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DOCKING, SYNTHESIS AND ANTITUBERCULAR EVALUATION OF ISONICOTINOYL HYDRAZINO-AMINO ACIDS

M. Himaja*[1], Rajesh K[1], Venkateshwarreddy M[1], Karigar Asif[2], Ramana M V[2]
[1]Department of Pharmaceutical Chemistry, School of Advanced Sciences, VIT University, Vellore 632 014, India.
[2]Maratha Mandal's College of Pharmacy, Belgaum 590016, Karnataka, India

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***Corresponding author**
Email: dr_himaja@yahoo.com

ABSTRACT
Novel series of isonicotinoylhydrazino-amino acids were designed based on docking with a tuberculosis target protein target protein 3OEI using HEX software. The title compounds with good dock score were synthesized by solution phase technique. The synthesized compounds were characterized by FTIR, ^{1}H NMR and mass spectral analysis and evaluated for their antitubercular activity. The compounds exhibited significant antitubercular activity.

KEYWORDS: Isoniazid, amino acids, antitubercular activity.

INTRODUCTION

Isoniazid found to be associated with biological activity such as antimicrobial activity and antitubercular activity[1,2]. Amino acids are well known to be penetration enhancers when attached to drug molecules, thereby increasing the bioavailability of the drug molecules. In view of the activity associated with isoniazid we wish to report the synthesis and antitubercular activity of amino acids incorporated with isoniazid. Isoniazid derivatives of amino acids were synthesized by EDC/Et_3N mediated solution phase technique. The amino group of amino acids was protected by Boc_2O to form Boc-amino acids. The Boc- amino acids were coupled with isoniazid using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as a coupling reagent and triethylamine (Et_3N) as a base to get Boc-protected isonicotinoyl hydrazine-amino acids, followed by hydrolysis of Boc- group with Trifluoroacetic acid (TFA) to form the title compounds..

MATERIALS AND METHODS

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The amino acids used are L-amino acids purchased from Spectrochem Private Limited, Mumbai, India. Solvents and reagents were purified by standard methods. Boc-amino acids were prepared by standard procedures[3] (Scheme 1). Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by capillary method. The completion of reaction and purity of compounds were checked by thin layer chromatography. IR spectra were recorded on Nicolet impact 400 FT/IR spectrometer using KBr pressed pellet technique. ^{1}H NMR spectra were recorded on GEOL-JMS D-300 (MHz) NMR spectrometer. Mass spectra of were Shimandzu GC-MS (at 70ev) using Mass spectrometer using Xenon as a carrier gas.

Docking

Docking was done by using Hex software with a tuberculosis target protein.

Molecular docking involves the following steps using Hex 4.5 software:

1. Identify a target protein 3OEI from the Protein data Bank.
2. Download PDB FILE (text) and save in Example Folder of Hex 4.5.
3. Draw all the ligands using Chem Sketch.
4. Generate 3-D view (SDF format), convert it into MOL file.
5. Convert into PDB format by using Swiss PDB viewer and save it.
6. Open Hex 4.5 software, select appropriate protein and ligand and perform Docking.
7. Tabulation of the docking score of all the ligands (Table-1).

Preparation of Isoniazid-amino acids

Boc-amino acid (5 mmol) was dissolved in DMF (30ml). To this, isoniazid (5 mmol) in DMF (5mL) was added followed by 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) reagent (5 mmol) at 0^0C and Et_3N (2mmol). The resulting reaction mixture was stirred at room temperature for 24hr. Then the reaction mass was diluted with ice cold water (10mL), then compound was extracted with ethyl acetate (30mL), washed with little water, dried over anhydrous sodium sulfate and evaporated to get a semisolid mass. The byproduct EDU was water soluble and could be easily removed by washing with water[3,4] (Scheme 2). The physical data of synthesized compounds is mentioned in Table-2.

