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Research Article
Multicomponent Biginelli's Synthesis, Antimycobacterial Activity and Molecular Docking Studies of Dihydropyrimidine Derivatives as Thymidylate Kinase Protein Targets

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Abstract

Background and Objective: Various biginelli compounds (dihydropyrimidinones) have been synthesized efficiently and in high yields under mild, solvent free and eco-friendly conditions in a one pot reaction of 1, 3-dicarbonyl compounds, aldehydes and urea/thiourea using Sodium Dodecyl Sulphate (SDS) as a novel catalyst under two experimental conditions. **Methodology:** The obtained products have been identified by spectral data (^{1}H-NMR, IR and Mass) and their melting points. The dihydropyrimidinone derivatives were evaluated for their *in vitro* antimycobacterial activity against H_{37}Rv strain by using alamar blue dye method. **Results:** The synthesized compounds exhibited promising activity (MIC: 6.25-100 μg mL^{-1}) against mycobacterium tuberculosis H_{37}Rv strain. Docking studies were carried out on synthesized dihydropyrimidines (DHPMs) using GOLD software, with the crystal structure of thymidylate kinase (1G3U) to gain some structural insights on the binding mode and possible interactions with the active site. **Conclusion:** Among the tested compounds, IV_f was found to be most potent with Minimum Inhibitory Concentration (MIC): 6.2±0.36 μg mL^{-1} with least minimal toxicity some of them were found to possess significant activity, when compared to standard drug. The docking results revealed useful information to understand the interaction mode between dihydropyrimidine derivatives and thymidylate kinase (TMPK) and will facilitate the next cycle of drug design to explore the newer lead molecules.

Key words: Pyrimidine, antitubercular activity, thymidylate kinase, molecular docking

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Multi drug resistant TB cases (MDRTB) have increased due to lack of an effective vaccine[1] and it seriously demands the development of new drugs which can control against drug resistant TB and duration[2,3] of treatment may be shorten. The *M. tuberculosis* H_{37}Rv strain[4] complete genome sequencing led to the development of new mycobacterial genetic tools which facilitated the identification of targets essential in bacterial growth, metabolism and viability[5,6]. Enzyme thymidylate kinase represents a promising target for developing drugs against tuberculosis[7,8]. Kinases are responsible for the activation of nucleosides to nucleotide triphosphates (NTPs), the building units of RNA and DNA. Kinases represent effective candidates and have been subjected to extensive structural studies[9]. The importance of kinases in controlling essential processes in gene regulation, signal transduction and metabolism make these enzymes attractive targets for the development of drugs[10].

Important biological and pharmacological activities were exhibited by DHPMs and form the basis for the several activities i.e., antihypertensive[11], antimycobacterial[12], calcium channel blockers[13], α1-adrenergic antagonist[14], anti-inflammatory[15] and antitumor[16]. Many alkaloids obtained from marine sources contain DHPM moiety and exhibited various interesting biological activities[17,18]. Based on the literature the study was proceeded for identification of bioactive agents i.e., synthesis of various 4-aryl-5-carboxyl-6-methyl-3,4-dihydropyrimidine-2(1H)-ones/thiones. These novel synthesized compounds were screened for their *in vitro* antimycobacterial activity based on molecular docking studies performed by docking various DHPMs with TMPK protein.

MATERIALS AND METHODS

The purity of the compounds was checked by TLC using ethyl acetate, benzene (4:6) as solvent system and iodine vapours for visualization. Melting points were detected in open capillaries using Bachi melting point apparatus and are uncorrected. The IR spectras were recorded on Perkin- Elmen RX1-FTIR. The ^{1}H-NMR spectra on a JEOL 400 spectrometer using TMS as an internal standard and mass spectra in JEOL DX 300 in EI ionization made at 70 eV. The MW reactions were carried out in BPL-SANYO domestic micro-wave oven. The elemental analysis of the compounds were recorded on Perkin-Elmer series 2400 analyzer.

Chemistry

Synthesis of 4-(substituted aryl)-3,4-dihydropyrimidine-2-(1H)-ones/thiones[19,20]

Micro-wave irradiation method: To a mixture of β-ketoester (0.01 mol, I), substituted aromatic aldehyde (0.01 mol, II), urea or thiourea (0.01 mol, III) and sodium dodecyl sulphate (10% w/v in water) was subjected to microwave irradiation at 220 W for 5-6 min. The completion of the reaction was monitored by TLC. After cooling to room temperature, the reaction mixture was poured into 100 mL of cold water and stirred for 5 min. The separated solid was filtered under reduced pressure, washed with cold water and then recrystalised from ethanol to afford the pure product.

