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A Novel Approach for Synthesis of Pyrimidine Chalcones Against Thymidylate Kinase Protein Targets And Evaluation of Antimycobacterial Activity

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ABSTRACT

Introduction: Infectious diseases are affecting the world with their morbidity and mortality. Globally, more than one-third of the world population is infected with the bacteria that cause tuberculosis (TB) and each year approximately 9 million people affected with the disease and each year 2 million of those die.

Aim: To synthesize a series of novel 3,4-dihydropyrimidine chalcones which have anti-mycobacterial activity and to perform docking studies for active compounds.

Methods: A series of 6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one derivatives were synthesized and evaluated their anti-mycobacterial activity against M. tuberculosis H37Rv strain using Microplate Alamar Blue dye Assay (MABA). The docking calculations were done on protein, thymidylate kinase (PDB ID: 1G3U) employing in Genetic Optimization for Ligand Docking (GOLD v4.0.1 2008) software using Genetic algorithm.

Results: Among all the synthesized derivatives, 5-(3-(2-thienyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one was exhibited potent activity compared with reference standards pyrazinamide and streptomycin. The potent compound forms H-bonding with Tyrosine39, Glutamic acid 166 and Arginine160 amino acids of the active site, a 2-thienaldehyde derivative which undoubtedly accounts for their superior activity associated with other analogs of the series. The docking results exposed useful information to understand the interaction mode between 3,4-dihydropyrimidine chalcone derivatives and thymidylate kinase will simplify the next cycle of drug design to reconnoitre the newer lead molecules.

Conclusion: The interaction model between dihydropyrimidine chalcone derivatives and thymidylate kinase will facilitate the next cycle of drug design to explore the newer lead molecules.

Key Words: Antimycobacterial activity, Chalcone, Dihydropyrimidine, Docking studies, MABA, Thymidylate kinase

INTRODUCTION

Infectious diseases are affecting the entire world with their morbidity and mortality. Globally, more than one-third of the world population is infected with the bacteria that cause tuberculosis (TB) and each year approximately 9 million people affected with the disease and each year 2 million of those die.[1-4] The widespread of TB is due to the following major factors: the susceptibility of people infected with the acquired immune deficiency syndrome (AIDS), which enhances the risk of developing TB in 100 times, and increasing resistance to the existing drugs.[5-7] Treatment of TB is a complex process because of various factors which include patient's inability to persist with the combined treatment regimen, the spreading ability of non-tubercular mycobacteria (NTM) like *M. avium* complex (MAC), the ineffectiveness of the drugs on immunosuppressed patients, and multidrug resistance (MDR).[8-10] Due to increase in significance resistance of the pathogenic strain towards the existing antibiotics, there is a requirement to of design newer antibiotics.

Chalcones are a diverse group of compounds which could be synthesized as well as obtained from natural sources. Chalcones are known to possess different types of biologi-

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cal activity: anti-leishmanial, anti-inflammatory, antimitotic, anti-invasive, anti-fungal, cysteinyl leukotriene1 (CyLT1) receptor antagonism, anti-malarial, anti-plasmodial, immunosuppressive, cytotoxic, anti-tumour, and anti-oxidant properties, and modulation of P-glycoprotein-mediated multi-drug resistance.[11-13] To the best of our knowledge, there has been no previous report of analogous dihydropyrimidine chalcones as anti-tuberculosis agents. However, there are numerous examples of nitrogen containing heterocycles being used to treat TB, for example Clofazimine, Isoniazid and Pyrazinamide. These compounds provide structural precedence that our dihydropyrimidinones chalcone analogues may lead to the generation of novel anti-TB therapeutics.

Herein the synthesis and *in vitro* anti-mycobacterial activity of novel dihydropyrimidine chalcone derivatives are described. Further, we propose the molecular interactions and the binding of the synthesized compounds using the X-ray crystal structure of thymidylate kinase (PDB ID: 1G3U) through docking studies[14]. We hope it will help to further development of new cheap and effective anti-mycobacterial medicines so much needed by the contemporary medicine.

MATERIALS AND METHODS

Experimental

Melting points were recorded in open capillaries on melting point apparatus (MEPA MP08050204) and were uncorrected. Infra red (IR) spectra were recorded on Perkin Elmer FT-IR Spectrometer (Spectrum RX I) using KBr pellet technique. ^{1}H NMR spectra were recorded on Bruker Advance II 400 MHz spectrometer in $CDCl_3$ using Tetra Methyl Silane (TMS) as internal standard. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro and elemental analyses were performed using Thermo EA 2110 series elemental analyser. All chemicals used were of analytical grade and commercially available from E.Merck, Mumbai. Solvents were used without further purification. Silica gel (100–200 mesh; E. Merck, Mumbai) was used for column chromatography. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh) (E. Merck, Mumbai) and spots were visualized under UV light (254 nm).

