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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

An efficient synthesis, characterization, and antimicrobial screening of tetrahydropyrimidine derivatives

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Abstract Several new substituted 1,2,3,4-tetrahydropyrimidine derivatives **2a–h** have been synthesized by the modified Biginelli and Hantzsch reactions and compounds were characterized by spectral techniques. All compounds were evaluated for their in vitro antimicrobial activity against different strains of Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *S. pyogenus*) bacteria and selected fungi *C. albicans*, *A. niger*, and *A. clavatus* using serial Broth dilution method (Mueller–Hinton broth dilution method). Compound **2e** was found active against both Gram-negative and -positive bacterial strains used for present study, while compound **2b** was good active against Gram-negative and *S. pyogenus* bacterial strains. Compounds **2a–h** were not that much significant against (*C. albicans*, *A. niger*, and *A. clavatus*) selected fungal strains.

Keywords Tetrahydropyrimidine · Biginelli reaction · Hantzsch reaction · Antibacterial activity · Antifungal activity

Introduction

The pyrimidine has attracted increasing interest in the synthetic organic chemistry because of their diverse therapeutic and pharmacological properties (Kappe, 1993). These tetrahydropyrimidines have emerged as antihypertensive agents, mitotic kinesis inhibitors (Kappe *et al.*, 2000; Kappe, 2002), α1a-adrenergic receptor antagonists (Barrow *et al.*, 2000) and hepatitis B virus replication inhibitors (Deres *et al.*, 2003).

These compounds are known to possess wide spectrum of biological and therapeutic properties such as antibacterial, antiviral, antitumor, and anti-inflammatory activities, which represents the important of tetrahydropyrimidine compounds as the class of multi-channel blockers (Fabian and Semones, 1997; Eisner and Kuthan, 1972; Stout and Meyers, 1982). Tetrahydropyrimidines (THPMs) with other types of bioactivity, cerebrocrast (Klusa, 1995) has been introduced as a neuroprotectant and cognition enhancer. Recently, Desai et al. (2011) reported potent in vitro anti-cancer activity of THPMs.

The biological as well as medicinal importance of 1,2,3,4-tetrahydropyrimidine prompted us for further modification in the heterocyclic frame work for synthesis of new THPMs. The product one-pot synthesis that precipitated as pyrimidine compounds on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2(1*H*)-one (Biginelli, 1893). This has led to the development of multi-step approaches that produce overall higher yield, but need the simplicity of Biginelli synthesis.

During the past decades, the scope of the original cyclocondensation reaction is gradually extended by variation of all three building blocks, allowing access to a large number of structurally diverse multi-functionalized dihydropyridines (DHPs) and dihydropyrimidines (DHPMs). These heterocyclic compounds exist in a variety of natural and synthetic organic compounds. These developments led to the preparation and pharmacological evaluation of

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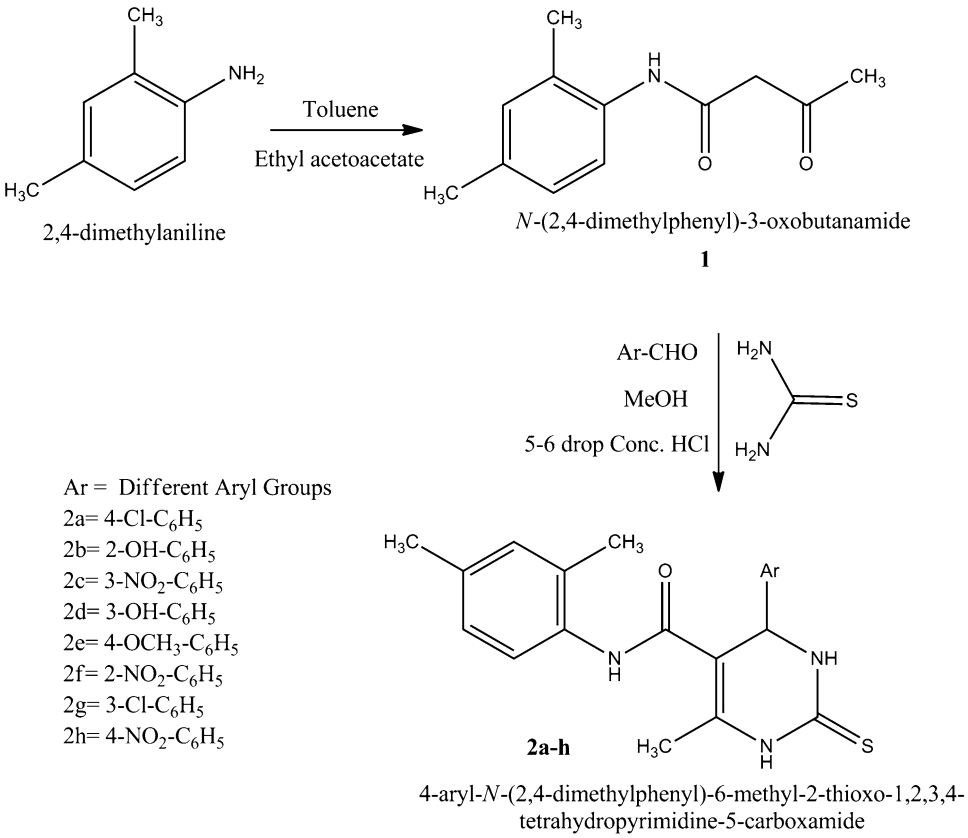


