	% RSD: <2.0	% RSD: <2.0

Intra-day precision:

Table 15: intra-day precision

S.No	Peak area		
	9:00 AM	1:00 PM	5:00 PM
1	851434	864641	860668
2	860248	864577	860871
3	866432	865808	856037
4	859099	860968	863200
5	863340	862654	866271
6	860504	862118	867955
Average	860176.17	863461.00	862500.33
STDEV	5036.74	1833.91	4295.29
% RSD	0.59	0.21	0.50
Limits	% RSD <2.0	% RSD <2.0	% RSD <2.0

LIMITOF DETECTION:

From the theoretical value LOD is found to be
Standard deviation= 6778.733
Slope from linearity=16496
LOD=3.3×Standard deviation/slope =3.3×6778.733/16496 =1.3µg/ml
Remarks: The concentration of LOD was calculated by using standard deviation and slope.

LIMIT OF QUANTIFICATION:

From the theoretical value LOQ was found to be
Standard deviation= 6778.733
Slope from linearity=16496
LOQ=10×Standard deviation/slope =10×6778.733/16496 =4.10 µg/ml
Remarks: The concentration of LOQ was calculated by using standard deviation and slope. The values obtained from the LOD and LOQ are mentioned in the table no 16

Table no 16: results of LOD and LOQ

Parameters	Slope from Linearity	SD of peak from lowest concentration
	16496	6778.73
LOD = 3 x SD/slope	1.350µg/ml	
LOQ = 10 x SD/slope	4.100µg/ml	

ROBUSTNESS:

The values obtained for robustness are mentioned in table no 17.

Table 17 results of robustness

Robustness						
Parameter	Condition	Condition	RT	Peak area	Theoritical plates	% Assay
Flow (ml/min) min±0.5 ml	Less flow	0.8ml/min	4.104	1099540	5241	89.62
	Optimized	1.0ml/min	3.284	861880	4786.00	70.25
	More flow	1.2ml/min	2.75	732315	4554	59.69
Temp (°C) min±5°C	Less temp	35	3.268	833531	4754	67.94
	Optimized	40	3.525	861880	4786.00	70.25
	More temp	45	3.26	835985	4994	68.14
Wave length (nm) min±5 nm	Low WL	249	3.299	779589	4886	63.54
	Optimized	254	3.525	861880	4786.00	70.25
	High WL	259	3.299	802192	4295	65.39
Mobile phase (ratio) min±5 ratio	Low	85:15:00				0.00
	Optimized	90:10:00	3.620	861880	2364.00	70.25
	High	95:05:00				0.00

DEGRADATION STUDIES OR STABILITY STUDIES:

Table 18 results of degradation studies

Forced Degradation studies				
S.No	Condition	Peak Area	% Assay	% Degradation
1	Acid	1083033	88.278	11.722
2	Base	1130909	92.180	7.820
3	Temperature	1119270	91.231	8.769
4	Peroxide	1135693	92.570	7.430
5	UV	1093938	89.166	10.834
Average		-		
STDEV				
% RSD				
Limits	**Average Assay : 90; % Degradation:10%**			

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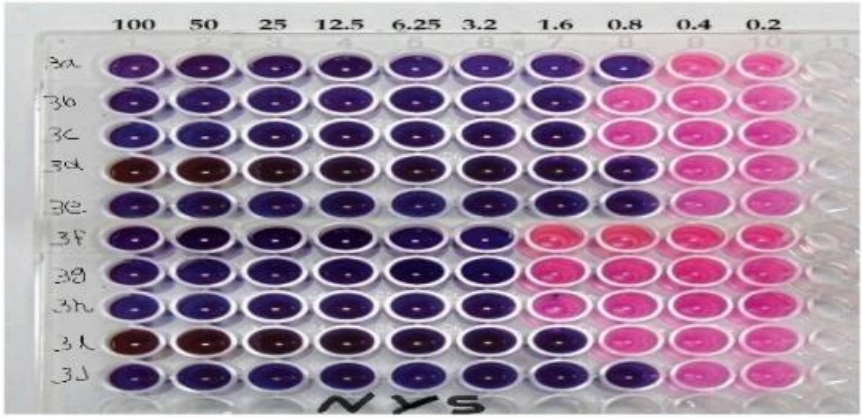
3.6 Biological evaluation

Preliminary biological screening indicated that the synthesized compound exhibited improved activity compared with the parent drug, supporting the docking results (Table:19).