General experimental procedure

Synthesis of 5-acetyl-4-phenyl-6-methyl-3,4-dihydropyrimidine-2-(1H)-ones (4)

A mixture of benzaldehyde, acetylacetone and urea (0.1 mol. each) was taken into a 250 ml dry beaker and add soy lecithin (0.1 mol.) as a catalyst. An inverted glass funnel was placed over the beaker and subjected to microwave irradiation in a microwave oven at 220 W for 1-2 min, reaction progress was monitored by TLC, after completion of reaction triturated with 150 ml of cold water and dried. Purified by recrystallization from ethanol to afford 3,4-dihydropyrimidine-2-(1H)-ones.[15]

Yield: 96%; mp 210°C; IR (KBr) cm^{-1}: 3241 (N-H), 3095 (C-H,Ar), 1713 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.1 (s, 3H, -CH_3), 2.29 (s, 3H, -CH_3), 5.26 (s, 1H, H of pyrimidine ring), 7.24 (m, 5H, Ar-H), 7.82 (s, 1H, -NH), 9.17 (s, 1H, -NH). Mass (ESI-MS): m/z 231 (M+1). Elemental analysis: For $C_{13}H_{14}N_2O_2$ calculated C, 67.81%; H, 6.12%; N, 12.16%; found C, 67.82%; H, 6.08%; N, 12.17%.

6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one derivatives (6a-t)

A mixture of substituted aromatic aldehyde and compound **4** (0.1 mol. each) in 20 ml of absolute ethanol was taken into a 250 ml dry beaker to the clean reaction mixture 10% NaOH was added. An inverted glass funnel was placed over the beaker and subjected to microwave irradiation at 180 W in a *BPL-SANYO* microwave oven for 2-5 min reaction was monitored by TLC. After completion of reaction neutralized with dil. HCl the precipitated product is filtered, washed with water, dried and recrystallized from absolute ethanol.

5-(3-(4-chlorophenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6a)

Yield: 95%; mp 186°C; IR (KBr) cm^{-1}: 3348 (NH), 1628 (C=O), 1571 (C=C), 1123 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.29 (s, 3H, -CH_3), 5.49 (s, 1H, H of pyrimidine ring), 6.54 (d, 1H, *J*= 17.6 Hz, -CH=CH-), 6.70 (d, 1H, *J*= 7.2 Hz, -CH=CH-), 7.22 (m, 5H, Ar-H), 7.46 (d, 2H, *J*= 8.8 Hz), 7.70 (d, 2H, *J*= 8.8 Hz), 7.89(s, 1H, -NH), 9.26 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_2O_2Cl$: calculated: C, 68.08%; H, 4.85%; N, 7.94%; found: C, 68.18%; H, 4.82%; N, 7.95%.

5-(3-(4-dimethylaminophenyl)acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6b)

Yield: 88%; mp 160°C; IR (KBr) cm^{-1}: 3321 (NH), 1642 (C=O), 1629 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.22 (s, 3H, -CH_3), 3.43 (s, 6H, -N(CH_3)$_2$), 5.19 (s, 1H, H of pyrimidine ring), 6.24 (d, 1H, *J*= 6.8 Hz, -CH=CH-), 6.44 (d, 1H, *J*= 17.6 Hz, -CH=CH-), 8.15 (m, 5H, Ar-H), 8.43 (d, 2H, *J*= 8.4 Hz), 8.76 (d, 2H, *J*= 8.4 Hz), 8.33 (s, 1H, -NH), 9.95 (s, 1H, -NH). Elemental analysis for $C_{22}H_{22}N_2O_4$: calculated: C, 73.10%; H, 6.41%; N, 11.62%; found: C, 73.13%; H, 6.37%; N, 11.63%.