Antitubercular activity

The antimycobacterial activity of compounds was assessed against *M. Tuberculosis* using micro plate Alamar Blue assay (MABA)[5,6]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. The isoniazid- phenylalanine compound showed good activity. This compound showed activity even in low concentrations. The isoniazid- valine, isoniazid- proline compounds showed moderate activity. These compounds showed activity at higher concentrations. The isoniazid- phenylalanine showed good activity because it contained benzene ring along with heterocyclic ring. The results are presented in Table-3

Spectral data

Compound-1: Isoniazid- leucine (LAC):

FTIR- 3040 cm^{-1} (Ar-CH stretch), 2872 cm^{-1} (Aliphatic- CH), 3223 cm^{-1} (NH stretch), 1708 cm^{-1} (C=O stretch), 1662 cm^{-1} (NH-C=O stretch); ^{1}H NMR**:** DMSO δ8.2-8.8(4H, d, aromatic-H), δ10.2 (1H, s, NH), δ10.4 (1H, s, NH) δ7.7 (1H, s, NH), 5.2-5.3 (1H,m, CH), 2.05-2.19 (2H, m, CH_2), 1.72-1.79(1H, m, CH), 1.59 (9H, s, Boc), 1.11-1.22 (6H, d, CH_3); MS m/z (rel intensity) 351.5 (M+1)

Compound-2: Isoniazid- valine (VAC) :

FTIR- 3033 cm^{-1} (Ar-CH stretch), 2848 cm^{-1} (Aliphatic- CH), 3153 cm^{-1} (NH stretch), 1695 cm^{-1} (C=O stretch), 1636 cm^{-1} (NH-C=O stretch);

Fig: 14 ^{1}H NMR**:** DMSO δ8.6 (1H, s, NH), δ8.3 (1H, s, NH), δ8.1 (1H, s, NH_2), δ7.88-7.83 (2H, d, Ar-H), δ7.38-7.35 (2H, d, Ar-H), δ4.5-4.3 (1H, m, CH), δ 2.4-2.8 (1H, m, CH), 1.18 (6H. d, CH_3).

Compound-3: Isoniazid- proline (PRC) : FTIR- 3010 cm^{-1} (Ar-CH stretch), 2798 cm^{-1} (Aliphatic- CH), 3335 cm^{-1} (NH stretch), 1768 cm^{-1} (C=O stretch), 1686 cm^{-1} (NH-C=O stretch);

Fig: 17 ^{1}H NMR: DMSO δ9.7 (1H, s, NH), δ8.3 (1H, s, NH), 8.2 (1H, s, NH), 7.1-7.3 (9H, t, aromatic-H), δ 4.3 (1H, t, CH), δ2.5-2.7 (2H, t, CH_2), δ1.0-1.3 (9H. m, BOC).

RESULTS AND DISCUSSION

Structural modification of isoniazid was carried out by coupling different amino acids with carboxylic acid group of isoniazid and the synthesized compounds were characterized by FTIR, ^{1}H NMR and Mass spectral analysis. The compounds were subjected to antitubercular activity by Microplate Alamar Blue Assay Method. All compounds shows potential antitubercular activity against the *M. Tuberculosis*. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. All the compounds showed moderate antitubercular activity at higher concentrations.

CONCLUSION

Present investigation describes successful synthesis of title compounds via coupling reaction in good yields. EDC proved to be effective coupling agent both economically and yield wise, in comparison to DCC and DIPC. All the compounds showed potent antitubercular activity as compared to the standard drug. The isoniazid-phenylalanine showed good activity. This compound showed activity even in low concentrations. The isoniazid- valine, isoniazid- proline showed moderate activity. These compounds showed activity at higher concentrations. The isoniazid-phenylalanine showed good activity because it contains benzene ring along with heterocyclic ring.

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Table 1: Docking score of isoniazid-amino acids

