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SYNTHESIS AND EVALUATION OF ANTITUBERCULAR ACTIVITY OF SOME LAMIVUDINE BASED HYBRID DRUGS

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ABSTRACT
A series of Lamivudine based hybrid drugs (1-4) were synthesized by solution phase peptide synthesis. The docking studies of the designed compounds were carried out against tuberculosis target protein 3OEI using Hex software resulting in a good dock score. Synthesis of these compounds is carried out by using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and Dicyclohexyl carbodiimide (DCC) as coupling agents. Structure of synthesized compounds was confirmed by spectroscopic techniques and the compounds were evaluated for antitubercular property by Micro Plate Alamar Blue (MABA) assay method. The synthesized compounds possesed moderate antitubercular activity.
Keywords: Lamivudine, Solution phase peptide synthesis, Hex-software (Docking), Antitubercular activity.

INTRODUCTION

Tuberculosis (TB) is the most opportunistic Infection (OI) for the people who are suffering from Acquired Immuno Deficiency Syndrome (AIDS). A patient suffering from tuberculosis is susceptible to AIDS. Hence, it was planned to design drug hybrids which can exhibit Antitubercular as well as anti-HIV activities. Lamivudine is 2,3-dideoxy-3-thiacytidine, commonly known as 3TC. This is used for Human Immunodeficiency Virus type-1(HIV-1) and Hepatitis-B (HBV). A series of hybrid drugs were designed by coupling lamivudine with the precursors of Antitubercular agents.

Lamivudine was coupled with pyrazine-2-carboxylic acid and isonicotinic acid using EDC and DCC and triethylamine in DMF to get compounds 1 and 2 respectively. Lamivudine was also coupled with pyrazine-2-carboxylic acid and isonicotinic acid using phenylalanine as handle to get compounds 3 and 4 respectively.

MATERIALS AND METHOD

Analytical grade solvents and commercially available reagents were used without further purification. Anhydrous conditions for all reactions were conducted in oven-dried apparatus. All the reactions were magnetically stirred unless otherwise stated. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by open capillary method. Amino acids, Tetrahydrofuran (THF), EDC, DCC, Trifluroacetic acid and Chloroform were obtained from Spectrochem Ltd, Mumbai. IR spectra in KBr disk were recorded from 4000 to 400 cm^{-1} on Avatar 330.FT-IR spectrometer equipped with DTGS detector. ^{1}HNMR spectra were recorded on GEOL-JMS D-300(MHz).In NMR using $CDCl_3$ as the solvent with Trimethyl silane (TMS) as internal standard. MASS spectra were recorded on Schimadzu GC-MS (at 70ev) mass spectrometer using xenon as the carrier gas.

General Procedure for the Synthesis of Lamivudine-based Hybrid Molecules

Lamivudine[5-8] (10 mmol) and isonicotinic acid/ pyrazine 2-carboxylic acid (10 mmol) were dissolved separately in DMF and then mixed. To the mixture, triethylamine (Et_3N) was added at 0^{o}C and DCC (Dicyclohexyl carbodiimide) (2.2gm, 10 mmol) was added with stirring. Reaction mixture was allowed to stir for 24 hrs. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate, which was washed with 5%HCl (10 ml), 5% $NaHCO_3$ (10 ml) and water. The organic layer was dried with anhy Na_2SO_4 and evaporated to get the title compound. Physical data of the compounds are given in Table 2.

Antitubercular Activity

All the synthesized compounds were evaluated for anti-TB activity by using Micro Plate Alamar Blue assay (MABA)[9] method. In this method, 200µl of sterile deionzed 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. Different concentrations of compounds were tested (0.2, 0.4, 0.6, 0.8, 1.6, 3.12, 6.2, 12.5, 25, 50 and 100µl/ml).

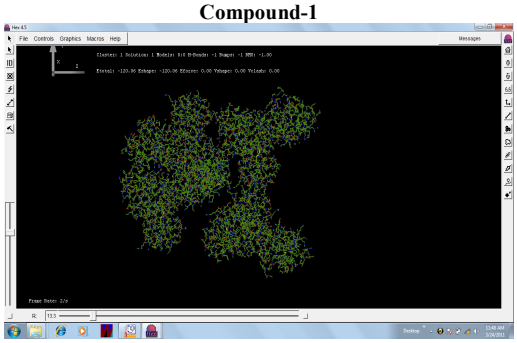
Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULTS AND DISCUSSION

Docking

A preliminary docking study was initially carried out with the HEX-software for the designed compounds tried to dock with the antitubercular protein (3OEI) from Protein Data Base (PDB). The docked compounds showed good dock values when compared with the standard drugs which used for the treatment of TB. With this study it was able to predict that the designed ligands were able to bind with the protein (3OEI). The docking scores are shown in Table 1.