5-(3-(4-hydroxyphenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6c)

Yield: 83%; mp 192°C; IR (KBr) cm^{-1}: 3526 (OH), 3305 (NH), 1617 (C=O), 1575 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.30 (s, 3H, -CH_3), 5.19 (s, 1H, H of pyrimi-

dine ring), 6.32 (d, 1H, *J*= 8.8 Hz,-CH=CH-), 6.53(d,1H, *J*= 19.2 Hz, -CH=CH-), 7.17 (m, 5H, Ar-H), 7.53 (d, 2H, *J*= 8.4 Hz), 7.67(d, 2H, *J*= 8.4 Hz) 8.77 (s, 1H, -NH), 10.16 (s, 1H, -NH), 10.35 (s, 1H, -OH). Elemental analysis for $C_{20}H_{18}N_2O_3$: calculated: C, 71.84%; H, 5.42%; N, 8.38%; found: C, 71.85%; H, 5.38%; N, 8.38%.

5-(3-(3,5-dimethoxyphenyl)acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6d)

Yield: 87%; mp 202°C; IR (KBr) cm^{-1}:3327 (NH), 1673 (C=O), 1469 (C=C), 1193 (C-O-C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.35 (s, 3H, $-CH_3$), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 5.21 (s, 1H, H of pyrimidine ring), 6.91 (d, 1H, *J*= 8.8 Hz, -CH=CH-), 7.24 (d, 1H, *J*= 17.2 Hz, -CH=CH-), 7.32 (m, 8H, Ar-H). Elemental analysis for $C_{22}H_{22}N_2O_4$: calculated: C, 69.83%;H, 5.85%;N, 7.40%; found: C, 69.84%; H, 5.82%; N, 7.40%.

5-(3-(3-nitrophenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6e)

Yield: 92%; mp 201°C; IR (KBr) cm^{-1}: 3356 (NH), 1652 (C=O), 1584 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.53 (s, 3H, $-CH_3$), 4.92 (s, 1H, H of pyrimidine ring), 6.75 (d, 1H, *J*= 17.6 Hz, -CH=CH-), 6.85 (d, 1H, *J*= 7.6 Hz, -CH=CH-), 7.12 (m, 5H, Ar-H), 7.27 (m, 3H, Ar-H), 7.46 (s, 1H, -NH), 8.02 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_3O_4$: calculated: C, 66.11%; H, 4.71%; N, 11.56%; found: C, 66.11%; H, 4.68%; N, 11.57%.

5-(3-(3,4,5,-trimethoxyphenyl)acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6f)

Yield: 91%; mp 182°C; IR (KBr) cm^{-1}: 3327 (NH), 1633 (C=O), 1592 (C=C), 1230 (C-O-C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.29 (s, 3H, $-CH_3$), 3.61 (s, 3H, $-OCH_3$), 3.91 (s, 6H, $-OCH_3$), 5.19 (s, 1H, H of pyrimidine ring), 6.24 (d, 1H, *J*= 5.6Hz, -CH=CH-), 6.45 (d, 1H, *J*= 16.8 Hz, -CH=CH-), 7.95 (m, 7H, Ar-H), 8.53 (s, 1H, -NH), 8.76 (s, 1H, -NH). Elemental analysis for $C_{23}H_{24}N_2O5$: calculated: C, 67.63%; H, 5.92%; N, 6.86%; found: C, 67.64%; H, 5.88%; N, 6.86%.

5-(3-(2-hydroxyphenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6g)

Yield: 88%; mp 187°C; IR (KBr) cm^{-1}: 3436 (OH), 3320 (NH), 1720 (C=O), 1623 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.35 (s, 3H, $-CH_3$), 5.42 (s, 1H, H of pyrimidine ring), 6.46 (d, 1H, *J*= 8.8 Hz, -CH=CH-), 6.62 (d, 1H, *J*= 19.2 Hz, -CH=CH-), 7.16 (m, 9H, Ar-H), 8.92 (s, 1H, -NH), 9.13 (s, 1H, -NH), 9.38 (s, 1H, -OH). Elemental analysis for $C_{20}H_{18}N_2O_3$: calculated: C, 71.84%; H, 5.42%; N, 8.38%; found: C, 71.85%; H, 5.38%; N, 8.38%.

5-(3-(4-methylphenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6h)

Yield: 87%; mp 179°C; IR (KBr) cm^{-1}: 3328 (NH), 1618 (C=O), 1522 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.33 (s, 3H, $-CH_3$), 2.77 (s, 3H, $-CH_3$), 5.18 (s, 1H, H of pyrimidine ring), 6.33 (d, 1H, *J*= 7.6 Hz, -CH=CH-), 6.52 (d, 1H, *J*= 16.4 Hz, -CH=CH-), 7.18 (m, 5H, Ar-H), 7.56(d, 2H, *J*= 6 Hz), 7.67 (d, 2H, *J*= 6 Hz) 8.77 (s, 1H, -NH), 10.16 (s, 1H, -NH). Elemental analysis for $C_{21}H_{20}N_2O_2$: calculated: C, 75.88%; H, 6.06%; N, 8.43%; found: C, 75.90%; H, 6.02%; N, 8.43%.