Fig. 1 Reaction scheme of 4-aryl-*N*-(2,4-dimethylphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

1,4-dihydropyridines (DHPs) and 3,4-dihydropyrimidines (DHPMs) of biological interest (Loev *et al.*, 1972; Meyer *et al.*, 1981, 1992; Kappe *et al.*, 2000; Dallinger *et al.*, 2004). However, in spite of their potential utility many of these reported one-pot protocols suffer from drawbacks such as the use of expensive reagents, strong acidic conditions, and long reaction times. THPMs, a variety of different combinatorial protocols based on the classical Biginelli microwave reaction have been reported. Syntheses of THPMs by using over silica sulfuric acid as a reusable catalyst under solvent-free conditions are reported (Armstrong *et al.*, 1996; Salehi *et al.*, 2003).

In extension of our efforts to understand the effect of functional groups on the conformational and features in the "Biginelli" class of compounds, we present herein a systematic study of THPMs-containing thiol group (Nayak *et al.*, 2010) moiety as depicted in reaction scheme (Fig. 1).

Experimental

Materials and methods

Melting points are determined on an electro-thermal melting point apparatus and are uncorrected. Completion of reaction and purity of all compounds were checked on aluminum-coated TLC plates 60 F_{245} (E. Merck) using benzene: ethyl acetate (8:2 V/V) as mobile phase and visualized under ultraviolet (UV) light or iodine vapor. A Perkin-Elmer 2400 CHN analyzer is used for elemental analysis of carbon, hydrogen, and nitrogen. IR spectra of compounds were recorded on Thermo-Nicolet FT-IR-200 spectrophotometer in KBr disk (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker (200 MHz) spectrometer using $CDCl_3$ as a solvent and TMS as an internal standard. Chemical shifts are reported in parts per million (δ_{ppm}). Mass spectral study of synthesized compounds (**2a**–**h**) was carried out using the Shimadzu GC–MS (Shimadzu 2010 plus) direct probe method. Compounds **2a**–**h** were synthesized using random synthesizer Syrris IKA-RCA with safety control under similar experimental conditions for each batch of synthesis.

General procedure

*General procedure for synthesis N-(2,4-dimethylphenyl)-3-oxobutanamide (**1**)*

A brief summary of method adopted for preparation of compound **1** is described here (Banik *et al.*, 2007; Desai *et al.*, 2012). A mixture of substituted aniline (0.01 mol) and ethyl acetoacetate (0.012 mol) in 40 mL toluene was refluxed for 12 h in the presence of few drops of NaOH in water. The reaction was monitored with the help of TLC.

Table 1 Analytical data of the prepared compounds **2a–h**

Sr. no.	Ar	Molecular formula	% Yield	M.P. °C	Elemental analysis (%)					
					C		H		N	
					Req.	Obs.	Req.	Obs.	Req.	Obs.
2a	4-Cl–C_6H_4	$C_{20}H_{20}ClN_3Os$	60	175	62.25	62.23	5.22	5.21	10.89	10.89
2b	2-OH–C_6H_4	$C_{20}H_{21}N_3O_2s$	60	140	65.37	65.36	5.76	5.75	11.44	11.44
2c	3-NO_2–C_6H_4	$C_{20}H_{20}N_4O_3s$	61	166	60.59	60.57	5.08	5.07	14.13	14.12
2d	3-OH–C_6H_4	$C_{20}H_{21}N_3O_2s$	69	152	65.37	65.36	5.76	5.75	11.44	11.42
2e	4-OCH_3–C_6H_4	$C_{21}H_{23}N_3O_2s$	63	165	66.12	66.11	6.08	6.06	11.01	11.00
2f	2-NO_2–C_6H_4	$C_{20}H_{20}N_4O_3s$	55	172	60.59	60.58	5.08	5.06	14.13	14.13
2 g	3-Cl–C_6H_4	$C_{20}H_{20}ClN_3Os$	58	130	62.25	62.24	5.22	5.20	10.89	10.88
2 h	4-NO_2–C_6H_4	$C_{20}H_{20}N_4O_3s$	67	170	60.59	60.57	5.08	5.07	14.13	14.12

The excess of toluene was distilled off and the reaction mixture was taken in hexane with continuous mechanical starring at constant RPM. The product was isolated in hexane and filtered off. The crude product was dried. The dried product was dissolved in aqueous NaOH solution so that, impurity of raw material can be removed using filtration. The crystallization of compound **1** was carried out in ethanol.

