



Synthesis and antimicrobial activity of some ethyl [6-methyl-2-methoxy-3-(substituted phenylethanone)-4-(substituted phenyl)]-1,2,3,4-tetrahydropyrimidine-5-carboxylates

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Abstract

Fifteen new ethyl 6-methyl-2-methoxy-3-(substituted 1-phenylethanone)-4-(Substituted phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylates (**6.a-o**) have been synthesized in a two step reaction. In first step ethyl acetoacetate, s-methylisourea and appropriate benzaldehydes reacted in a single step reaction to obtain ethyl 6-methyl-2-methoxy-4-(substituted phenyl) -1, 4-dihydropyrimidine-5-carboxylates (**4.a-e**). Second step involves synthesis of reaction between substituted phenacyl bromides and 1-4 dihydropyrimidine-5-carboxylates (**6.a-o**). Their structures are confirmed by IR, ¹H- NMR, Mass and elemental analysis. Out of the total screened compounds, 6 compounds have shown antitubercular activity against *Mycobacterium tuberculosis* H₃₇Rv in concentration range of 1-2 µg/mL. Eight compounds have shown good activity against gram positive (*S. aureus* and *B. subtilis*) and gram negative organisms (*E. coli* and *S. typhi*), ranging in the concentration from 1-25 µg/mL.

Keywords: Pyrimidines; Biginelli reaction; Antitubercular; Antimicrobial

Introduction

Microbial infection is a very common disease since the man is known to live on earth. Even with the advancement in the field of medicine there is no perfect solution to many of the deadly diseases caused by bacteria. Here in this effort we have tried to develop a lead in a potent antimicrobial agent. We know that similar groups/structures often exhibit similar biological activities. However, they usually exhibit different potency. The traditional structure activity relationship (SAR) is a useful tool in the search for new drugs. However, SAR is usually determined by making minor changes to the structure of the existing compound and assessing the effect on its biological activity. Similarly, structural analogy has played vital role in designing compounds with higher potency. One of such structural analogy is seen between 4-aryl-1,4-dihydropyridines (DHPs) of the nifedipine type and dihydropyrimidines (DHPMs). In 1893

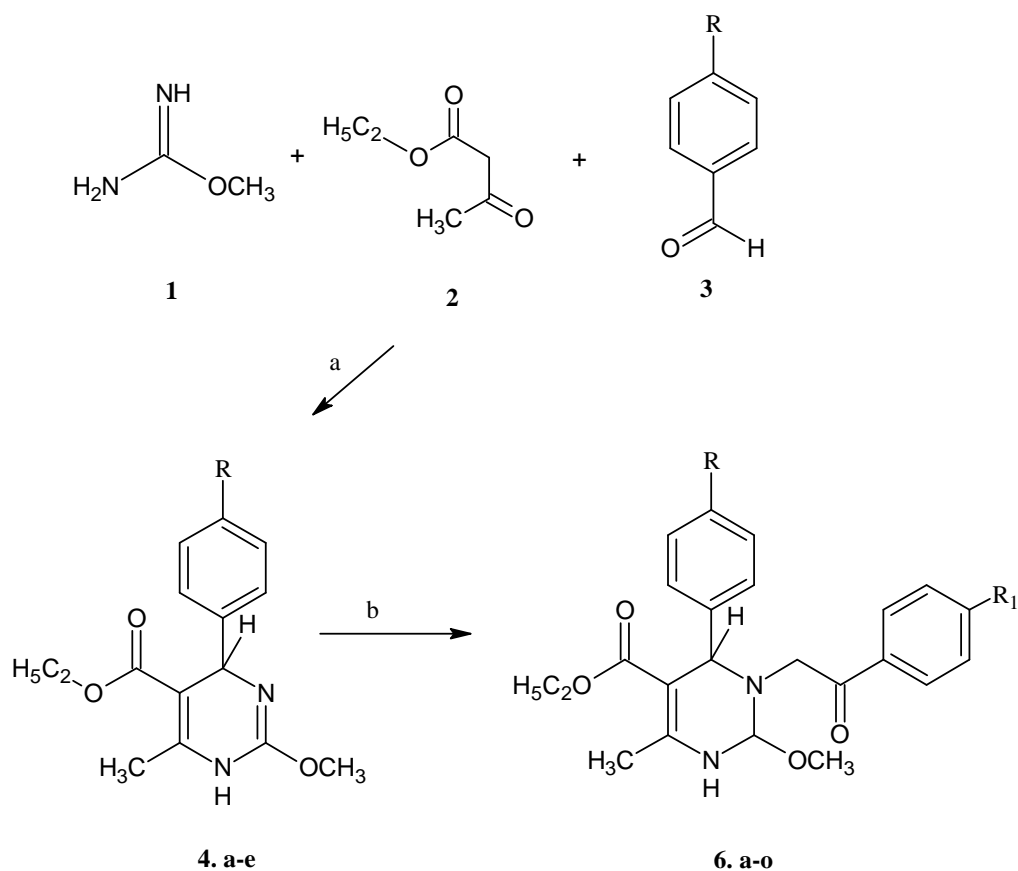
Italian chemist Pietro Biginelli reported on the acid-catalyzed cyclocondensation reaction of ethyl acetoacetate, benzaldehyde and urea. The reaction was carried out simply by heating a mixture of three components dissolved in ethanol with a catalytic amount of hydrochloric acid at reflux temperature. The product of this novel one pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3, 4-dihydropyrimidine-2(1H)-one [1].