Conventional method: To a mixture of β-ketoester (0.01 mol, I), substituted aromatic aldehyde (0.01 mol, II), urea or thiourea (0.01 mol, III) and sodium dodecyl sulphate (10% w/v in water) were heated under reflux for 4-5 h with magnetic stirring. After completion of the reaction as monitored by TLC, the reaction mixture was poured into ice-cold water and stirred for 10-15 min. The contents of the flask were then filtered, washed with cold water (20 mL) to remove excess urea/thiourea. The solid so obtained was the corresponding dihydropyrimidines (IV) and recrystallized by hot ethanol to get the pure products (Fig. 1). Physical data of synthesized compounds are presented in Table 1.

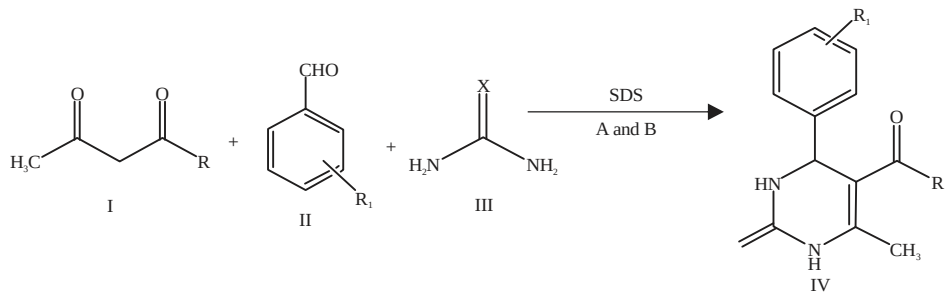

Fig. 1: Dihydropyrimidines and recrystallzed by hot enthanol, X: O, S, R: -CH_3, -OC_2H_5, -OCH_3, R1: -H, 4-OCH_3, 4-OH, 2-OH, 4-Cl, 3-OCH_3-4-OH, -4-NO_2, A: Micro-wave irradiation method, B: Conventional method and SDS: Sodium dodecy1 sulphate

Table 1: Physical properties of synthesized dihydropyrimidines

Componds code	R_1	R	X	Mol. Formula	M.P (°C)	Yield (%)	
						Conventional	MWI
IV_a	H	CH_3	O	$C_{13}H_{14}N_2O_2$	200-02	93	95
IV_b	H	OC_2H_5	O	$C_{14}H_{16}N_2O_3$	208-210	94	96
IV_c	4-OCH_3	OC_2H_5	O	$C_{15}H_{18}N_2O_4$	199-201	87	89
IV_d	4-OH	OC_2H_5	O	$C_{14}H_{16}N_2O_4$	226-229	90	92
IV_e	2-OH	OC_2H_5	O	$C_{14}H_{16}N_2O_4$	199-200	92	94
IV_f	4-Cl	OC_2H_5	O	$C_{14}H_{15}N_2O_3Cl$	209-211	95	95
IV_g	4-NO_2	OC_2H_5	O	$C_{14}H_{15}N_3O_5$	206-08	90	94
IV_h	H	OC_2H_5	S	$C_{14}H_{16}N_2O_2S$	208-210	94	96
IV_i	H	CH_3	S	$C_{13}H_{14}N_2OS$	210-211	93	95
IV_j	4-(OCH_3)	CH_3	O	$C_{14}H_{13}N_2O_3$	190-191	92	96
IV_k	4-Cl	OC_2H_5	S	$C_{14}H_{15}N_2O_2SCl$	209-211	95	95
IV_l	4-OH	OC_2H_5	S	$C_{14}H_{16}N_2O_3S$	227-228	88	89
IV_m	4-OH,3-OCH_3	OC_2H_5	O	$C_{15}H_{18}N_2O_5$	233-235	82	84
IV_n	2-OH	OC_2H_5	S	$C_{14}H_{16}N_2O_3$	220-223	85	85
IV_o	4-Cl	OCH_3	O	$C_{13}H_{13}N_2O_3Cl$	203-205	90	90

Spectral data of synthesized compounds

5-(acetyl)-4-phenyl-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_a): The IR (KBr) cm^{-1}: 3241 (N-H), 3095 (C-H, Ar), 1713 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 2.1 (3H, s, -CH_3), 2.29 (3H, s, -$COCH_3$), 5.26 (1H, s, H of pyrimidine ring), 7.24 (5H, m, Ar-H), 7.82 (1H, s, -NH) and 9.17 (1H, s, -NH). Mass (ESI-MS): m/z 231 (M+1). Elemental analysis: For $C_{13}H_{14}N_2O_2$ calculated 67.81% C, 6.12% H, 12.16% N; found 67.82% C, 6.08% H and 12.17% N.