*Elemental and characterization data of N-(2,4-dimethylphenyl)-3-oxobutanamide (**1**)*

White crystallized powder; m.p.: 225 °C yield 78 % IR (KBr): cm^{-1}: 2921 (C–H-stretching asymmetric), 2866 (C–H-stretching asymmetric), 1644 (C=O-stretching of amide), 1382 (–CH_3-bending vibration), 3058 (C–H-stretching aromatic), 1511 (C=C-stretching ring skeletal), 1091 (C–H in-plane bending), 1267 (C–N stretching), 3245 (N–H stretching), 1010 (C–O stretching). ^{1}H NMR (200 MHz $CDCl_3$ δ_{ppm}) 2.24 (s, 6H, dimethylphenyl ring –CH_3), 1.74 (s, 3H, –CH_3), 6.45–7.21 (m, 3H, Ar–H), 7.25 (s, 1H, –C–NH, amide), 7.32 and 7.21 (s, 2H, Ar–H). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.4–143.0 (Ar–C, 6C, C-1-6), 21.5 and 17.3 (Ar–CH_3, 2C, C-7, C-8), 165.6 and 201.6 (–C=O, 2C, C-9, C-11), 51.4 (C–C, 1C, C-10, attached of oxobutamide), 27.9 (O=C–CH_3, 1C, C-12). GC–MS: *m/z* $[M+1]^+$ 205.15. Anal. Calcd. for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82; Found C, 70.21; H, 7.36; N, 6.82 %.

*General procedure for synthesis of 4-(aryl)-N-(2,4-dimethylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2a–h**)*

A mixture of compound (**1**) (0.01 mol), 4-chlorobenzaldehyde (0.01 mol), substituted diketone (0.012 mol), and thiourea (0.015 mol) in methanol was placed in the round bottom flask and was refluxed about for 12 h in the presence of catalytic amount concentrated HCl. The reaction mixture was poured onto crushed ice. The solid obtained was filtered and dried. The crystallization of final compounds was carried out in ethanol as described elsewhere. (Desai *et al.*, 2011) and their analytical data are summarized in Table 1.

*Elemental and characterization data of 4-(4-chlorophenyl)-N-(2,4-dimethylphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2a**)*

White crystalline powder; m.p.: 175 °C yield 60 % IR (KBr): cm^{-1}: 2920 (C–H-stretching asymmetric), 2855 (C–H-stretching symmetric), 1630 (C=O-stretching of amide), 1383 (–CH_3-bending vibration), 1514 (C=C-stretching ring skeletal), 1089 (C–H inplane bending), 1270 (C–N stretching), 3251 (N–H stretching), 1013 (C–O stretching), 1488 (–CH def asymmetric), 750 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 811 (C–H o.o.p. bending 1,2,4-trisubtituted benzene ring). ^{1}H-NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.23 (s, 6H, attached phenyl ring, –CH_3), 1.72 (s, 1H, tetrahydropyrimidine ring, –SH), 1.81 (s, 3H, Ar–CH_3), 5.41 (d, 1H, tetrahydropyrimidine ring, –CH), 6.63–7.50 (m, 7H, Ar–H) 7.86 and 8.23 (s, 2H, tetrahydropyrimidine ring, –NH and C–NH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.6–159.3 (Ar–C, 12C, C-8-13, C-16-20, C-5), 16.3, 21.5, and 17.3 (Ar–CH_3, 3C, C-14-15, C-6), 163.2 (–C=O, 1C, C-7), 174.2 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 57.4 (Ar–C, 1C, C-4), 106.2 and 159.4 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 369. Anal. Calcd. for $C_{20}H_{20}ClN_3OS$: C, 62.25; H, 5.22; N, 10.89; Found C, 62.23; H, 5.21; N, 10.89 (Table 1).

*Elemental and characterization data of N-(2,4-dimethylphenyl)-4-(2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2b**)*

White crystal powder; m.p.: 140 °C yield 60 % IR (KBr): cm^{-1}: 2918 (C–H-stretching asymmetric), 2855 (C–H-stretching symmetric), 1643 (C=O-stretching of amide),

1380 (–CH_3-bending vibration), 3057 (C–H stretching), 1504 (C=C-stretching ring skeletal), 1089 (C–H inplane bending), 1261(C–N stretching), 3242 (N–H stretching), 1009 (C–O stretching), 3315 (N–H stretching), 1458 (–CH def asymmetric), 750 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 815 (C–H o.o.p. bending 1,2,4-trisub). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.19 (s, 6H, attached phenyl ring, –CH_3), 1.74 (s, 1H, tetrahydropyrimidine ring, –SH), 1.86 (s, 3H, Ar–CH_3), 5.43 (s, 1H, tetrahydropyrimidine ring, –CH), 6.74–7.52 (m, 7H, Ar–H) 7.83 and 8.22 (s, 2H, –NH). 3.51 (s, 1H, phenol Ar–OH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 112.3–156.5 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.7, 21.5, and 17.6 (Ar–CH_3, 3C, C-14-15, C-6), 163.8 (–C=O, 1C, C-7), 174.4 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 59.5 (Ar–C, 1C, C-4), 108.7 and 159.7 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 338. Anal. Calcd. for $C_{20}H_{21}N_3O_2S$: C, 65.37; H, 5.76; N, 11.44; Found C, 65.36; H, 5.75; N, 11.44.