The synthetic potential of this new heterocyclic synthesis remained unexplored for quite some time. In the 1970s and 1980s interest slowly increased, and the scope of the original cyclocondensation reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multifunctionalized dihydropyrimidines [2-3].

In the past decades, a broad range of biological effects, including anti tumor [4], anti-inflammatory [5] and antibacterial [6] activities has been ascribed to these partly reduced pyrimidine derivatives. More recently, DHPMs have emerged as, for e.g., orally active antihypertensive agents [7]. A very recent highlight in this context been the identification of the structurally rather simple DHPM monastrol as a mitotic kinesin motor protein inhibitor and potential new lead for the development of anticancer drugs [8]. We have also reported the antihypertensive activity of DHPM's [9]. Appropriately functionalized DHPM derivatives have emerged as potent antimicrobial leads [10]. Apart from synthetic DHPM derivatives several marine natural products with interesting biological activities containing the dihydropyrimidine-5-carboxylate core have recently been isolated. Most among these are the batzelladine alkaloids A and B which inhibit the binding of HIV envelop protein gp-120 to human CD4 cells and therefore, are potential new leads for AIDs therapy [11].

1.1 Chemistry

The pyrimidine-5-carboxylate substituted at third position, i.e., on nitrogen in the pyrimidine ring gives good antimicrobial activity and potent antitubercular activity. It involves the application of Biginelli reaction and its modifications. [12-15] In the first step three component reaction involving *o*-methylisourea hydrogensulfate, ethyl acetoacetate and substituted benzaldehydes (**3.a-e**) reacted in presence of sodium bicarbonate and dimethylformamide to form the substituted ring nucleus compounds (**4.a-e**), on reaction with various substituted phenacyl bromides **5a-c**, they undergo nucleophilic substitution reaction in presence of a base such as pyridine, to form their respective derivatives (**6a-o**), (Scheme 1).



Comp.	6.a	6.b	6.c	6.d	6.e	6.f	6.g	6.h	6.i	6.j	6.k	6.l	6.m	6.n	6.o
R	H	H	H	Cl	Cl	OCH ₃	OH	OH	OH	CH ₃	CH ₃	CH ₃	OCH ₃	OCH ₃	OCH ₃
R₁	H	Cl	OCH ₃	H	Cl	Cl	H	Cl	OCH ₃	H	Cl	OCH ₃	H	Cl	OCH ₃

Scheme 1. Reagents and conditions: (a) Sodium bicarbonate, dimethyl formamide, reflux (b) Substituted phenacyl bromides, pyridine

1.2 Biological activity

The newly synthesized compounds were tested for their antimicrobial activity¹⁵ against gram positive microorganisms (*S. aureus* ATCC 3750 and *B. subtilis* 6633), gram negative microorganisms (*E. coli* ATCC 25922 and *S. typhi* NCTC 786), and for antitubercular activity¹⁶ (against *Mycobacterium tuberculosis* H₃₇Rv) (Table 1).

Materials and Methods

2.1. Chemistry

General Procedures

Chemicals were obtained from Fluka Chemical Co. (Germany). Melting points (m.p.) were detected with open capillaries using Thermo Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^1H NMR was obtained using a Varian EM 390 Spectrophotometer (Shimadzu, Japan) using CDCl_3 . All chemical shift values were recorded as δ (ppm). The purity of compounds was controlled by thin layer chromatography (Merck, silica gel, HF₂₅₄₋₃₆₁, type 60, 0.25 mm, Darmstadt, Germany). The elementary analysis was performed at RTM Nagpur University, India. Elementary analyses for C, H, N were within $\pm 0.4\%$ of theoretical values.