5-(3-(4-bromophenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6i)

Yield: 89%; mp 169°C; IR (KBr) cm^{-1}: 3328 (NH), 1684 (C=O), 1575 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.28 (s, 3H, $-CH_3$), 5.41 (s, 1H, H of pyrimidine ring), 6.83 (d, 1H, *J*= 7.2 Hz, -CH=CH-), 7.32 (d, 1H, *J*= 16.5 Hz, -CH=CH-), 8.12 (m, 9H, Ar-H), 7.83 (s, 1H, -NH), 8.92 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_2O_2Br$: calculated: C, 60.55%; H, 4.31%; N, 7.06%; found: C, 60.60%; H, 4.29%; N, 7.07%.

5-(3-(4-flurophenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6j)

Yield: 84%; mp 159°C; IR (KBr) cm^{-1}. 3329 (NH), 1635 (C=O), 1529 (C=C), 1192 (C-F). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.39 (s, 3H, $-CH_3$), 5.25 (s, 1H, H of pyrimidine ring), 6.68 (d, 1H, *J*= 8.8 Hz, -CH=CH), 7.12 (m, 5H, Ar-H), 7.25(d, 1H, *J*= 16 Hz, -CH=CH-), 7.53 (d, 2H, *J*= 8.8 Hz), 7.72 (d, 2H, *J*= 8.8 Hz), 8.01 (s, 1H, -NH), 9.74 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_2O_2F$: calculated: C, 71.42%; H, 5.09%; N, 8.33%; found: C, 71.64%; H, 5.07%; N, 8.35%.

5-(3-(3-chlorophenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6k)

Yield: 88%; mp 161°C; IR (KBr) cm^{-1}: 3356 (NH), 1674 (C=O), 1469 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.42 (s, 3H, $-CH_3$), 5.31 (s, 1H, H of pyrimidine ring), 6.54 (d, 1H, *J*= 16.4 Hz, -CH=CH-), 6.91 (d, 1H, *J*= 8.4 Hz, -CH=CH-), 7.32 (m, 6H, Ar-H), 7.53 (m, 3H, Ar-H), 7.63 (s, 1H, -NH), 8.12 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_2O_2Cl$: calculated: C, 68.08%; H, 4.85%; N, 7.94%; found: C, 68.18%; H, 4.82%; N, 7.95%.

5-(3-(4-nitrophenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6l)

Yield: 84%; mp 211°C; IR (KBr) cm^{-1}: 3332 (NH), 1790 (C=O), 1671 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.35 (s, 3H, $-CH_3$), 5.48 (s, 1H, H of pyrimidine ring), 6.97 (d, 1H, *J*= 7.2Hz, -CH=CH-), 7.19 (d, 1H, *J*= 16.5 Hz, -CH=CH-), 7.68 (m, 9H, Ar-H), 8.17 (s, 1H, -NH), 8.91 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_3O_4$: calculated: C,

66.11%; H, 4.71%; N, 11.56%; found: C, 66.11%; H, 4.68%; N, 11.57%.

5-(3-(phenyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6m)

Yield: 94%; mp 162°C; IR (KBr) cm^{-1}: 3320 (NH), 1652 (C=O), 1460 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.34 (s, 3H, $-CH_3$), 5.56 (s, 1H, H of pyrimidine ring), 6.16 (d, 1H, *J*= 9.2 Hz, -CH=CH-), 6.51 (d, 1H, *J*= 17.2 Hz, -CH=CH-), 7.00 (m, 10H, Ar-H), 7.80 (s, 1H, -NH), 8.90 (s, 1H, -NH). Elemental analysis for $C_{20}H_{18}N_2O_2$: calculated: C, 75.45%; H, 5.69%; N, 8.80%; found: C, 75.47%; H, 5.66%; N, 8.80%.