5-(ethoxycarbonyl)-4-phenyl-6-methyl-3,4-dihydropyrimidine -2(1H)-one (IV_b): The IR (KBr) cm^{-1}: 3249 (N-H), 3073 (C-H, Ar), 1738 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.04 (3H, t, -OCH_2CH_3), 2.23 (3H, s, -CH_3), 3.95 (2H, q, -OCH_2CH_3), 5.19 (1H, s, H of pyrimidine ring), 7.15 (5H, m, Ar-H), 7.77 (1H, s, NH) and 9.85 (1H, s, -NH). Mass (ESI-MS): m/z 261 (M+1). Elemental analysis: For $C_{14}H_{16}N_2O_3$ calculated 64.61% C, 6.19% H, 10.76% N; found 64.61% C, 6.15% H and 10.76% N.

5-(carboethoxy)-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_c): The IR (KBr) cm^{-1}: 3246 (N-H), 3042 (C-H, Ar), 1709 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.04 (3H, t, -OCH_2CH_3), 2.23 (3H, s, -CH_3), 3.79 (3H, s, -OCH_3), 3.94 (2H, q, -OCH_2CH_3), 5.15 (1H, s, H of pyrimidine ring), 6.97 (4H, m, Ar-H), 7.75 (1H, s, -NH), 9.73 (1H, s, -NH). Mass (ESI-MS): m/z 291 (M+1). Elemental analysis: For $C_{15}H_{18}N_2O_4$ calculated 62.06% C, 6.25% H, 9.65% N; found 62.06% C, 6.20% H and 9.65% N.

5-(carboethoxy)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_d): The IR (KBr) cm^{-1}: 3290 (N-H), 3092 (C-H, Ar), 1690 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.03 (3H, t, OCH_2CH_3), 2.22 (3H, s, -CH_3), 3.94 (2H, q, -OCH_2CH_3), 5.12 (1H, s, H of pyrimidine ring), 6.61 (4H, m, Ar-H), 7.73 (1H, s, -NH), 8.86 (1H, s, -NH) and 9.76 (1H, s, -OH). Mass (ESI-MS): m/z 277 (M+1). Elemental analysis: For $C_{14}H_{16}N_2O_4$ calculated 60.86% C, 5.83% H, 10.04% N; found 60.86% C, 5.79% H and 10.14% N.

5-(ethoxycarbonyl)-4-(2-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_e): The IR (KBr) cm^{-1}: 3224 (N-H), 3084 (C-H, Ar),1748 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.04 (3H, t, -OCH_2CH_3), 2.23 (3H, s, -CH_3), 3.95 (2H, q, -OCH_2CH_3), 5.15 (1H, s, H of pyrimidine ring), 6.74 (4H, m, Ar-H), 7.76 (1H, s, -NH), 8.27(1H, s, -NH) and 9.73 (1H, s, -OH). Mass (ESI-MS): m/z 277 (M+1). Elemental analysis: For $C_{14}H_{16}N_2O_4$ calculated 60.86% C, 5.83% H, 10.04% N; Found 60.86% C, 5.79% H and 10.14% N.

5-(carboethoxy)-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_f): The IR (KBr) cm^{-1}: 3242 (N-H), 3095 (C-H, Ar), 1723 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.04 (3H, s, -OCH_2CH_3), 2.24 (3H, s, -CH_3), 3.21 (2H, q, -CH_2CH_3), 5.21 (1H, s, H, of pyrimidinering), 7.16 (4H, m, Ar-H), 8.51 (1H, s, -NH), 9.46 (1H, s, -NH). Mass (ESI-MS): m/z 295 (M+1). Elemental analysis: For $C_{14}H_{15}N_2O_3Cl$ calculated 57.05% C, 5.13% H, 9.50% N; found 57.14% C, 5.10% H and 9.52% N.