Compound-1



Compound-2

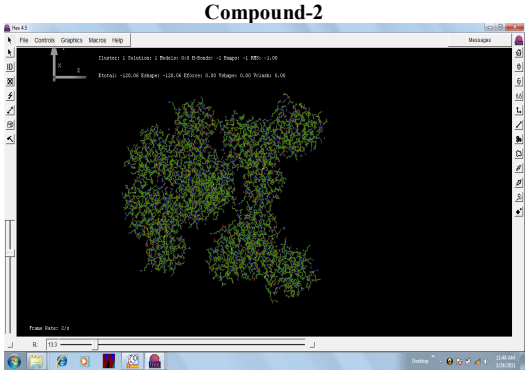


Figure 1: Visualizes of compounds 1, 2 docking against target Mycobacterium tuberculosis protein (3OEI)

Table 1: Docking Scores

S.No	Compound	Protein	Scores
1	compound-1	3OEI	-120.06
2	compound-2	3OEI	-120.06
3	compound-3	3OEI	-127.99
4	compound-4	3OEI	-127.99

Table 2: Physical properties

S.No	Compound	Nature	% of yield
1	compound-1	Solid	72%
2	compound-2	Solid	65%
3	compound-3	Semi Solid	60%
4	compound-4	Semi Solid	60%

Spectral Data

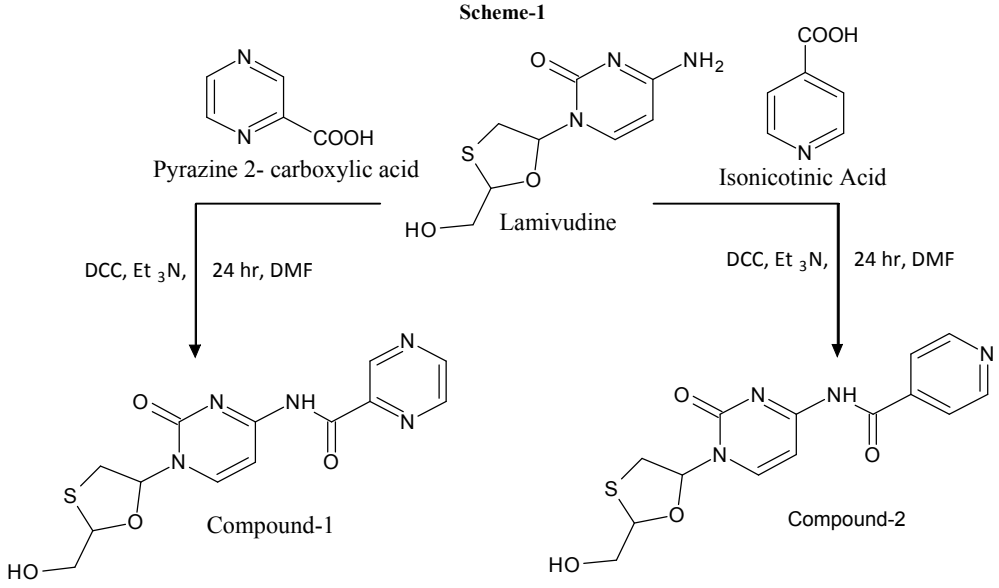
Compound-1: IR (KBr Pallets): 3278 (NH stretch), 3076 (Ar CH stretch), 2929 (aliphatic CH), 1651.07 (C=O stretch), 1731 (O=C-NH stretch) Cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 8-9 (3H, s, arom), δ 5.8(1H, br, NH), δ 4.2(1H, m, alip), δ 3.5(1H, m, alip), δ 1.9(1H, m, OH). **FAB Mass: m/z:** 335.33(M-1).

Compound-2: IR (KBr Pallets): 3298 (NH stretch), 3070 (Ar CH stretch), 2935 (aliphatic CH), 1651(C=O stretch), 1731 (O=C-NH stretch) Cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 8.62-7.36 (5H, m, arom), δ 5.5(1H, s, NH), δ 4.100(1H, d, CH_2), δ 3.12(1H, d, alip), δ 1.97(1H, m, OH). **FAB Mass: m/z:** 358(M + metal ion).

Compound-3: IR (KBr Pallets): 3298 (NH stretch), 3284 (NH str), 3098 (Ar CH stretch), 2976 (aliphatic CH), 1690.5 (C=O stretch), 1679 (C=O str), 1725 (O=C-NH stretch), 1708(O=C-NH str) Cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 8-9 (3H, s, arom), δ 5.8(1H, br, NH), δ 4.2(1H, m, alip), δ 3.5(1H, m, alip), δ 1.9(1H, m, OH). **FAB Mass: m/z:** 467.48(M-1).