5-(3-(2-bromophenyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6n)

Yield: 86%; mp 167°C; IR (KBr) cm^{-1}: 3342 (NH), 1644 (C=O), 1627 (C=C), 1170 (C-Br). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.52 (s, 3H, $-CH_3$), 5.29 (s, 1H, H of pyrimidine ring), 6.13 (d, 1H, *J*= 9.2 Hz, -CH=CH-), 6.47 (d, 1H, *J*= 15.6 Hz, -CH=CH-), 7.21 (m, 9H, Ar-H), 7.62 (s, 1H, -NH), 8.32 (s, 1H, -NH). Elemental analysis for $C_{20}H_{17}N_2O_2Br$: calculated: C, 60.46%; H, 4.31%; N, 7.05%; found: C, 60.60%; H, 4.29%; N, 7.07%.

5-(3-(4-diethylaminophenyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6o)

Yield: 92%; mp 195°C; IR (KBr) cm^{-1}: 3324 (NH), 1678 (C=O), 1531 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 1.04 (t, 6H, N-CH_2CH_3)$_2$), 2.28 (s, 3H, $-CH_3$), 3.95 (m, 4H, -N(CH_2CH_3)$_2$, 5.21 (s, 1H, H of pyrimidine ring), 6.27 (d, 1H, *J*= 7.2Hz, -CH=CH-), 6.45 (d, 1H, *J*= 14.4 Hz, -CH=CH), 8.12 (m, 5H, Ar-H), 8.32 (s, 1H, -NH), 8.43 (d, 2H, *J*= 8.4 Hz), 8.77 (d, 2H, *J*= 8.4 Hz), 9.89 (s, 1H, -NH). Elemental analysis for $C_{24}H_{27}N_3O_2$: calculated: C, 74.01%; H, 6.98%; N, 10.79%; found: C, 74.03%; H, 6.94%; N, 10.79%.

5-(3-(2, 4-dichlorophenyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6p)

Yield: 91%; mp 192°C; IR (KBr)cm^{-1}: 3364 (NH), 1656 (C=O), 1612 (C=C), 1185 (C-Cl). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.48 (s, 3H, $-CH_3$), 5.46 (s, 1H, H of pyrimidine ring), 6.90 (d, 1H, *J*= 8.8 Hz, -CH=CH-), 7.15 (d, 1H, *J*= 17.2 Hz, -CH=CH-), 7.34 (m, 8H, Ar-H), 8.77 (s, 1H, -NH), 9.62 (s, 1H, -NH). Elemental analysis for $C_{20}H_{16}N_2O_2Cl_2$: calculated: C, 62.03%; H, 4.16%; N, 7.23%; found: C, 62.17%; H, 4.14%; N, 7.25%.

5-(3-(4-methoxyphenyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6q)

Yield: 82%; mp 170°C; IR (KBr) cm^{-1}: 3332 (NH), 1668 (C=O), 1520 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.32 (s, 3H, $-CH_3$), 3.79 (s, 3H, $-OCH_3$), 5.26 (s, 1H, H of pyrimidine ring), 6.97 (d, 1H, *J*= 9.04 Hz, -CH=-CH-), 6.97 (d, 1H, *J*= 16.8 Hz, -CH=CH-), 7.26 (m, 9H, Ar-H), 7.89 (s, 1H, -NH), 9.26 (s, 1H, -NH). Elemental analysis for $C_{21}H_{20}N_2O_3$: calculated: C, 72.39%; H, 5.78%; N, 8.04%; found: C, 72.41%; H, 5.74%; N, 8.04%.

5-(3-(3, 4-dimethoxyphenyl) acryloyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (6r)

Yield: 87%; mp 202 °C; IR (KBr) cm^{-1}: 3332 (NH), 1647 (C=O), 1607 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.34 (s, 3H, $-CH_3$), 4.863 (s, 6H, -OCH3), 5.15 (s, 1H, H of pyrimidine ring), 6.51 (d, 1H, *J*= 8.8 Hz, -CH=-CH-), 6.89 (d, 1H, *J*= 16.8 Hz, -CH=CH-), 8.27 (m, 8H, Ar-H), 8.65 (s, 1H, -NH), 9.44 (s, 1H, -NH). Elemental analysis for $C_{22}H_{22}N_2O_4$: calculated: C, 69.83%; H, 5.85%; N, 7.40%; found: C, 69.84%; H, 5.82%; N, 7.40%.