2.1.1. General Procedure for Preparation of Compounds (4.a-e)

A mixture of o-methylisourea hydrogensulfate (60 mmol), ethyl acetoacetate (55 mmol), substituted benzaldehydes (50 mmol), were mixed together with sodium bicarbonate (200 mmol) and (100 mL) of dimethylformamide, reaction mixture was heated at 70° for 12 h. After cooling to room temperature, the mixture was diluted with 150 mL of brine and extracted with ether (2 x 150 mL), dried over magnesium sulfate and excess of solvent was removed under reduced pressure. After complete drying product was collected and recrystallized from ethanol. The structure of compounds was confirmed by IR, ^1H -NMR, Mass and element analysis.

2.1.1.1. Ethyl 6-methyl-2-methoxy-4-(phenyl)-1,4-dihydropyrimidine-5-carboxylate (4.a)

Yield: 64.47%, m.p. 202–204 °C. R_f : 0.56 (chloroform–benzene 50:50). IR (KBr) $\nu = 1687.6\text{ cm}^{-1}$ (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^1H NMR (CDCl_3) δ 8.32 (s, 1H, N₁–H), 8.15 (d, J = 7.9 Hz, 1H, aromatic), 8.11 (s, 1H, aromatic), 7.64 (d, J = 7.9 Hz, 1H, aromatic), 7.51 (t, J = 7.9 Hz, 1H, aromatic), 7.32 (m, 5H, aromatic), 5.98, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.19 (m, 2H, ethyl ester), 2.39 (s, 3H, methyl) and 1.40–1.70 (d, J = 7.38 Hz, 2H, ethyl ester). ESMS: m/z (MH^+) 274. Anal. ($\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_3$) C(65.68/65.66), H(6.61/6.50), N(10.21/10.24).

2.1.1.2. Ethyl 6-methyl-2-methoxy-4-(4-hydroxyphenyl)-1,4-dihydropyrimidine-5-carboxylate (4.b)

Yield: 64%, m.p. 216–218 °C. R_f : 0.71 (chloroform–benzene 20:80). IR (KBr) $\nu = 1687.6\text{ cm}^{-1}$ (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ^1H NMR (CDCl_3) δ 8.32 (s, 1H, N₁–H), 8.15 (d, J = 7.9 Hz, 1H, aromatic), 8.13 (s, 1H, aromatic), 7.64 (d, J = 7.9 Hz, 1H, aromatic), 7.51 (t, J = 7.9 Hz, 1H, aromatic), 7.42 (m, 5H, aromatic), 5.90, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.11 (m, 2H, ethyl ester), 2.49 (s, 3H, methyl) and 1.18 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH^+) 289. Anal. ($\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_4$) C(62.06/62.09), H(6.25/6.22), N(9.65/9.69).

2.1.1.3. Ethyl 6-methyl-2-(methylsulfanyl)-4-(4-methylphenyl)-1,4-dihydropyrimidine-5-carboxylate (4.c)

Yield: 76%, m.p. 144–147 °C. *R_f*: 0.78 (chloroform–benzene 20:80). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 8.34 (s, 1H, N₁–H), 8.15 (d, J = 7.9 Hz, 1H, aromatic), 8.17 (s, 1H, aromatic), 7.74 (d, J = 7.9 Hz, 1H, aromatic), 7.51 (t, J = 7.9 Hz, 1H, aromatic), 7.32 (m, 5H, aromatic), 5.98, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.10 (m, 2H, ethyl ester), 2.59 (s, 3H, methyl) and 1.18 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH⁺)288. Anal. (C₁₆H₂₀N₂O₃) C(66.65/66.68), H(6.99/6.96), N(9.72/9.70).

2.1.1.4. Ethyl 6-methyl-2-methoxy-4-(4-methoxyphenyl)-1,4-dihydropyrimidine-5-carboxylate (4.d)

Yield: 75%, m.p. 157–159 °C. *R_f*: 0.64 (chloroform–benzene 20:80). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 8.33 (s, 1H, N₁–H), 8.14 (d, J = 7.9 Hz, 1H, aromatic), 8.16 (s, 1H, aromatic), 7.66 (d, J = 7.9 Hz, 1H, aromatic), 7.54 (t, J = 7.9 Hz, 1H, aromatic), 7.33 (m, 5H, aromatic), 5.96, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.11 (m, 2H, ethyl ester), 2.342 (s, 3H, methyl) and 1.14 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH⁺) 305. Anal. (C₁₆H₂₀N₂O₄) C(63.14/63.16), H(6.62/6.63), N(9.20/9.21)