Compound-4: IR (KBr Pallets): 3278 (NH stretch), 3256 (N-H str), 3065 (Ar CH stretch), 2954 (aliphatic CH), 1651.07 (C=O stretch),1669 (C=O str) 1731 (O=C-NH stretch) Cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 8-9 (3H, s, arom), δ 5.8(1H, br, NH), δ 4.2(1H, m, alip), δ 3.5(1H, m, alip), δ 1.9(1H, m, OH). **FAB Mass: m/z:** 482.5(M+1).

Scheme-1



Scheme-2

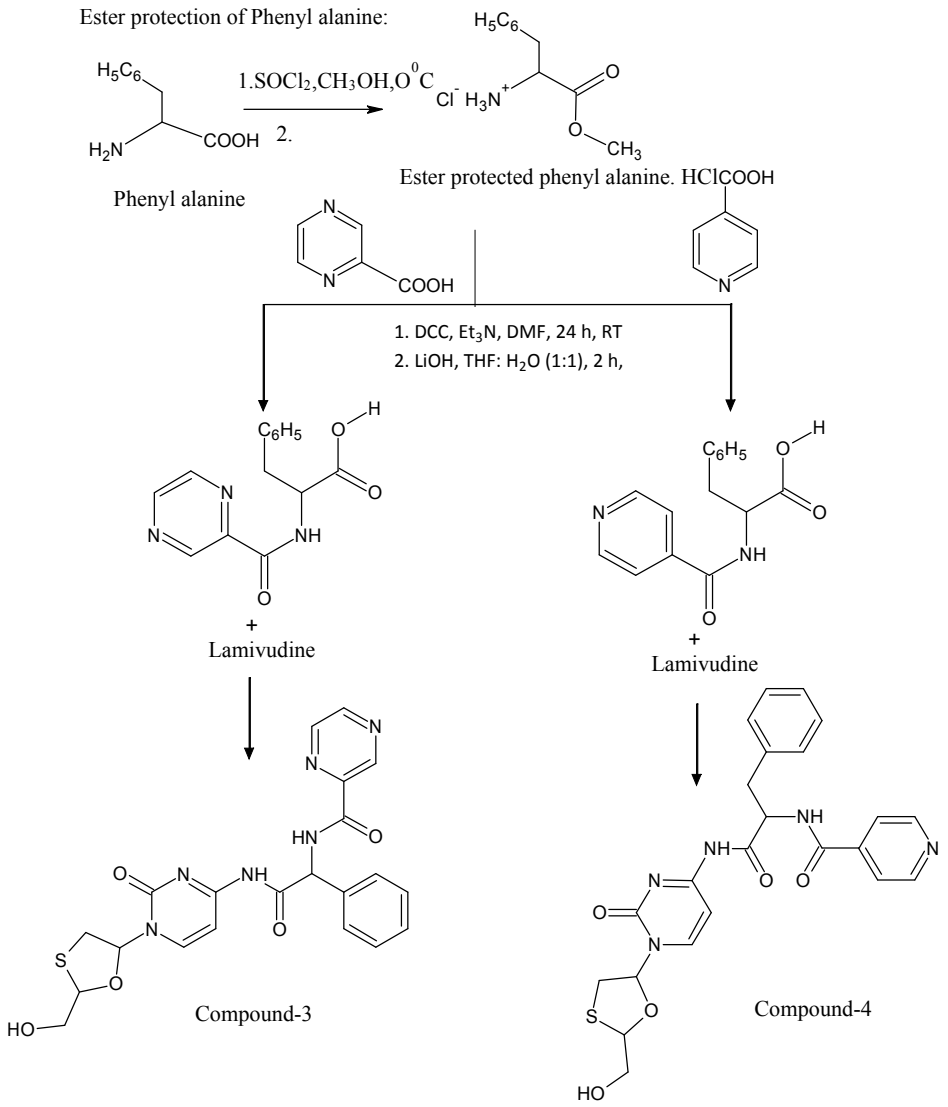


Table 3: Antitubercular activity (Concentrations µl / ml)

Compounds	100	50	25	12.5	6.2	3.12	1.6	0.8	0.4	0.2
compound-1	+	+	+	-	-	-	-	-	-	-
compound-2	+	+	+	+	-	-	-	-	-	-
compound-3	+	+	+	-	-	-	-	-	-	-
compound-4	+	+	+	+	-	-	-	-	-	-

CONCLUSION

All the synthesized compounds (1-4) were characterized by FT-IR, ^{1}H NMR, FAB-MASS spectral studies. All the 4 molecules showed good docking scores against 3OEI and in vitro antitubercular activity at a minimum concentration of 25 µl/ml.

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