*Elemental and characterization data of N-(2,4-dimethylphenyl)-6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2c**)*

Yellow amorphous powder; m.p.: 166 °C yield 61 % IR (KBr): cm^{-1}: 2929 (C–H-stretching asymmetric), 2850 (C–H-stretching symmetric), 1640 (C=O-stretching of amide), 1378 (–CH_3-bending vibration), 3052 (C–H stretching), 1506 (C=C-stretching ring skeletal), 1088 (C–H inplane bending), 1243 (C–N stretching), 3233 (N–H stretching), 1008 (C–O stretching), 1447 (–CH def asymmetric), 750 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 813 (C–H o.o.p. bending 1,2,4-trisubstituted), 1218 (N–O stretching). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.21 (s, 6H, attached with phenyl ring, –CH_3), 1.76 (s, 1H, tetrahydropyrimidine ring, –SH), 1.88 (s, 3H, Ar–CH_3), 5.46 (s, 1H, tetrahydropyrimidine ring, Ar–H), 6.83–7.54 (m, 7H, Ar–H) 7.85 and 8.24 (s, 2H, amide in tetrahydropyrimidine ring, –NH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.7–159.1 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.9, 21.6, and 17.6 (Ar–CH_3, 3C, C-14-15, C-6), 163.1 (–C=O, 1C, C-7), 174.1 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 57.9 (Ar–C, 1C, C-4), 106.4 and 159.1 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 380. Anal. Calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; Found C, 60.57; H, 5.07; N, 14.12.

*Elemental and characterization data of N-(2,4-dimethylphenyl)-4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2d**)*

White crystalline powder; m.p.: 152 °C yield 69 % IR (KBr): cm^{-1}: 2918 (C–H-stretching asymmetric), 2856 (C–H-stretching symmetric), 1638 (C=O-stretching of amide), 1378 (–CH_3-bending vibration), 3048 (C–H stretching), 1515 (C=C-stretching ring skeletal), 1088 (C–H inplane bending), 1263 (C–N stretching), 3237 (N–H stretching), 1017 (C–O stretching), 3318 (N–H stretching), 1455 (–CH def asymmetric), 753 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 817 (C–H o.o.p. bending 1,2,4-trisub). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.17 (s, 6H, attached phenyl ring, –CH_3), 1.73 (s, 1H, tetrahydropyrimidine ring, –SH), 1.87 (s, 3H, Ar–CH_3), 5.44 (s, 1H, tetrahydropyrimidine ring,–CH), 6.54–7.54 (m, 7H, Ar–H) 7.87 and 8.26 (s, 2H, amide tetrahydropyrimidine ring, –NH). 3.53 (s, 1H, phenol Ar–OH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 112.6–156.7 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.8, 21.6, and 17.5 (Ar–CH_3, 3C, C-14-15, C-6), 163.4 (–C=O, 1C, C-7), 174.2 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 59.2 (Ar–C, 1C, C-4), 108.4 and 159.3 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 342. Anal. Calcd. for $C_{20}H_{21}N_3O_2S$: C, 65.37; H, 5.76; N, 11.44; Found C, 65.36; H, 5.75; N, 11.42.

*Elemental and characterization data of N-(2,4-dimethylphenyl)-4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2e**)*

White powder; m.p.: 165 °C yield 63 % IR (KBr): cm^{-1}: 2918 (C–H-stretching asymmetric), 2870 (C–H-stretching. symmetric), 1643 (C=O-stretching of amide), 1388 (–CH_3-bending vibration), 3058 (C–H stretching), 1517 (C=C-stretching ring skeletal), 1083 (C–H-inplane bending), 1258 (C–N stretching), 3245 (N–H stretching), 1010 (C–O stretching), 3311 (N–H stretching) 1455 (–CH def. asymmetric), 756 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 811 (C–H o.o.p. bending 1,2,4-trisubtituted benzene ring). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.24 (s, 6H, attached phenyl ring, –CH_3), 1.76 (s, 1H, tetrahydropyrimidine ring, –SH), 1.82 (s, 3H, Ar–CH_3), 5.44 (s, 1H, tetrahydropyrimidine ring,–CH), 6.73–7.57 (m, 7H, Ar–H) 7.86 and 8.27 (s, 2H, amide tetrahydropyrimidine ring, –NH), 2.34 (s, 3H, attached phenyl ring, Ar–OCH_3). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.3–157.4 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.8, 21.8, and 17.6 (Ar–CH_3, 3C, C-14-15, C-6), 163.4 (–C=O, 1C, C-7), 174.4 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 57.7 (Ar–C, 1C, C-4), 106.3 and 158.3 (C=C, 2C, tetrahydropyrimidine ring, C-1-2), 56.2 (Ar–OCH_3, 1C, C-21). GC–MS: *m/z* $[M+1]^+$ 364. Anal. Calcd. for $C_{21}H_{23}N_3O_2S$: C, 66.12; H, 6.08; N, 11.01; Found C, 66.11; H, 6.06; N, 11.00.