2.1.1.5. Ethyl 4-(4-chlorophenyl)-6-methyl-2-methoxy-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4.e)

Yield: 64%, m.p. 240–245 °C. *R_f*: 0.74 (chloroform–benzene 20:80). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 8.31 (s, 1H, N₁–H), 8.17 (d, J = 7.9 Hz, 1H, aromatic), 8.14 (s, 1H, aromatic), 7.66 (d, J = 7.9 Hz, 1H, aromatic), 7.54 (t, J = 7.9 Hz, 1H, aromatic), 7.30 (m, 5H, aromatic), 5.96, 4.18 (d, J = 5.29 Hz, 2H, benzylic), 6.00 (s, 1H, methine), 4.11 (m, 2H, ethyl ester), 2.49 (s, 3H, methyl) and 1.18 (t, J = 7.38 Hz, 3H, ethyl ester). ESMS: m/z (MH⁺)307. Anal. (C₁₅H₁₇ClN₂O₃) C(58.35/58.40), H(5.55/5.51), N(9.07/9.09).

2.1.2. General Procedure for Preparation of Compounds (6.a-o)

A mixture of ethyl 6-methyl-2-methoxy-4-(substituted phenyl)-1, 4- dihydropyrimidine-5-carboxylates **4.a-e** (0.01 mmol), substituted phenacyl bromides **5.a-c** (0.01 mmol) were taken in dichloromethane (20 mL) as solvent and pyridine (1.5 mL) as a catalyst. The resultant mixture was refluxed for 7 h. Then it was cooled to room temperature and poured on to crushed ice. It was kept overnight and filtered to obtain solid, which was dried and recrystallized from ethanol.

2.1.2.1. Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(phenyl)-1, 2, 3, 4-tetrahydro pyrimidine-5-carboxylate (6.a)

Yield: 80.88%, m.p. 96–99 °C. *R_f*: 0.67 (ethanol–benzene 40:60). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1392.5, 3176 (N–H), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 7.78 (d, J = 7 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1 H), 7.37 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.11 (s, 1H), 4.8–4.6 (m, 1H), 3.42 (q, J = 6 Hz, 2H), 3.7 (s, 3H), 2.46 (s, 3H), 0.96 (t, J = 6 Hz, 3H). ESIMS: m/z (MH⁺)394. Anal. (C₂₃H₂₆N₂O₄) C(70.03/ 70.06), H(6.64/6.66), N(7.10/7.09).

2.1.2.2. *Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(phenyl)-1, 2, 3,4-tetrahydropyrimidine-5-carboxylate (6.b)*

Yield: 45%, m.p. 158–161 °C. *R_f*: 0.63 (ethanol–benzene 40:60). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1392.51, 3203 (N–H), 1325.01 (C–Cl) 1292.22 (C–N). ¹H NMR (CDCl₃) δ 7.76 (d, J = 7 Hz, 1H), 7.72 (t, J = 7.0 Hz, 1H), 7.56 (t, J = 7 Hz, 1H), 7.39 (d, J = 7.0 Hz, 1H), 7.05 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.89 (s, 1H), 4.9–4.7 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.8 (s, 3H), 2.45 (s, 3H), 0.98 (t, J = 6 Hz, 3H). ESIMS: m/z (MH⁺)429. Anal. (C₂₃H₂₅ClN₂O₄) C(64.41/64.44), H(5.88/5.90), N(6.5/6.2).

2.1.2.3. *Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.c)*

Yield: 64%, m.p.108–110 °C. *R_f*: 0.67 (ethanol–benzene 40:60). IR (KBr) $\nu = 1691.6 \text{ cm}^{-1}$ (C=O), 1390.58, 3245 (N–H), 1355.86 (O–CH₃), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 7.76 (d, J = 7 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1H), 7.39 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.94 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.9 (s, 3H), 2.45 (s, 3H), 0.96 (t, J = 6 Hz, 3H). ESIMS: m/z (MH⁺)424. Anal. (C₂₄H₂₈N₂O₅) C(67.91/67.93), H(6.65/ 6.68), N(6.60/6.63).