Standard values for the Anti-Tb test which was performed (figure:6)
Isoniazid-1.6 µg/ml
Ethambutol-1.6 µg/ml
Pyrazinamide- 3.125 µg/ml
Rifampicin-0.8 µg/ml
Streptomycin-0.8µg/ml

Streptomycin-0.8µg/ml



Note: S-Sensitive, R-Resistant

Date: 26.12.2025

Place: Belgaum

Dr. Preeti Ingalagi

Microbiologist

Figure:6 Representative microplate image showing biological activity of 5-oxo imidazoline derivatives at different concentrations

Table:19 biological activity of synthesized 5-oxo imidazoline derivative at different concentrations

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	3a	S	S	S	S	S	S	S	R
02	3b	S	S	S	S	S	S	S	S
03	3c	S	S	S	S	S	S	S	S
04	3d	S	S	S	S	S	S	S	R
05	3e	S	S	S	S	S	S	S	R
06	3f	S	S	S	S	S	S	R	R
07	3g	S	S	S	S	S	S	R	R
08	3h	S	S	S	S	S	S	R	R
09	3i	S	S	S	S	S	S	S	R
10	3j	S	S	S	S	S	S	S	S

4.CONCLUSION

The present study successfully accomplished the chemical modification of secnidazole in to a novel 5-oxo imidazoline derivative through a suitable synthetic procedures under optimized conditions. The synthesized compound was isolated and purified by column chromatography technique, structurally conformed using FTIR,

1HNMR, 13CNMR, and mass spectrometric analysis which supports the successful formation of 5-oxo imidazoline derivatives, molecular docking studies reveled the successful ligand-receptor interactions, indicating promising binding affinity towards the selected biological target. The validated RP-HPLC method proved reliable for quantitative estimation. Preliminary biological evaluation indicated promising activity, suggesting the potential of the synthesized compound for further investigation.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

7. AUTHOR CONTRIBUTION

8. REFERENCES

1. Khan MS, Baig MH, Ahmad S, Siddiqui SA, Srivastava AK, et al. (2013) Design, synthesis, evaluation and thermodynamics of 1-substituted pyridyl imidazo[1,5-a] pyridine derivatives as cysteine protease inhibitors. PLoS One 8: e69982.
2. Khan AU, Baig MH, Wadhwa G (2011) Molecular docking analysis of new generation cephalosporins interactions with recently known SHVvariants. Bioinformation 5: 331-335.
3. Shan Y, Kim ET, Eastwood MP, Dror RO, Seeliger MA, et al. (2011) How does a drug molecule find its target binding site? J Am ChemSoc 133: 9181-9183.
4. Merad M, Soufi W, Ghalem S, Boukli F, Baig MH, et al. (2014) Molecular interaction of acetylcholinesterase with carnosic Acid derivatives: a neuroinformatics study. CNS NeurolDisord Drug Targets 13: 440-446.
5. Terstappen GC, Reggiani A (2001) Insilico research in drug discovery. Trends PharmacolSci 22: 23-26.
6. Blake RA (2007) Target validation in drug discovery. Methods MolBiol 356: 367-377.
7. Ekins S, Freundlich JS, Hobrath JV, Lucile White E, Reynolds RC (2014) Combining computational methods for hit to lead optimization in Mycobacterium tuberculosis drug discovery.Pharm Res 31: 414-435.
8. Smith C (2003) Drug target validation: Hitting the target. Nature 422: 341, 343, 345 passim.
9. Green DV, Segall M (2014) Chemoinformaticsin Lead Optimization. Chemoinformatics for Drug Discovery 149-178.
10. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46: 3-26.
11. Vacca J P, Condra J H (1997) Clinicaly effective HIV-1 protease inhibitors. Drug Discovery Today 2: 261-72.
12. Baig MH, Shakil S, Khan AU (2012) Homology modeling and docking study of recent SHV type ß-lactamses with traditional and novel inhibitors: an in silico approach to combat problem of multiple drug resistance in various infections. Medicinal Chemistry Research 21: 2229-2237.
13. Doman TN, McGovern SL, Witherbee BJ, Kasten TP, Kurumbail R, et al. (2002) Molecular docking and high-throughput screening for novel inhibitors of protein tyrosine phosphatase-1B. J Med Chem 45: 2213-2221.
14. Danishuddin M, Khan A, Faheem M, Kalaiarasan P, Hassan Baig M, et al. (2014) Structure-based screening of inhibitors against KPC-2: designing potential drug candidates against multidrug-resistant bacteria. J BiomolStructDyn 32: 741-750.
15. Baig MH, Danishuddin M, Khan S, Khan AU (2012) Screening of inhibitors for S130G inhibitor resistant mutants of TEM type betalactamase. Bioinformation 8: 1225-1229.
16. Laskowski RA, Hutchinson EG, Michie AD, Wallace AC, Jones ML, et al. (1997) PDBsum: a Web-based database of summaries and analyses of all PDB structures. Trends BiochemSci 22: 488-490.
17. Traxler P M (1997) Protein tyrosine kinase inhibitors in cancer treatment. Expert Opinion on Therapeutic Patents 7: 571-88.
18. Brady SF, Stauffer KJ, Lumma WC, Smith GM, Ramjit HG, et al. (1998) Discovery and development of the novel potent orally active thrombin inhibitor N-(9-hydroxy-9-fluorenecarboxy)prolyl trans-4-aminocyclohexylmethyl amide (L-372,460): coapplication of structurebased design and rapid multiple analogue synthesis on solid support. J Med Chem 41: 401-406.
19. Anshul Chawla, Ashu Sharma and Anil Kumar Sharma, *Der Pharma Chemica,* 4 (1):116-140 (2012).
20. Kidwai AR, Dewasia GMV. A New Method for the Synthesis of Amino Acids. Synthesis of Amino Acids and Their Derivatives through 2, 4- Disubstituted 2-Imidazolin-5-ones. *Journal of the American Chemical Society*; 1962; **27**:4527