5-(3-(2-furfuryl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6s)

Yield: 94%; mp 192°C; IR (KBr) cm^{-1}: 3329 (NH), 1629 (C=O), 1469 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.98 (s, 3H, $-CH_3$), 4.92 (s, 1H, H of pyrimidine ring), 7.13 (d, 1H, *J*= 16 Hz, -CH=CH-), 7.18 (m, 9H, Ar-H), 7.60 (s, 1H, -NH), 8.02 (s, 1H, -NH). Elemental analysis for $C_{18}H_{16}N_2O_3$: calculated: C, 70.12%; H, 6.12%; N, 9.08%; found: C, 70.12%; H, 5.19%; N, 9.09%.

5-(3-(2-thienyl) acryloyl)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one (6t)

Yield: 91%; mp 201°C; IR (KBr) cm^{-1}: 3364 (NH), 1720 (C=O), 1472 (C=C). ^1H NMR (400 MHz, $CDCl_3$, 25°C, ppm) δ: 2.54 (s, 3H, $-CH_3$), 5.21 (s, 1H, H of pyrimidine ring), 6.35 (d, 1H, *J*= 15.6 Hz, -CH=CH-), 7.08 (d, 1H, *J*= 3.7 Hz, -CH=CH-), 7.28 (m, 5H, Ar-H), 7.59 (m, 3H), 7.19 (s, 1H, -NH), 8.32 (s, 1H, -NH). Elemental analysis for $C_{18}H_{16}N_2O_2S$: calculated: C, 66.64%; H, 4.96%; N, 8.63%; found: C, 66.66%; H, 4.93%; N, 8.64%.

The scheme and Various aldehyde used were shown in Figure 1

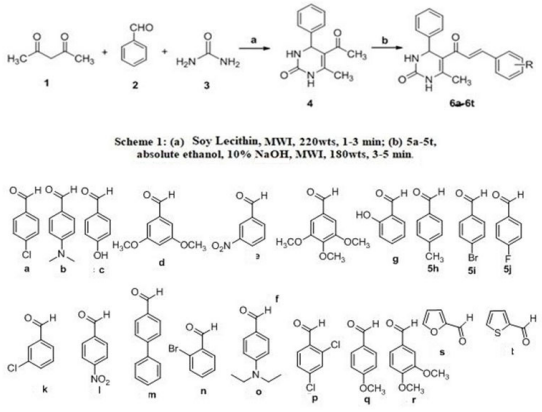

Figure 1: Various aldehyde used in the present study 6a-6t.

Anti-Tubercular Activity

The anti-mycobacterial activity of compounds was assessed against *M. tuberculosis* $H_{37}Rv$ strain using MABA.[16] Briefly, 200µl volume of deionized water priory sterilized was added to all outside boundary wells of the sterile 96 plate to diminish the evaporation of medium in the test wells while in incubation. The 96 wells plate collected 100 µl of the middle brook 7H9 broth and serial dilution of derivatives were prepared directly on plate. The final drug concentrations tested were 100 to 0.8 µg/ml and compound with standards pyrazinamide 3.125µg/ml and streptomycin 6.25µg/ml plates were covered and sealed with parafilm and incubated at 37°c for five days. After this time 25 µl freshly prepared 1:1 mixture of Almar blue reagent and 10% tween 80 was added to the plate and incubated for 24h. No bacterial growth was represented by the blue colour formation in the well, and pink colour formation has represented the growth of bacteria. The minimum effective concentration (MIC) was the lowest drug concentration which prevents the change in colour from blue to pink.

Molecular Docking

Genetic optimization for ligand docking (GOLD v4.0) molecular docking program used in this study, which was obtained from the Cambridge Crystallographic Data Centre (CCDC), Cambridge (GOLD v4.0.1 2008). The crystal structure of thymidylate kinase was taken from the Protein Data Bank (PDB ID: 1G3U)[14] for protein preparation using Schrodinger protein preparation wizard tool. This performs the following steps: assigning of bond orders, the addition of hydrogens, and optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex. All the bound water molecules, ligands, and cofactors were removed (preprocess) from the proteins which were taken in '.mol' format. 3D structures of compounds were generated by using ISIS DRA 3.2 (a software to get the 3D structures of compounds) and hydrogen was added in all the ligand structure.[17-18]

GOLD is a ligand-docking application that utilizes a genetic algorithm (GA) to explore ligand conformation flexibility and orientation with partial flexibility of the protein and satisfy ligand-binding requirements. For each of the 10 independent GA runs, a maximum number of 100 GA operations were performed. The standard set parameters were used in all the calculations.[19] Astex statistical potential (ASP) score recorded on each binding model using a fitness function that accounts for the frequency of interactions between ligand and receptor atoms. ASP score is a database to measure the protein-ligand complexes by the potential derived from atom to atom. In ASP score measurement rate of interaction between receptor and ligand atoms is gained by analyzing existing ligand-protein structures in the protein database (PDB) and this information is used to generate statistical potentials.