*Elemental and characterization data of N-(2,4-dimethylphenyl)-6-methyl-4-(2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2f**)*

Yellow crystalline powder; m.p.: 172 °C yield 55 % IR (KBr): cm^{-1}: 2922 (C–H-stretching asymmetric), 2856 (C–H-stretching symmetric), 1646 (C=O-stretching of amide), 1383 (–CH_3-bending vibration), 3051 (C–H stretching),

1515 (C=C-stretching ring skeletal), 1081 (C–H-inplane bending), 1266 (C–N stretching), 3245 (N–H stretching), 1010 (C–O stretching), 1456 (–CH def asymmetric), 753 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 814 (C–H o.o.p. bending 1,2,4-trisub), 1221 (N–O stretching). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.23 (s, 6H, dimethylphenyl attached phenyl ring, $–CH_3$), 1.77 (s, 1H, tetrahydropyrimidine ring, –SH), 1.89 (s, 3H, Ar–CH_3),5.42 (s, 1H, tetrahydropyrimidine ring, –CH), 6.78–7.54 (m, 7H, Ar–H) 7.85 and 8.24 (s, 2H, amide tetrahydropyrimidine ring, –NH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.4–159.1 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.8, 21.7, and 17.8 (Ar-CH_3, 3C, C-14-15, C-6), 163.4 (–C=O, 1C, C-7), 174.2 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 57.8 (Ar–C, 1C, C-4), 106.3 and 159.3 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 348. Anal. Calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; Found C, 60.58; H, 5.06; N, 14.13.

*Elemental and characterization data of 4-(3-chlorophenyl)-N-(2,4-dimethylphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2g**)*

White crystalline powder; m.p.: 130 °C yield 58 % IR (KBr): cm^{-1}: 2923 (C–H-stretching asymmetric), 2855 (C–H-stretching symmetric), 1644 (C=O-stretching of amide), 1384 (–CH_3-bending vibration), 3066 (C–H stretching), 1517 (C=C-stretching ring skeletal), 1089 (C–H-inplane bending), 1263 (C–N stretching), 3243 (N–H stretching), 1011 (C–O stretching), 1453 (–CH def. asymmetric), 755 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 815 (C–H o.o.p. bending 1,2,4-trisubtituted benzene ring). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.27 (s, 6H, attached phenyl ring–CH_3), 1.74 (s, 1H, tetrahydropyrimidine ring, –SH), 1.88 (s, 3H, Ar–CH_3), 5.44 (s, 1H, tetrahydropyrimidine ring,–CH), 6.89–7.57 (m, 7H, Ar–H) 7.84 and 8.21 (s, 2H, amide tetrahydropyrimidine ring, –NH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.2–159.2 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.4, 19.6 and 17.4 (Ar–CH_3, 3C, C-14-15, C-6), 163.3 (–C=O, 1C, C-7), 174.3 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 57.8 (Ar–C, 1C, C-4), 106.3 and 159.4 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 235. Anal. Calcd. for $C_{20}H_{20}ClN_3OS$: C, 62.25; H, 5.22; N, 10.89; Found C, 62.24; H, 5.21; N, 10.87.

*Elemental and characterization data of N-(2,4-dimethylphenyl)-6-methyl-4-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2h**)*

White–yellow crystalline powder; m.p.: 170 °C yield 67 % IR (KBr): cm^{-1}: 2911 (C–H-stretching asymmetric), 2871 (C–H-stretching symmetric), 1665 (C=O-stretching of amide), 1384 (–CH_3-bending vibration), 3060 (C–H stretching), 1511 (C=C-stretching ring skeletal), 1088 (C–H-inplane bending), 1276 (C–N stretching), 3245 (N–H stretching), 1015 (C–O stretching), 1455 (–CH def asymmetric), 753 (C–H o.o.p. bending 1,4-disubtituted benzene ring), 817 (C–H o.o.p. bending 1,2,4-trisub), 1223 (N–O stretching). ^{1}H NMR (200 MHz, $CDCl_3$ δ_{ppm}): 2.26 (s, 6H, attached with phenyl ring, $–CH_3$), 1.75 (s, 1H, tetrahydropyrimidine ring, –SH), 1.84 (s, 3H, Ar–CH_3), 5.41 (s, 1H, tetrahydropyrimidine ring, –CH), 6.71–7.56 (m, 7H, Ar–H) 7.84 and 8.23 (s, 2H amide tetrahydropyrimidine ring, –NH). ^{13}C NMR (200 MHz, $CDCl_3$ δ_{ppm}): 114.3–159.1 (Ar–C, 12C, C-8-13, C-16-20, C-5), 17.7, 21.6, and 17.4 (Ar–CH_3, 3C, C-14-15, C-6), 163.3 (–C=O, 1C, C-7), 174.5 (–C–S, 1C, tetrahydropyrimidine ring, C-3), 57.4 (Ar–C, 1C, C-4), 106.4 and 159.4 (C=C, 2C, tetrahydropyrimidine ring, C-1-2). GC–MS: *m/z* $[M+1]^+$ 360. Anal. Calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; Found C, 60.57; H, 5.07; N, 14.12.