5-(ethoxycarbonyl)-4-(4-nitrophenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_g): The IR (KBr) cm^{-1}: 3274 (N-H), 3052 (C-H, Ar), 1758 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.06 (3H, t, -OCH_2CH_3), 2.26 (3H, s, -CH_3), 3.95 (2H, q, -OCH_2CH_3), 5.23 (1H, s, H of pyrimidine ring), 7.47 (m, 4H, Ar-H), 7.84 (1H, s, -NH), 9.35 (1H, s, -NH). Mass (ESI-MS): m/z 306

(M+1). Elemental analysis: For $C_{14}H_{15}N_3O_5$ calculated 55.08% C, 4.95% H, 13.76% N; found 55.08% C, 4.91% H and 13.77% N.

5-(ethoxycarbonyl)-4-phenyl-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (IV_h): The IR (KBr) cm^{-1}: 3265 (N-H), 3086 (C-H, Ar), 1742 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.12 (3H, t, $-OCH_2CH_3$), 2.31 (3H, s, CH_3), 4.01 (2H, q, $-OCH_2CH_3$), 5.22 (1H, s, H of pyrimidine ring), 7.29 (5H, m, Ar-H), 9.61 (1H, s, -NH), 10.27 (1H, s, -NH). Mass (ESI-MS): m/z 277 (M+1). Elemental analysis: For $C_{14}H_{16}N_2O_2S$ calculated 60.84% C, 5.83% H, 10.14% N; found 60.86% C, 5.79% H and 10.14% N.

5-(acetyl)-4-phenyl-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (IV_i): The IR (KBr) cm^{-1}: 3283 (N-H), 3099 (C-H, Ar), 1715 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.90 (3H, s, $-CH_3$), 2.02 (3H, s, $-COCH_3$), 5.07 (1H, s, H of pyrimidine ring), 7.06 (5H, m, Ar-H), 9.51 (1H, s, -NH), 10.05 (1H, s, -NH). Mass (ESI-MS): m/z 247 (M+1). Elemental analysis: For $C_{13}H_{14}N_2OS$ calculated 67.81% C, 6.12% H, 12.16% N; found 67.82% C, 6.08% H and 12.17% N.

5-(acetyl)-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_j): The IR (KBr) cm^{-1}: 3213 (N-H), 3057 (C-H, Ar), 1715 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 2.06 (3H, s, CH_3), 2.27 (3H, s, $COCH_3$), 3.71 (3H, s, $-OCH_3$), 5.24 (1H, s, H of pyrimidine ring), 6.86 (4H, m, Ar-H), 7.7 (1H, s, -NH), 9.10 (1H, s, -NH). Mass (ESI-MS): m/z 258 (M+1). Elemental analysis: For $C_{13}H_{14}N_2O_2$ calculated 63.38% C, 5.72% H, 11.37% N; Found 63.41% C, 5.69% H and 11.38% N.

5-(ethoxycarbonyl)-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (IV_k): The IR (KBr) cm^{-1}: 3204 (N-H), 3080 (C-H, Ar), 1698 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 0.97 (t, 3H, $-OCH_2CH_3$), 2.21 (3H, s, CH_3), 3.82 (2H, q, $-OCH_2CH_3$), 5.43 (1H, s, H of pyrimidine ring), 7.31 (m, 4H, Ar-H), 7.96 (1H,s,-NH), 8.81(1H, s, -NH). Mass (ESI-MS): m/z 279 (M+1). Elemental analysis: For $C_{14}H_{15}N_2O_2SCl$ calculated 60.32% C, 5.42% H, 10.05% N; found 60.43% C, 5.39% H and 10.07% N.

5-(ethoxycarbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)thione (IV_l): The IR (KBr) cm^{-1}: 3422 (O-H), 3219 (N-H), 3074 (C-H, Ar), 1672 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.14 (t, 3H, $-OCH_2CH_3$), 2.28 (3H, s, $-CH_3$), 3.97 (2H, q, $-OCH_2CH_3$), 5.18 (1H, s, H of pyrimidine ring), 6.84 (4H, m, Ar-H), 7.67 (1H, s, -NH), 9.18 (1H, s, -NH), 9.87 (1H, s, OH). Mass (ESI-MS): m/z 293 (M+1). Elemental analysis: For $C_{14}H_{16}N_2O_3S$ calculated 57.52% C, 5.51% H, 9.58% N; found 57.53% C, 5.47% H and 9.58% N.