The top 10 ranked solutions of the ligands were taken for further observation of binding orientation and H-bond interactions.

RESULTS AND DISCUSSION

Chemistry

To the formation of products **(6a-6t)**, the reaction took place between acetylacetone **(1)**, benzaldehyde **(2)** and urea **(3)** by one-pot condensation method (Biginelli reaction) leads to formation of 5-acetyl-4-phenyl-6-methyl-3,4-dihydropyrimidine-2-(1H)-ones **(4)** and **4** was subjected to Claisen–Schmidt condensation using different substituted aromatic aldehydes **(5a–5t)** in the presence of sodium hydroxide and absolute ethanol keeping the MicroWave Irradiation (MWI) results in the formation of different chalcones **(6a–6t)**, Scheme 1. The synthesized compounds were purified by column chromatography. All of the derivatives were characterized by FT-IR, ^{1}H NMR, and mass spectral data. The chemical structures were confirmed through physical and spectral data.

Antimycobacterial activity

Based on docking study results, we have selected synthesized compounds were evaluated for their inhibition potential against *M. tuberculosis* $H_{37}Rv$ strain which shows better docking scores. The current research program is the design and development of dihydropyridines and the objective is to identify some lead molecules with $H_{37}Rv$ strain inhibitory activity. The minimum inhibitory concentrations (MIC) of the compounds were compared with Streptomycin and Pyrazinamide as standards. The results obtained from in vitro screening of test compounds are summarized in Figure 2.

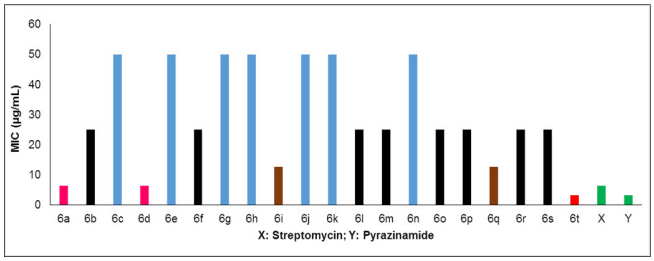

Figure 2: Inhibitory activity of synthesized DHPM chalcone derivatives 6a-6t against *M.tuberculosis* H_{37} Rvstrain.

Docking

The biological active compounds were evaluated molecular docking to recognize their hypothetical binding mode using the X-ray crystal structure of thymidylate kinase using GOLD molecular docking program (21). All the compounds were docked well into the active site of thymidylate kinase.

The potent compounds **6a**, **6d**, **6i**, **6q** and **6t** forming H-bonding with Tyr 39, Glu 166, Arg 153 and Asp 163 amino acids of the active site. (**Figure 3 & 4**)

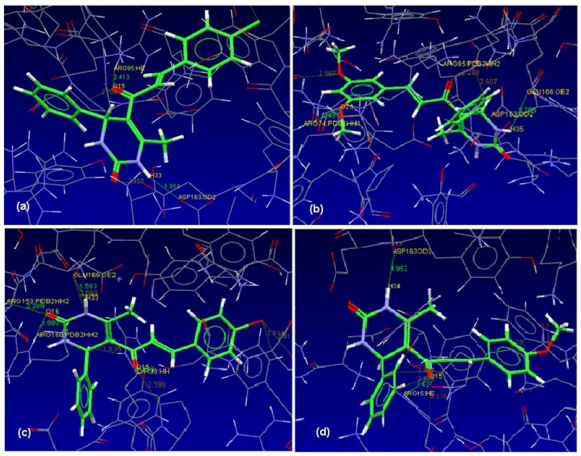

Figure 3: Binding mode of potent dihydropyrimidine chalcones 6a, 6d, 6i and 6q with the active site of thymidylate kinase. a) H-bonding of compound 6a with Arg 95, Asp 163; b) H-bonding of compound 6d with Glu 166, Arg 74; c) H-bonding of compound 6i with Tyr 39, Glu 166, Arg 153; d) H-bonding of compound 6q with Arg 95 and Asp 163.