2.1.2.4. *Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone) -4-(4-chlorophenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.d)*

Yield: 60%, m.p. 115–120 °C. *R_f*: 0.67 (ethanol–benzene 40:60). IR (KBr) $\nu = 1690.6 \text{ cm}^{-1}$ (C=O), 1390.58, 3245 (N–H), 1355.86 (O–CH₃), 1325.01 (C–Cl), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 7.77 (d, J = 7 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.58 (t, J = 7 Hz, 1H), 7.35 (d, J = 7.0 Hz, 1H), 7.03 (d, J = 10 Hz, 2H), 6.52 (d, J = 10 Hz, 2H), 5.91 (s,1H), 4.8–4.6 (m,1H), 3.93 (q, J = 6 Hz, 2H), 3.3 (s, 3H), 2.47 (s, 3H), 0.95 (t, J = 6Hz, 3H). ESIMS: m/z (MH⁺)429. Anal. (C₂₃H₂₅ClN₂O₄) C(64.41/64.38), H(5.88/5.89), N(6.53/6.50).

2.1.2.5. *Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-chlorophenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.e)*

Yield: 55%, m.p. 110–115 °C. *R_f*: 0.67 (ethanol–benzene 40:60). IR (KBr) $\nu = 1690.6 \text{ cm}^{-1}$ (C=O), 1390.58, 3245 (N–H), 1355.86 (O–CH₃), 1330, 1325.01 (C–Cl), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 7.75 (d, J = 7 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.45 (t, J = 7 Hz, 1H), 7.38 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.67 (d, J = 10 Hz, 2H), 5.95 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.9 (s, 3 H), 2.44 (s, 3H), 0.99 (t, J = 6 Hz, 3H). ESIMS: m/z (MH⁺)464. Anal. (C²³H²⁴Cl²N²O⁴) C(59.62/59.60), H(5.22/5.27), N(6.05/6.08).

2.1.2.6. *Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-methoxyphenyl) -1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.f)*

Yield: 65%, m.p. 115–120 °C. *R_f*: 0.67 (ethanol–benzene 40:60). IR (KBr) $\nu = 1690.6 \text{ cm}^{-1}$ (C=O), 1390.58, 3245 (N–H), 1355.86 (O–CH₃), 1325.01 (C–Cl), 1292.22 (C–N). ¹H NMR (CDCl₃) δ 7.76 (d, J = 7 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7 Hz, 1H), 7.34 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 10 Hz, 2H), 6.57 (d, J = 10 Hz, 2H), 5.91 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, J = 6 Hz, 2H), 3.7 (s, 3H), 2.45 (s, 3H), 0.96 (t, J = 6 Hz, 3H). ESIMS: m/z (MH⁺)459. Anal. (C₂₄H₂₇ClN₂O₅) C(62.81/62.85), H(5.93/5.90), N(6.10/6.15).

2.1.2.7. *Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-hydroxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.g)*

Yield: 78%, m.p. 131–134 °C. R_f : 0.57 (ethanol–benzene 40:60). IR (KBr) $\nu = 1691.6 \text{ cm}^{-1}$ (C=O), 1390.51, 3176 (N–H), 1296.22 (C–N). $^1\text{H NMR}$ (CDCl_3) δ 7.74 (d, $J = 7 \text{ Hz}$, 1H), 7.62 (t, $J = 7.0 \text{ Hz}$, 1H), 7.55 (t, $J = 7 \text{ Hz}$, 1H), 7.44 (d, $J = 7.0 \text{ Hz}$, 1H), 7.06 (d, $J = 10 \text{ Hz}$, 2H), 6.57 (d, $J = 10 \text{ Hz}$, 2H), 5.91 (s, 1H), 4.8–4.4 (m, 1H), 3.97 (q, $J = 6 \text{ Hz}$, 2H), 3.7 (s, 3H), 2.47 (s, 3H), 0.95 (t, $J = 6 \text{ Hz}$, 3H). ESIMS: m/z (MH^+)411. Anal. ($\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_5$) C(67.30/67.35), H(6.38/6.35), N(6.82/6.85).

2.1.2.8. *Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-hydroxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.h)*

Yield: 57%, m.p. 136–138 °C. R_f : 0.57 (ethanol–benzene 40:60). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1390.51, 3203 (N–H), 1342.01 (C–Cl), 1296.22 (C–N). $^1\text{H NMR}$ (CDCl_3) δ 7.76 (d, $J = 7 \text{ Hz}$, 1H), 7.68 (t, $J = 7.0 \text{ Hz}$, 1H), 7.56 (t, $J = 7 \text{ Hz}$, 1H), 7.35 (d, $J = 7.0 \text{ Hz}$, 1H), 7.04 (d, $J = 10 \text{ Hz}$, 2H), 6.57 (d, $J = 10 \text{ Hz}$, 2H), 5.91 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, $J = 6 \text{ Hz}$, 2H), 3.7 (s, 3H), 2.45 (s, 3H), 0.99 (t, $J = 6 \text{ Hz}$, 3H). ESIMS: m/z (MH^+)444. Anal. ($\text{C}_{23}\text{H}_{25}\text{ClN}_2\text{O}_5$) C(62.09/62.11), H(5.66/5.65), N(6.30/6.34).