COMPOUND	PDB CODE	E SCORE (KJ/ Mol)
INH	3OEI	-98.88
PHC	3OEI	-115.53
LEC	3OEI	-111.11
VAC	3OEI	-108.81
PRC	3OEI	-110.18

Table 2: physical data of isoniazid-amino acid coupled compounds

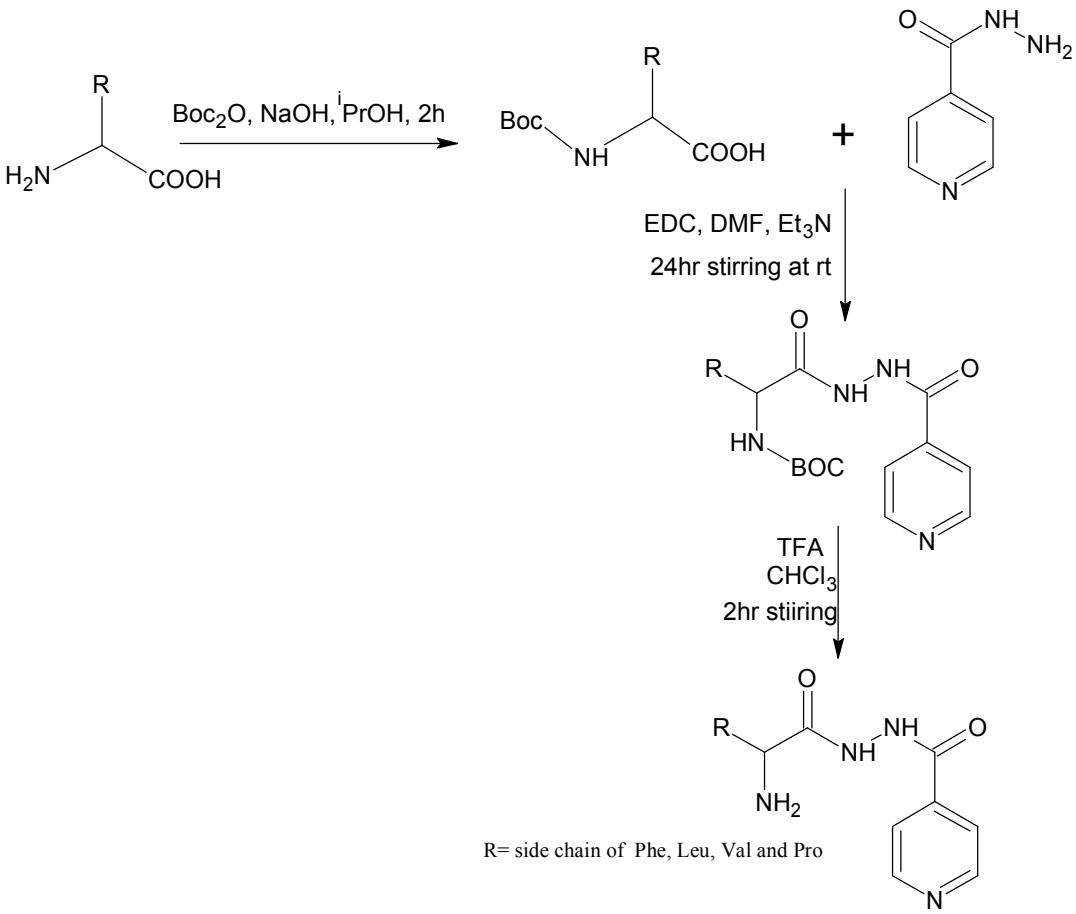
Title compounds	Physical state	Yield (%)
Isoniazid- phenylalanine	Semisolid	55
Isoniazid- leucine	White solid	53
Isoniazid- valine	Semi solid	58
Isoniazid-proline	semisolid	45

Table 3: Results of antitubercular activity

Compound	Concentrations(µl/ml)									
	100	50	25	12.5	6.2	3.12	1.6	0.8	0.4	0.2
PAC	+	+	+	+	+	+	+	+	+	+
PRC	+	+	+	+	+	-	-	-	-	-
VAC	+	+	+	+	+	+	-	-	-	-
LAC	+	+	+	+	+	+	+	-	-	-

(+) = showing activity & (-) = not showing activity, PAC (CN-1) = Isoniazid- phenylalanine, VAC (CN-2) = Isoniazid- valine, PRC (CN-4) = Isoniazid- proline, LAC (CN-3) = Isoniazid-leucine