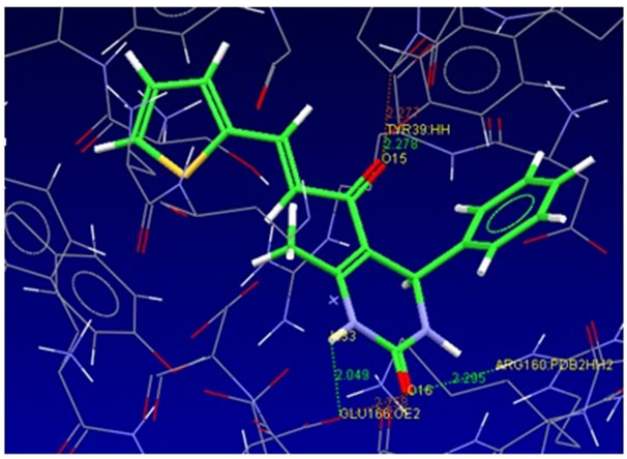

Figure 4: H-bonding of compound compound 6t with Tyr 39, Glu 166, Arg 160 Green dotted lines indicates Hydrogen bond interaction

The complete spectral and elemental analytical data of the products confirmed the formation of novel 6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1*H*)-one derivatives **(6a-6t)**. Initially, compound **4** was confirmed by the FT-IR spectra of the compound revealed absorption bands in the region around 3241, 2985, and 1713 cm^{-1} regions, resulting from the -NH, Ar-H and C=O functions respectively. Further, in the ^{1}H NMR spectra, the signal derived from acetyl group (-$COCH_3$) was observed at 2.29 ppm (singlet), the aromatic protons are shown in multiplets around 7.24 ppm and broad singlets in the range 7.82 ppm and 9.17 ppm due to –NH protons. The LC-MS showed its molecular ion peak at 231 (M+H).

The chemical structures of chalcones **(6a–6t)** were confirmed through physical and spectral data. The ^{1}H NMR spectrum **6a** showed signal 6.719 ppm (*J*= 7.2 Hz) and 6.592 ppm (*J*= 17.6 Hz), the aromatic protons are shown in multiplets around 7.22 - 7.78 ppm. The LC-MS showed its molecular ion peak at 353 (M+H), the higher magnitude of coupling constants (*J* value) for both protons indicate *trans* configuration.

In the biological evaluation of all the synthesized compounds against M. tuberculosis H_{37}Rv strain using MABA exhibits one potent compound **6t** with MIC value of 3.12 µg/ml and four moderate activity compounds **6a, 6d, 6i** and **6q** with MIC values of 6.24 µg/ml, 6.24 µg/ml, 12.48 µg/ml and 12.48 µg/ml respectively, **Figure 3 and 4**. The five bioactive compounds were evaluated molecular docking studies to recognize their hypothetical binding mode using the X-ray crystal structure of thymidylate kinase using GOLD molecular docking program illustrates forming H- bonding with Tyrosine 39, Glutamicacid 166, Arginine 153 and Aspertic acid 163 amino acids, which were shown in Table 1. Biological and docking studies reveal that the heterocyclic aldehyde (2-thienaldehyde) derivative which probably accounts for their better activity compared to other analogues of the current synthesized series.

Table 1: GOLD score and amino acids involved in hydrogen binding with synthesized compounds

Compound	GOLD score	Residues involved in hydrgoen binding
6a	53.10	Arginine 95, Aspertic acid 163
6d	49.42	Glutamic acid 166, Arginine 74
6i	51.27	Tyrosine 39, Glutamic acid 166, Arginine 153
6q	52.82	Arginine 95 and Aspertic acid 163
6t	55.12	Tyrosine 39, Glutamic acid 166, Arginine 160

CONCLUSION

A series of novel dihydropyrimidine chalcones were synthesized, characterized, and evaluated for *invitro* antimycobacterial $H_{37}Rv$ strain. Compound **6t** exhibited potent MIC value compared with the reference standard. Further docking studies were undertaken to gain an insight into the molecular interactions and binding mode of the target compounds into thymidylate kinase. The most active compound **(6t)** may form an H-bonding with Tyr 39, Glu 166, Arg 160 amino acids of the

active site, heterocyclic aldehyde derivative which probably accounts for their better activity compared to other analogues of the series. The docking results revealed useful information to understand the interaction mode between dihydropyrimidine chalcone derivatives and thymidylate kinase will facilitate the next cycle of drug design to explore the newer lead molecules. Efforts are currently being taken up to optimize the lead structure and the results of which will be the basis of our future research endeavour.

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Conflict of Interest

No conflict of interest from all the authors

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