5-(ethoxycrbonyl)-4(3-methoxy-4-hydroxylphenyl)-6-methyl-3,4-tetrahydropyrimidine-2(1H)one (IV_m): The IR (KBr) cm^{-1}: 3401 (-OH), 3221 (N-H), 3016 (C-H, Ar), 1673 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.17 (3H, t, $-OCH_2CH_3$), 2.35 (3H, s, -CH3), 3.86 (3H, s, $-OCH_3$), 4.09 (2H, q, $-OCH_2CH_3$), 5.33 (1H, s, H of pyrimidine ring), 6.77 (3H, m, Ar-H), 7.161 (1H, s, -NH), 7.676 (1H, s, -NH), 9.738 (1H,s,OH). Mass (ESI-MS): m/z 307 (M+1). Elemental analysis: For $C_{15}H_{18}N_2O_5$ calculated 58.82% C, 5.92% H, 9.14% N; found 58.92% C, 5.88% H and 9.15% N.

5-(ethoxycarbonyl)-4-(2-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidine-2(1H)-one (IV_n): The IR (KBr) cm^{-1}: 3224 (N-H), 3084 (C-H, Ar), 1748 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 1.04 (3H, t, $-OCH_2CH_3$), 2.23 (3H, s, $-CH_3$), 3.95 (2H, q, $-OCH_2CH_3$), 5.15 (1H, s, H of pyrimidine ring), 6.74 (4H, m, Ar-H), 7.76 (1H, s, -NH), 8.27 (1H, s, -NH), 9.73 (1H, s, -OH). Mass (ESI-MS): m/z 277 (M+1). Elemental analysis: For $C_{14}H_{16}N_2O_4$ calculated 60.86% C, 5.83% H,10.04% N; found 60.86% C, 5.79% H and 10.14% N.

5-(methoxycarbonyl)-4-(4-chlorophenyl)-6-methyl-3,4-tetrahydropyrimidine-2(1H)-thione (IV_o): The IR (KBr) cm^{-1}: 3204 (N-H), 3080 (C-H, Ar), 1698 (C=O). The ^{1}H-NMR (DMSO-d_6) ppm: δ 0.97 (t, 3H, $-OCH_2CH_3$), 2.21 (3H, s, CH_3), 3.82 (2H, q, $-OCH_2CH_3$), 5.43 (1H, s, H of pyrimidine ring), 7.31 (m, 4H, Ar-H), 7.96 (1H, s, -NH), 8.81 (1H, s, -NH). Mass (ESI-MS): m/z 279 (M+1). Elemental analysis: For $C_{14}H_{15}N_2O_2SCl$ calculated 60.32% C, 5.42% H, 10.05% N; found 60.43% C, 5.39% H and 10.07% N.

Anti-tubercular activity: All the synthesized compounds were tested against *Mycobacterium tuberculosis* $H_{37}Rv$ strain for antitubercular activity using microplate alamar blue dye assay (MABA) method[21]. The results were shown in Table 2.

Docking: The x-ray crystal structure of thymidylate kinase obtained from the protein data bank (PDB ID: 1G3U)[22]. The 3D structures of the derivatives were constructed with the ChemBioDraw Ultra11.0 and hydrogen was added in all the ligand structures. Docking studies were performed by GOLD 3.0.1 (Genetic Optimization for Ligand Docking) software, the final corrector PDB file of the protein and synthesized analogous were submitted to GOLD 3.0.1 software tools in order to run docking process and all the parameters set as default. At the final stage through the docked structures of all analogous, best conformations were selected and preparing figures and running protein ligand interactions.

RESULTS AND DISCUSSION

Chemistry: The biginelli protocol for the preparation of DHPMs consisted of heating a mixture of three components which included β-ketoester, aldehyde and urea in ethanol containing a catalytic amount of HCl[23]. The major drawback associated with this protocol was the use of strong acid as well as the low yields in the case of substituted aromatic and aliphatic aldehydes. To enhance the efficiency of the biginelli reaction, various catalysts and reaction conditions have been studied including classical conditions with ultrasound[24] or microwave-assisted irradiations[25] solid-support[26], ionic liquids[27], Lewis acid catalysts such as LiBr[28], NH_4Cl[29], $MgBr_2$[30] and CaF_2[31]. On the other hand, a number of the reported protocols for the synthesis of DHPMs required solvents and catalysts which are not acceptable in the context of green synthesis, utilization of reagents and catalysts which are either toxic or expensive and stoichiometric use of reagents with respect to reactant. Here the capacity of SDS as potential catalyst for one-pot synthesis of 3,4-dihydropyrimidinones was reported.