Statistical analysis

Standard deviation value is expressed in terms of ±SD. Based on calculated value by using one-way ANOVA method followed by independent two-sample Turkey test; it has been observed that differences below 0.001 levels are considered as statistically significant.

Result and discussion

Methodology adopted for preparation of tetrahydropyrimidines

Several methodologies are taking on for the preparation of tetrahydropyrimidines. Summary of adopted approaches are given here.

Messer *et al.*, (1992) and Dunbar *et al.*, (1993) used pyrimidine ring as initiator and reduced in the presence of Pd catalyst to yield the target compound of tetrahydropyrimidine. Sawant and Sarode (2011) prepared tetrahydropyrimidine-using $AlCl_3$ and con. HCl. Both methods are not efficient enough to get significant yield of final targeted compounds. Therefore, we have modified Biginelli reaction in which we used diketone, aromatic aldehyde and thiourea in methanol medium. This modified reaction helps us to get significant yield of our targeted moieties.

Chemistry of 4-(4-chlorophenyl)-*N*-(2,4-dimethylphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**2a**)

IR spectra

The IR spectrum of the compound **2a** (molecular formula $C_{20}H_{20}ClN_3O_2$ m.w. 369) clearly shows a C=O has given

absorption peak appear in the range of 1,630 cm^{-1} is due to stretching vibration, while ring skeletal C=C shows medium intensity absorption stretching at 1,514 cm^{-1}, respectively. C–N-stretching band at 1,270 cm^{-1} and high frequency region of IR spectra of this compound contains vibration N–H-stretching vibration band indicate at 3,251 cm^{-1} indicates ring closure of the pyrimidine ring corresponding secondary amine. The presence of 1,2,4-trisubstituted benzene ring is confirmed due to absorption bands at 750 and 811 cm^{-1}, respectively. The presence of substituted (C–Cl) benzene ring is also confirmed with weak intensity absorption peak corresponding to low frequency region at 650 cm^{-1} of synthesized compound **2a**.

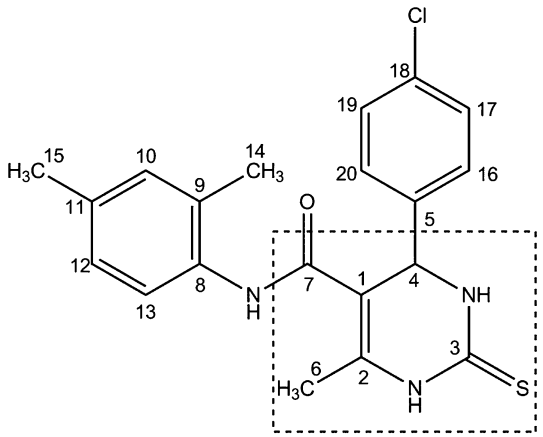
^{1}H NMR spectra

Proton-NMR spectra of compound **2a** suggests that C-10, C-12, C-13, C-16, C-17, C-19, and C-20, seven protons are attached to chemically equivalent environment in compound **2a**, which is appeared at $\delta = 6.63$–7.50 ppm. Secondary amide (attached to C-8) proton which is appeared as singlet at $\delta = 8.23$ ppm. Proton of –SH group in pyrimidine ring appeared as a singlet at $\delta = 1.72$ ppm. Protons of methyl group (C-14 and C-15) appeared as singlet at $\delta = 2.23$ ppm, while methyl group (C-6), which is appeared as singlet at $\delta = 1.81$ ppm. C–NH of pyrimidine ring is appeared as a singlet at $\delta = 7.86$ ppm and protons of C-4 of pyrimidine ring is appeared as a doublet at $\delta = 5.41$ ppm.