2.1.2.9. *Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(4-hydroxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.i)*

Yield: 60%, m.p. 106–108 °C. R_f : 0.57 (ethanol–benzene 40:60). IR (KBr) $\nu = 1691.6 \text{ cm}^{-1}$ (C=O), 1390.51, 3245 (N–H), 2914.24 (O–H), 1296.22 (C–N). $^1\text{H NMR}$ (CDCl_3) δ 8.76 (d, $J = 7 \text{ Hz}$, 1H), 7.62 (t, $J = 7.0 \text{ Hz}$, 1H), 7.55 (t, $J = 7 \text{ Hz}$, 1H), 7.34 (d, $J = 7.0 \text{ Hz}$, 1H), 7.04 (d, $J = 10 \text{ Hz}$, 2H), 6.57 (d, $J = 10 \text{ Hz}$, 2H), 6.0 (s, 1H), 4.8–4.6 (m, 1H), 3.93 (q, $J = 6 \text{ Hz}$, 2H), 3.9 (s, 3H), 2.45 (s, 3H), 0.99 (t, $J = 6 \text{ Hz}$, 3H). ESIMS: m/z (MH^+)441. Anal. ($\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_6$) C(65.44/65.47), H(6.41/6.45), N(6.36/6.32).

2.1.2.10. *Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-methylphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.j)*

Yield: 62.5%, m.p. 116–118 °C. R_f : 0.76 (ethanol–benzene 40:60). IR (KBr) $\nu = 1691.6 \text{ cm}^{-1}$ (C=O), 1390.51, 3176 (N–H), 1296.22 (C–N). $^1\text{H NMR}$ (CDCl_3) δ 9.75 (s, 1H), 8.5 (br s, 1H), 8.2 (s, 1H), 8.10 (d, $J = 8.4 \text{ Hz}$, 1H), 7.72 (d, $J = 7.4 \text{ Hz}$, 1H), 7.48 (t, $J = 7.91 \text{ Hz}$, 1H), 6.7 (s, 1H), 6.4 (br s, 1H), 5.05 (t, $J = 6.3 \text{ Hz}$, 1H), 2.4 (s, 3H), 1.29 (d, $J = 5.8 \text{ Hz}$, 3H), 1.16 (d, $J = 6.3 \text{ Hz}$, 3H). ESIMS: m/z (MH^+)409. Anal. ($\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_4$) C(70.57/70.55), H(6.91/6.96), N(6.86/6.85).

2.1.2.11. *Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-methylphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.k)*

Yield: 45.5%, m.p. 104–107 °C. R_f : 0.68 (ethanol–benzene 40:60). IR (KBr) $\nu = 1687.6 \text{ cm}^{-1}$ (C=O), 1390.51, 3203 (N–H), 1342.01 (C–Cl), 1296.22 (C–N). $^1\text{H NMR}$ (CDCl_3) δ 9.75 (s, 1H), 8.5 (br s, 1H), 8.2 (s, 1H), 8.10 (d, $J = 8.4 \text{ Hz}$, 1H), 7.72 (d, $J = 7.4 \text{ Hz}$, 1H), 7.48 (t, $J = 7.91 \text{ Hz}$, 1H), 6.7 (s, 1H), 6.4 (br s, 1H), 5.05 (t, $J = 6.3 \text{ Hz}$, 1H), 2.4 (s, 3H), 1.29 (d, $J = 5.8 \text{ Hz}$, 3H), 1.16 (d, $J = 6.3 \text{ Hz}$, 3H). ESIMS: m/z (MH^+)443. Anal. ($\text{C}_{24}\text{H}_{27}\text{ClN}_2\text{O}_5$) C(65.08/65.06), H(6.14/6.11), N(6.32/6.29).

2.1.2.12. *Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(4-methylphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.l)*

Yield: 66%, m.p. 157–159 °C. R_f : 0.68 (ethanol–benzene 40:60). IR (KBr) $\nu = 1693.6 \text{ cm}^{-1}$ (C=O), 1390.51, 3245 (N–H), 1402(CH₃–O–C), 1296.22 (C–N). ¹H NMR (CDCl₃) δ 9.71 (s, 1H), 8.4 (br s, 1H), 8.2 (s, 1H), 8.1 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.91 Hz, 1H), 6.8 (s, 1H), 6.4 (br s, 1H), 5.05 (t, J = 6.3 Hz, 1H), 2.6 (s, 3H), 1.29 (d, J = 5.8 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH⁺)439. Anal. (C₂₅H₃₀N₂O₅) C(68.47/68.50), H(6.90/6.92), N(6.35/6.38).