The 4-(substituted phenyl-)-3,4-dihydropyrimidine-2-(1H)-ones/thiones (IV_a-IV_o) were prepared using one pot Biginelli reaction using sodium doceyl sulphate as catalyst and water as solvent as depicted in Fig. 1. The IR spectra of the compound IV_a showed the absorption bands at 3241, 2985 and 1713 cm^{-1} due to presence of -NH, Ar-H and C=O groups respectively. The ^{1}H-NMR spectra showed signals at δ 2.29 (s, $-COCH_3$), 7.24 (m, Ar-H), 7.82 and 9.12 (br, -NH) and the mass spectra showed M+1 peak at 231 with its molecular formula $C_{13}H_{14}N_2O_2$.

Antimycobacterial activity: Based on docking study results, the compounds which showed better docking scores were selected for *in vitro* antimycobacterial screening. The compounds IV_b, IV_c, IV_f, IV_g, IV_i, IV_k, IV_l, IV_m and IV_n were screened against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and the results were summarized in Table 2. The compound IV_f exhibited significant antimycobacterial activity with MIC 6.2±0.36 μg mL^{-1} compared to pyrazinamide 3.1±0.35 μg mL^{-1}. Compounds IV_k, IV_l and IV_n showed moderate activity with MIC 12.5±0.55, 12.5±0.62 and 50.0±2.15 μg mL^{-1}, respectively and the compounds IV_c and IV_i exhibited MIC at 50±3.40 and 50±3.12 μg mL^{-1}. The significant activity of compound IV_f might be due to the presence of electron withdrawing substituents i.e., 4-chlorophenyl at C-4 and oxygen at C-2 in DHPM ring.

Table 2: *In vitro* evaluation of the antimycobacterial activity and docking scores of DHPMs

Compound	MIC (μg mL^{-1})*	Docking score
IV_b	100.0±6.13	46.39
IV_c	50.0±3.40	50.38
IV_f	6.2±0.36	53.24
IV_g	100.0±5.86	46.29
IV_i	50.0±3.12	48.23
IV_k	12.5±0.55	50.38
IV_l	12.5±0.62	52.20
IV_m	100.0±8.14	45.38
IV_n	50.0±2.15	42.91
Pyrazinamide	3.1±0.35	55.26

MIC: Minimum inhibitory concentration, *Values are expressed in Mean±Standard Deviation

Docking studies: Docking analysis revealed that hydrogen bonding interactions were the crucial factors affecting inhibitory action of the compounds. Amino acids Asp-9, Asp-163, Thy-39, Asp-94, Arg-95, Glu-166, Asp-183, Asn-100 and Thy-103 of TMPK protein were found to be directly interacting with the synthesized DHPMs. Bioisosteric replacement of thiourea 'S' with urea 'O' in the synthesized compounds (IV_a and IV_i) appeared to be oriented in similar fashion. Co-crystallized pyrazinamide when redocked in the active site of thymidylate kinase (1G3U) attained a score of 55.26. It displayed crucial H-bond interactions with the residues Arg-153, Gly-12, Asp-9, Lys-13 and Arg-95 (Fig. 2d). The most active compound IV_f on $H_{37}Rv$ strain (Table 2), fitted best in the active site of TMPK protein inhibition and attained the score of 53.24 (Fig. 2a). It retained all the prime interactions to anchor well in the active sites of the receptor. Moreover, the active compounds IV_k and IV_l of DHPM series were oriented in the active site of the protein in a way that places the aromatic ring into the pocket comprising the residues Asp-9, Glu-166, Asp-16, Asn-100 and Asp-183 (Fig. 2b, c).