^{13}C NMR spectra

Looking at, chemical shifts of final compound **2a** vary from $\delta = 16.3$–174.2 ppm. Carbon nuclei under the influence of a strong electronegative environment appeared in the downfield, e.g., carbon of tetrahydropyrimidine ring C-7 has chemical shift value of $\delta = 163.2$ ppm due to the influence of oxygen atom. Similarly, C-3 in tetrahydropyrimidine (–C–S) ring has chemical shift at $\delta = 174.2$ ppm due to influence of sulfur atom, while C-1 of same ring is appeared at $\delta = 106.5$ ppm due to influence of nitrogen atom presence in the tetrahydropyrimidine ring. Carbons are chemically equivalent in nature for C-5, C-8-13, and C-16-20 of compound **2a** show chemical shift at around $\delta = 114.6$–159.3 ppm, which suggest the presence of phenyl rings in the structure, while chemical shifts of C-6, C-14, and C-15 are appeared at $\delta = 17.3$–21.5 ppm indicates the presence of three methyl groups. GC–MS data suggest that molar mass of compound **2a** is *m/z* $[M+1]^{+}$ 369.

Based on ^{1}H NMR and ^{13}C NMR and mass spectrum data, carbon enumeration of compound **2a** is described in Fig. 2.



Structure of compound 2a

Fig. 2 Compound **2a** showed the presence of tetrahydropyrimidine moiety in its structure

Antimicrobial activity

The newly synthesized compounds **2a**–**h** were screened for their antimicrobial activity against Gram-positive bacteria *S. aureus* (MTCC-96), *S. pyogenus* (MTCC-442) and Gram-negative (*E. coli* (MTCC-443), *P. aeruginosa* (MTCC-1688), and fugal pathogens *C. albicans* (MTCC 227) *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323) All MTCC were collected from Institute of Microbial Technology, Chandigarh. The activity of compounds was determined at Microcare Laboratory, Surat using standard protocol of Mueller–Hinton Broth (Becton–Dickinson, USA). The compounds were dissolved in dimethyl sulfoxide (DMSO) for the disk-diffusion test, and minimal inhibitory concentrations (MIC) were determined by making a series of dilutions in an appropriate substrate ranging from 16.23 to 811.75 μM/L. The final concentration of DMSO did not exceed 2 %. The sensitivity of microorganisms to the investigated species was tested by measuring the zone of inhibition of a given concentration of the extract by the disk-diffusion method and by determining the minimal inhibitory concentration (MIC) (Clinical and Laboratory Standards Institute, 2008a, b). Bacterial inoculum were obtained from bacterial cultures incubated for 24 h at 37 °C on Mueller–Hinton agar substrate and diluted to approximately 10^{8} CFU/mL, according to the 0.5 McFarland standard. Suspensions of fungal spores were prepared from fresh mature (3–7 days old) cultures that grew at 30 °C on a PDA substrate (Gein, 2012). Spores were rinsed with sterile distilled water, in order to determine turbidity spectrophotometrically at 530 nm. The set was further diluted to approximately 10^{6} CFU/mL according to the procedure recommended by the NCCLS (CLSI; formerly NCCLS). The standard disk-diffusion method was used to study antimicrobial activity. The

appropriate inoculums were seeded in Müeller–Hinton agar (for bacteria) or SD agar (for fungi) in Petri dishes. Paper disks (7 mm in diameter) were laid on the inoculated substrate after being soaked with 15 μL of different concentration of 16.23–811.75 μM/L. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition around the disk. As a positive control of growth inhibition, ampicillin and chloramphenicol were used in the case of bacteria, nystatin in the case of fungi. A DMSO solution was used as a negative control for the influence of solvents and all experiments were performed in duplicate. The minimal inhibitory concentration (MIC) was determined by the broth tube dilution method. A series of dilutions with concentrations ranging from 16.23 to 811.75 μM/L was used in the experiment with each extract for every microorganism tested. The starting solutions of extracts with a concentration of 16.23 μM/L were obtained by measuring of a certain quantity of extract and dissolving it in DMSO. Twofold dilutions of extracts were prepared in Mueller–Hinton bullion for bacterial cultures and SD bullion for fungal cultures in test tubes. The boundary dilution without any visible growth was defined as the minimal inhibitory concentration (MIC) for the tested microorganism at the given compounds concentration. All experiments were performed in duplicate.

Antimicrobial assay

Compounds (**2a**–**h**) were screened against Gram-positive, -negative bacteria and fungi strains. The compounds **2b**, **2g**, and **2h** showed good activity against *E. coli* bacteria (MIC = 324.7–405.7 μM/L), while compound **2e** showed excellent activity against *E. coli* (MIC = 202.93 μM/L). Similarly, compounds **2b** and **2e** showed good activity against *P. aeruginosa*. Compounds **2a**, **2b**, **2c**, **2d**, **2f**, **2g,** and **2h** showed moderate activity against *S. aureus* with MIC = ranging from 649.4 to 811.75 μM/L. Compounds **2b**, **2e**, and **2f** showed good activity against *S. pyogenus* (MIC = 324.7–405.87 μM/L). Results also suggest that compound **2e** was more effective against all bacterial strains studied in this work (Table 2). Results of antifungal activity were of suggestive that the synthesized compounds were not equally effective against three fungal pathogens. The data revealed that electron-releasing groups as nitro, hydroxyl, and chloro, present on phenyl ring were increased the antibacterial property. These results clearly demonstrated that when compounds **1** converted into corresponding final compounds **2a–h**, exhibited significant antibacterial activity. Based on MIC values, it is our observation that the presence of 4-OCH_3, 4-NO_2, 2-OH, and 4-Cl group at the position of tetrahydropyrimidines led to a significant variation in the antimicrobial activity. A series of compounds when substituted by electron-releasing