2.1.2.13. *Ethyl 6-methyl-2-methoxy-3-(1-phenylethanone)-4-(4-methoxyphenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (6.m)*

Yield: 81%, m.p. 134–137 °C. R_f : 0.58 (ethanol–benzene 40:60). IR (KBr) $\nu = 1687.9 \text{ cm}^{-1}$ (C=O), 1390.51, 3176 (N–H), 1402 (CH₃–O–C), 1296.22 (C–N). ¹H NMR (CDCl₃) δ 9.77 (s, 1H), 8.5 (br s, 1H), 8.4 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.6 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.91 Hz, 1H), 6.9 (s, 1H), 6.4 (br s, 1H), 5.15 (t, J = 6.3 Hz, 1H), 2.4 (s, 3H), 1.29 (d, J = 5.8 Hz, 3H), 1.17 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH⁺)425. Anal. (C₂₄H₂₈N₂O₅) C(67.91/67.94), H(6.65/6.64), N(6.60/6.64).

2.1.2.14. *Ethyl 6-methyl-2-methoxy-3-[1-(4-chlorophenyl) ethanone]-4-(4-methoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6.n)*

Yield: 74%, m.p. 155–158 °C. R_f : 0.78 (ethanol–benzene 40:60). IR (KBr) $\nu = 1689.8 \text{ cm}^{-1}$ (C=O), 1402.15, 3204 (N–H), 1346.01 (C–Cl), 1402 (CH₃–O–C), 1296.22 (C–N). ¹H NMR (CDCl₃) δ 9.55 (s, 1H), 8.4 (br s, 1H), 8.3 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.40 (t, J = 7.91 Hz, 1H), 6.5 (s, 1H), 6.3 (br s, 1H), 5.10 (t, J = 6.3 Hz, 1H), 2.4 (s, 3H), 1.20 (d, J = 5.8 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH⁺)459. Anal. (C₂₄H₂₇ClN₂O₅) C(62.81/62.85), H(5.93/5.95), N(6.10/6.11).

2.1.2.15. *Ethyl 6-methyl-2-methoxy-3-[1-(4-methoxyphenyl) ethanone]-4-(4-methoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6.o)*

Yield: 74%, m.p. 108–111 °C. R_f : 0.64 (ethanol–benzene 40:60). IR (KBr) $\nu = 1690.8 \text{ cm}^{-1}$ (C=O), 1402.15, 3240 (N–H), 1402 (CH₃–O–C), 1402 (CH₃–O–C), 1296.22 (C–N). ¹H NMR (CDCl₃) δ 9.78 (s, 1H), 8.7 (br s, 1H), 8.2 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.52 (t, J = 7.91 Hz, 1H), 6.7 (s, 1H), 6.4 (br s, 1H), 5.00 (t, J = 6.3 Hz, 1H), 2.2 (s, 3H), 1.25 (d, J = 5.8 Hz, 3H), 1.26 (d, J = 6.3 Hz, 3H). ESIMS: m/z (MH⁺)455. Anal. (C₂₅H₃₀N₂O₆) C(66.06/66.06), H(6.65/6.69), N(6.16/6.18).

2.2. Antimicrobial study

General procedure

All the test compounds were assayed in vitro for antibacterial activity [16] against described organisms. The MIC was determined by the test tube dilution technique using Mueller-Hinton nutrient broth (for antibacterial) and modified Kirchner's culture medium containing 0.5% sterilized horse serum for antimycobacterial activity [17]. The MIC values were also tested for

three well-known antibiotics (penicillin-G, ampicillin and chloramphenicol) to compare the antibacterial activity of our test compounds with the antibiotics which are currently in therapy. Rifampicin and isoniazid (INH) were used as reference standard for antimycobacterial activity. The stock solution (2-4 µg/mL) of the test compounds was prepared in sterile water. Further, the serial dilution of test compounds was carried out and the following concentration was used: 1000, 500, 250, 125, 62, 32, 16, 8, 4, 1 µg/mL. Test compounds at various concentrations were added to culture medium in a sterilized borosilicate test tube and different bacterial strains were inoculated at 10⁶ bacilli/mL concentration. The tubes were incubated at 37 °C for 24 h for antibacterial activity and 14 and 21 days for antimycobacterial activity and then examined for the presence or absence of growth of the test organisms. All experiments were performed in triplicate. The MIC values were obtained from the lowest concentration of the test compounds where the tubes remained clear, indicated that the bacterial growth was completely inhibited at this concentration. The MIC values were expressed in µg/mL.