Scheme 1 : PREPARATION OF ISONIAZID- AMINO ACID COMPOUNDS



R= side chain of Phe, Leu, Val and Pro

Fig: 1 PDB view of 3OEI protein

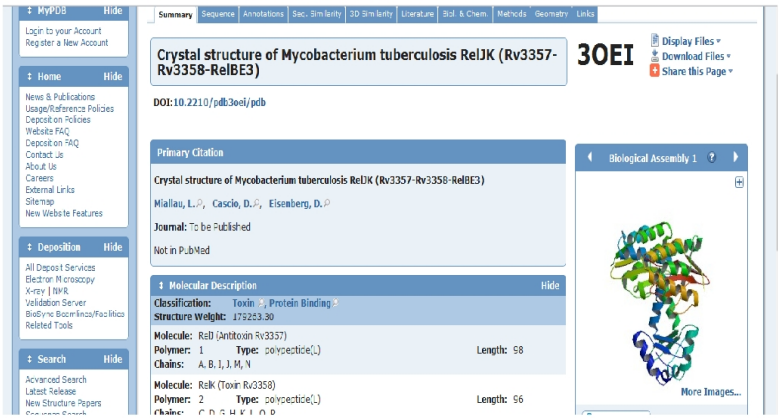
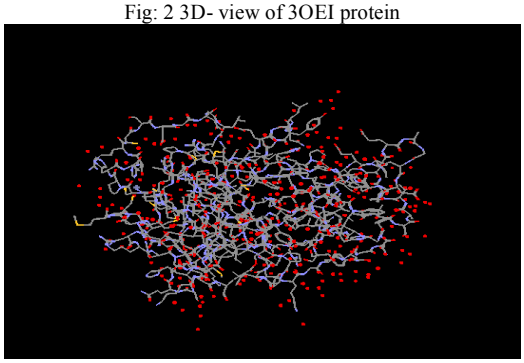
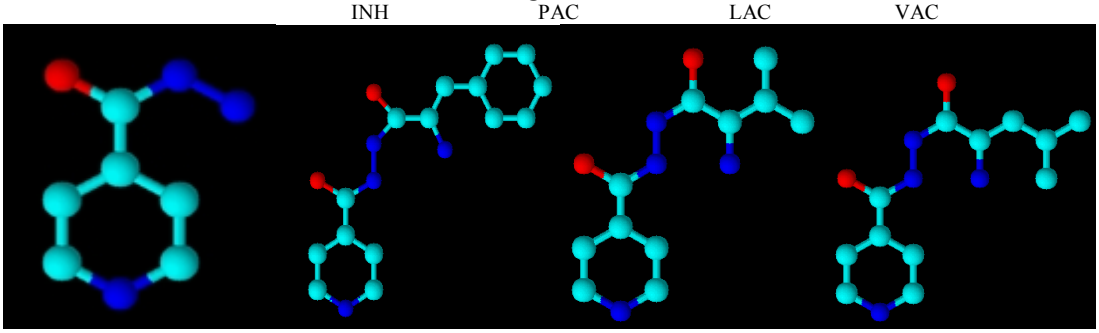


Fig: 2 3D- view of 3OEI protein

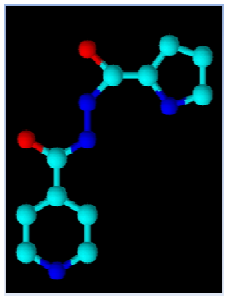


Ligands:

INH PAC LAC VAC

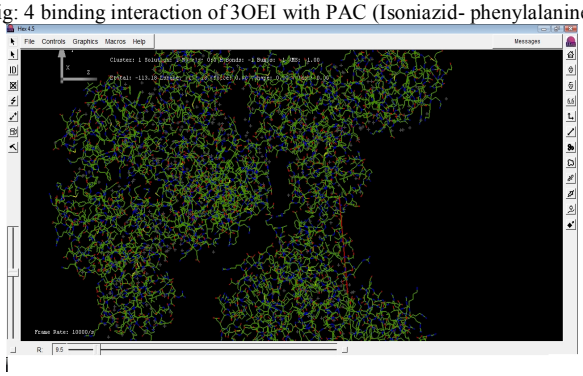


PRC



INH-Isoniazid, PAC-Isoniazid-phenylalanine, LAC-Isoniazid-leucine, VAC-Isoniazid-valine, PRC-Isoniazid-proline

Fig: 4 binding interaction of 3OEI with PAC (Isoniazid- phenylalanine)



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