Table 2 Antimicrobial activity of 4-aryl-*N*-(2,4-dimethylphenyl)-6-methyl-2-thioxo-1,2,3,4 tetrahydropyrimidine-5-carboxamide

Sr. No.	Ar	Minimal inhibition concentrations (bacteria) in μM/L				Minimal inhibition concentrations (fungicidal) in μM/L		
		E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
2a	4-Cl–C_6H_4	811.75 ± 2.346	649.4 ± 4.127	811.75 ± 1.827	649.4 ± 3.115	3247 ± 4.125	3247 ± 3.246	3247 ± 4.398
2b	2-OH–C_6H_4	**324.7** ± 3.114 **	**405.87** ± 2.487 **	811.75 ± 2.476	**324.7** ± 3.653 **	3247 ± 4.321	3247 ± 3.337	3247 ± 5.237
2c	3-NO_2–C_6H_4	649.4 ± 2.227	811.75 ± 3.754	811.75 ± 2.392	649.4 ± 3.225	1623.5 ± 4.574	3247 ± 4.451	3247 ± 3.447
2d	3-OH–C_6H_4	811.75 ± 2.356	649.4 ± 3.395	649.4 ± 3.614	**202.93** ± 3.774***	1623.5 ± 4.543	3247 ± 5.174	3247 ± 4.247
2e	4-OCH_3–C_6H_4	**202.93** ± 1.524 ****	**324.7** ± 2.448 **	**202.93** ± 2.555 ****	**405.87** ± 3.852 **	3247 ± 4.358	3247 ± 5.199	3247 ± 3.954
2f	2-NO_2–C_6H_4	649.4 ± 3.137	649.4 ± 2.341	649.4 ± 3.197	**405.87** ± 3.657 **	3247 ± 4.582	3247 ± 4.827	3247 ± 4.834
2g	3-Cl–C_6H_4	**324.7** ± 3.332 **	649.4 ± 1.476	811.75 ± 3.721	649.4 ± 2.634	1623.5 ± 4.357	3247 ± 4.357	3247 ± 3.442
2h	4-NO_2–C_6H_4	**405.87** ± 2.652 **	811.75 ± 3.662	811.75 ± 2.224	811.75 ± 2.542	1623.5 ± 5.152	3247 ± 4.443	3247 ± 4.887
Ampicillin chloramphenicol nystatin		100	100	250	100	–	–	–
		50	50	50	50	–	–	–
		–	–	–	–	100	100	100

Bold values indicate good antimicrobial activity

±SD standard deviation. All values are presented as mean of six experiments; (Replicate measurement, $n = 6$)

* $P < 0.05$ significant value; ** $P < 0.01$ moderately significant value; *** $P < 0.001$ extremely significant value. These results were calculated on one-way Bonferroni test

groups like nitro, hydroxyl, methoxy, and chloro present on aromatic ring enhanced the antimicrobial activity.

Conclusion

In this work, we have disclosed the Biginelli synthesis for tetrahydropyrimidines (THPMs). Using readily available ethyl acetoacetate as reaction mediator and Toluene as solvent, the yields in the one-pot Biginelli protocol can be increased on average by 40–55 % as compared with the traditional methods. This one-pot synthesis of tetrahydropyrimidines is therefore, superior in simplicity and yield compared to alternative multistep strategies that have been reported. The use of ethyl acetoacetate in related cyclization reactions is currently underway in our laboratories and will be reported in due course. A series of 4-aryl-*N*-(2,4-dimethylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide were synthesized successfully. The Antimicrobial activity profile of the title compounds were evaluated against Gram-positive bacteria, -negative bacteria, and three fungal pathogens. Looking to the structure–activity relationship, remarkable inhibition was observed in compounds bearing Ar = 4-chlorophenyl, 2-hydroxyphenyl, 3-nitrophenyl, 3-hydroxyphenyl, 4-methoxyphenyl, and 4-nitrophenyl substituents. Compound **2e**, which contains 4-methoxyphenyl group, was found very efficient against both Gram-negative and -positive bacteria. This is can be attributed to the fact that 4-methoxyphenyl derivative may interrupt the growth of all bacterial strains at one or more stages, studied in this study. This study also suggests less lipophilic, less bulky, and electron-withdrawing substituent may increase the potency, and also reveals that further in-depth study of 4-methoxyphenyl derivative will be extracted more relative information about the structure–activity relationship with antimicrobial screening.

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