Table 1. Biological activity of disubstituted compounds (6.a-o)

Compound	Antibacterial activity				Antimycobacterial activity ^a	
	Gram positive		Gram negative		<i>M. tuberculosis</i>	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	14 ^b	21 ^c
6.a	56	64	61	89	18	26
6.b	45	52	49	65	15	20
6.c	3	5	4	7	1.5	4
6.d	6	2	4	9	2	5
6.e	12	26	24	29	11	23
6.f	65	87	47	78	23	41
6.g	23	45	63	70	10	18
6.h	12	15	14	12	12	19
6.i	3	1	3	5	1	5
6.j	25	43	54	47	12	31
6.k	68	88	94	90	23	51
6.l	6	4	6	5	3	8
6.m	19	32	44	42	17	23
6.n	6	3	4	3	2	5
6.o	3	1	1	2	1.5	3
Std1 ^d	12.5	2.6	25	12.5	NA	NA
Std2 ^e	0.01	0.09	0.015	0.016	NA	NA
Std3 ^f	5	7	6	7	NA	NA
Std4 ^g	1	0.9	51	57	0.2	0.22
Std5 ^h	Inactive	Inactive	Inactive	Inactive	0.005	0.008

^aMIC: minimum inhibitory concentration.

^bAntimycobacterial activity was measured after 14 days of incubation.

^cAntimycobacterial activity was measured after 21 days of incubation.

^dStd1: Ampicillin, ^eStd2: Penicillin-G, ^fStd3: Chloramphenicol, ^gStd4: Rifampin,

^hStd5: Isoniazid(INH)

Results and Discussion

The dihydropyrimidine derivatives were obtained from the two step synthesis, their structures was confirmed by IR, NMR and elemental analysis. We have shown that 2-hetero-1, 4-dihydropyrimidines can be synthesized with selectively substitution of the para substituted electrophiles at N3 position. This selectivity is believed to be due to electron density at N3 and N1. The former being richer in electron density, is more reactive and produces products of exclusive functionalization at N3. The presence of various functional groups was detected by the absorption bands at 1687.6 cm^{-1} , 1392.5 cm^{-1} , 1292.22 cm^{-1} showing amide linkage, methoxy and ester respectively. The N3 substituted compound shows strong signal at around 6.0. The presence of ester group is determined by the presence of signal at 4.8, indicating stability of molecule.

Out of fifteen, 6 compounds (**6.c**, **6.d**, **6.i**, **6.l**, **6.n**, **6.o**) have shown good activity against *M. tuberculosis* H₃₇Rv. Four compounds (**6.d**, **6.i**, **6.n**, **6.o**) have shown activity in range of 1-2 $\mu\text{g/mL}$ at 14 days. In antibacterial activity against gram positive and gram negative organisms compounds showed similar trend as in case of antitubercular activity. Compound **6.a**, **6.g** and **6.j** showed activity at higher concentrations. Compound **6.c**, **6.d**, **6.e**, **6.l**, **6.n** and **6.o** exhibited activity at low concentrations at 1 to 9 $\mu\text{g/mL}$.

These compounds showed antitubercular activity at 1.5-3 $\mu\text{g/mL}$ concentrations. . Compounds having methoxy group (**6.o**), in general have shown good activity, while those with methyl group (**6.j**) have diminished activity. Compounds with chloro substitution at either position demonstrate better activity (**6.d**, **6.h**), Presence of hydroxyl group gave less activity (**6.g**). Eight compounds showed antibacterial activity, at a concentration range of 1-25 $\mu\text{g/mL}$.

In antibacterial activity against gram positive and gram negative organisms compounds showed similar trend as in case of antitubercular activity. Compound **6.a**, **6.g** and **6.j** showed activity at higher concentrations. Compound **6.c**, **6.d**, **6.e**, **6.l**, **6.n** and **6.o** exhibited activity at low concentrations at 1 to 9 $\mu\text{g/mL}$. Those compounds which had methoxy and/or chloro substitution at either position showed activity at lower concentrations.

Conclusion

These compounds have identified pyrimidines as new leads in the antibacterial, antitubercular chemotherapy. These molecules can be very useful for further optimization work in the antitubercular therapy. As these compounds are structurally similar to the dihydropyridines, these should be evaluated for their antihypertensive activity.

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