21. Devasia M, Pillai C. An improved method for the synthesis of cylamino acids through 2,4- disubstituted 2-imidazolin-5-ones. *Tetrahedron Letters*. 1975; **6**:4051
22. Joshi H, Upadhyay P, Karia D, Baxi AG. Synthesis of some novel imidazolinones as potent anticonvulsant agents. *European Journal of Medicinal Chemistry*. 2003;**38**:837
23. Fox SW. Chemistry of the biologically important Imidazoles. Chemical Reviews. 1943;**32**:479
24. A. W. Hoffmann, *Ber*., 21, 2332 (1888).
25. Ladenburg A., *ibid*. 27, 2952(1894).
26. A. Mystufa and A. H. E. Harshah, *J. Org. Chem.,* 21, 575 (1956).
27. Satya Prakash, Rastogi and Arora, *J. Indian Chem. Soc*., 43, 651 (1966).
28. A. F. M. Fahmy and M. O. A. Okabi, *Indian J. Chem*., 10, 961 (1972).
29. Isidor Greenwald, J. Am. Chem. Soc., 47, 1443 (1925); *Chem. Abstr.,* 19, 1853 (1925).
30. C. H. Granacher and G. Gulbas., *Helv. Chim. Acta*., 10, 819 (1927); *Chem. Abstr.*, 22, 781 (1928).
31. G. M. Devasia, Tetrahedron Lett., 46, 4051 (1975); *Chem. Abstr.,* 84, 74891z (1976).
32. Erlenmeyer and Matter, *Ann*., 337, 271 (1904).
33. Kore Srinivas, G.M. Sreenivasa, E. Jayachandran and D. Sreenivasa Rao. Synthesis and Pharmacological screening of bio-active molecule fluorobenzothiazole comprising sulfonamido imidazolinone derivatives. *Asian Journal of Chemistry,* 19(6):4193-4202; (2007).
34. Imidazolines | DrugBank
35. Secnidazole: Uses, Interactions, Mechanism of Action | DrugBank
36. T. Maneshwar, N.Vijetha, V.Balakrishna, Ch.Vijay Kumar, M.Suresh. Synthesis, characterization and anthelmintic activity of novel isatin substituted imidazoline derivatives. *International Journal of Pharmacy*, 4(1): 437-441; (2014).