Several publications have been reported on design and synthesis of new compounds as antitubercular agents[32,33]. It is observed that the synthesized dihydropyrimidine derivative by utilizing Biginelli reaction has shown prominent antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using Microplate Alamar Blue Assay (MABA). From these results, valuable data about the structure activity correlations of the tested compounds were deduced. Incorporation of chlorine atom at the 4-position of (compound IV_f) led to significant activity against *M. tuberculosis* (MIC = 6.2±0.36 μg mL^{-1}), suggesting that the presence of a strong electron withdrawing group was favourable to the activity. Incorporation of an unsubstituted phenyl group led to complete loss of activity. The compounds

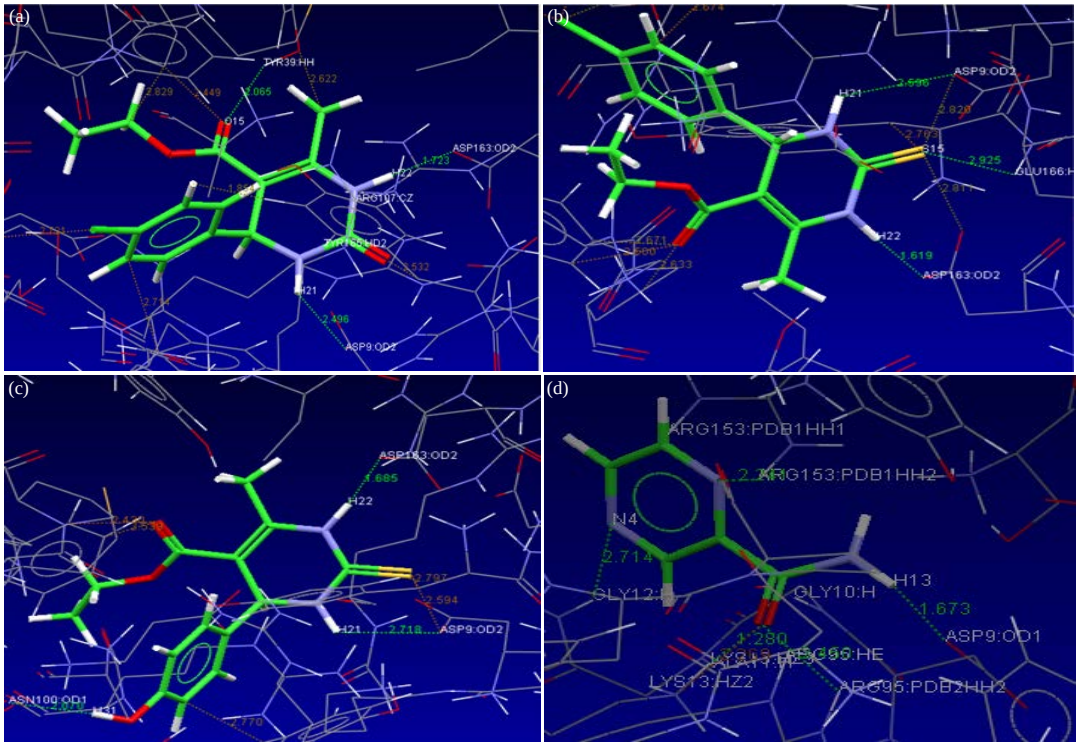

Fig. 2(a-d): (a) H-bond interactions (green) between compound IV_f and 1G3U, (b) Compound IV_k and 1G3U, (c) Compound IV_l and 1G3U and (d) Pyrazinamide and 1G3U

IV_k and IV_l bearing more lipophilic chlorine and hydroxy substituents at the same position, showed better activity (MIC = 12.5±0.55 and 12.5±0.62 μg mL^{-1}, respectively). On the basis of structure functional activities, the compounds IV_f and IV_k bearing the electron-withdrawing group may assist in binding to the active sites favorably.

CONCLUSION

The DHPM derivatives (IV_a-IV_o) were synthesized using SDS as novel catalyst under two experimental conditions. The synthesized compounds were characterized by FT-IR and ^{1}H-NMR. Synthesized compounds were evaluated for their *in vitro* antitubercular activity using alamar blue dye method. Docking studies were carried out on the crystal structure of thymidylate kinase to gain structural insights on the binding mode and possible interaction with the active site. The top ranked molecules were selectively evaluated, for their *in vitro* antimycobacterial activity. Among the tested compounds IV_f shows significant antitubercular activity with MIC 6.2±0.36 μg mL^{-1}, due to the presence of electron withdrawing chlorine group at C-4 phenyl ring in dihydropyrimidine ring. These studies showed that DHPM's scaffold can be utilized for designing of novel antitubercular agents.

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