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Synthesis of 1-phenyl-3, 4-dihydropyrimidine-2(1H)-ones derivatives under solvent free condition and their antimicrobial activity

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Abstract

We report herein the use of nickel nitrate hexahydrate [$Ni(NO_3)_2.6H_2O$] as a new catalyst for the one-pot Biginelli like reaction coupling of 1-phenyl thiourea, ethyl acetoacetate and aromatic aldehydes to afford the corresponding 1-phenyl-3,4-dihydropyrimidin-2(*1H*)-thiones under solvent free condition to avoid the usage of hazardous organic solvents. The synthesized compounds were evaluated for their antimicrobial activity by KIRBY-BAUER disk diffusion method. Most of the compounds showed good to moderate antimicrobial activity.

Keywords: Nickel nitrate hexahydrate; 1-Phenyl thiourea; Aldehydes; Ethyl acetoacetate; Biginelli reaction; Antimicrobial activity

Introduction

Pyrimidines and their derivatives play an important role in human vital functions. The pyrimidine skeleton is the component of a series of natural compounds (*vitamin* B1, nucleic acids), chemotherapeutic drugs (*Flurouracil*) and synthetic medicines (*Barbiturates*). The biological importance of pyrimidine derivatives caused a significant interest in their synthesis (Cho *et al*., 1989; Mirzaei *et al.,* 2001; Shaaban *et al*., 2008). Dihydropyrimidines (DHPMs) and their derivatives are pharmaceutically important as Calcium channel blockers, *α* 1-1-a-antagonists, antihypertensive agents, inhibitors of the fatty acid trans-porter, and mitotic kinesin inhibition (Rovnyak *et al*., 1995; Van Zandt *et al*., 2005). These compounds have also been found to posses antiviral, antitumor and antibacterial properties (Tsuruo *et al*., 1983). Moreover, the biological activity of some isolated marine natural products and alkaloids have been attributed to the Dihydropyrimidine moiety (Snider and Shi, 1993). For example, the anti-cancer agent monastrol (Fig. 1) has been shown to specifically affect mitosis *via* a new mechanism consisting of the specific and reversible inhibition of the motility of the motor protein mitotic kinesin (Dondoni *et al*., 2002). Since more than one hundred years, *Biginelli* suggested a dihydropyrimidine ring construction based on the use of *β*-dicarbonyl compounds as a source of two carbon fragment with aldehydes and urea or thiourea as an N-C-N fragment (Biginelli, 1893).

It is worth mentioning that *Biginelli* reaction is one of the most named reactions and his collaboration is still considered one of the important pyrimidine synthesis. In the past 10 years, several one-pot methodologies for the synthesis of DHPM derivatives were developed and several modifications have been introduced. Most of them are based on Lewis acid-catalyzed reactions (Paraskar *et al*., 2003; Sabitha *et al*., 2005; Azizian *et al*., 2006; Sadek *et al*., 2010; Shapiro and Vigalok, 2008; Chitra and Pandiarajan, 2009; Mandhane *et al*., 2010; Salim and Akamanchi, 2011) which permits the reaction to proceed under milder conditions and with higher yields, than those outlined by *Biginelli* in the original procedure. Microwave irradiation has also proved beneficial (Pasunooti *et al*., 2011).

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Natural acidic catalysts have been also utilized (Patil *et al*., 2011). Very recently, *Biginelli* reaction has been conducted under basic conditions. This involves the use of PPh_3, under solvent free conditions (Debache *et al*., 2008), *t*-BuOK at 70ºC (Shen *et al*., 2010), chiral primary amines (Ding *and Zhao,* 2010) and ammonium carbonate in water (Tamaddon *et al*. 2010). It is worth mentioning that many of these existing methods displayed drawbacks, such as environmental pollution caused by utilizing catalysts in stoichiometric quantities, exotic reaction conditions, unsatisfactory yields and complicated operations while other possess some advantages overcoming these drawbacks. Nickel nitrate hexahydrate is a convenient and widely used catalyst for affecting a broad spectrum of synthetic transformations because it has many advantages such as: solubility in water, inexpensive, eco-friendly nature, simplicity in handling and convenient work up (Boumoud *et al*., 2012).

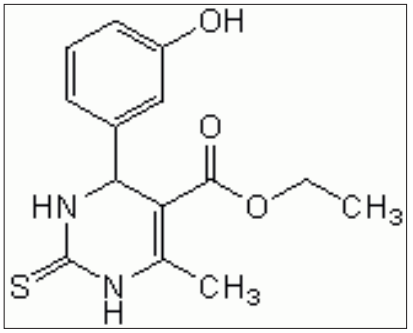


Fig. 1. Monastrol

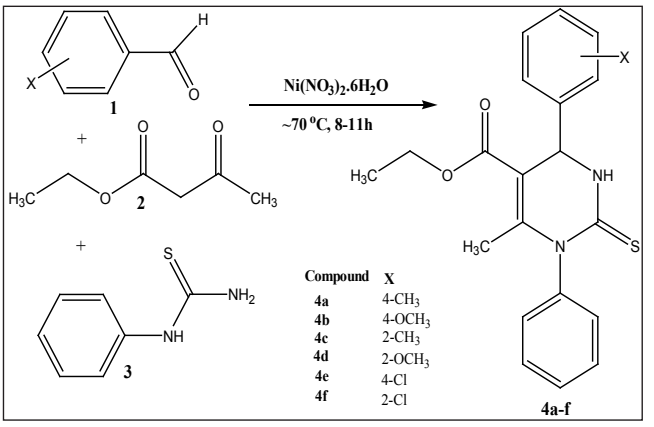
In continuation of our interest in the synthesis of fused pyrimidines (Akhter *et al*., 2015), we report herein a simple, efficient procedure for one-pot synthesis of 3,4 -dihydropyrimidin-2*(1H)*-thiones derivatives 4a-f using $Ni(NO_3)_2.6H_2O$ as a catalyst under solvent free conditions through Biginelli reactions (Scheme 1). The synthesized compounds were evaluated for *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus* (gram +ve), *Escherichia coli, Salmonella typhimerium* (gram-ve) using KIRBY-BAUER method **(**Bauer *et al*., 1966**)**. The primary purpose of the study was to evaluate antimicrobial potency of synthetic products against the particular bacteria.

Materials and methods

All products were characterized by their mp, IR, ^{1}H-NMR, ^{13}C-NMR and elemental analyses. Thin layer chromatography (TLC) was carried out on plates percolated with silica gel 60 F_{254} (E.Merck) and spots were detected with iodine vapour. Melting points were determined on an Electro thermal micro melting point apparatus and uncorrected. IR spectra were recorded as KBr pellet using Shimadzu IR-470A spectrophotometer. The ^{1}H-NMR was taken in DMSO-d_6 and ^{13}C-NMR spectra were taken in DMSO with TMS as an internal standard in Bruker 400 MHz spectrophotometer. The elemental analyses were done using PerkinElmer 2400 CHN Analyzer.

General procedure for the synthesis of derivatives, 4a–f

A mixture of an appropriate aldehyde, **1** (10 mmol), ethylacetoacetate, **2** (10 mmol) and 1-phenyl-2-thiourea, **3** (4.5 mmol) in the presence of $Ni(NO_3)_2.6H_2O$ (10 mol %) was heated (70ºC) within 8-11 h; after completion of the reaction as indicated by TLC (eluting solvent, $CHCl_3$), the reaction mixture was poured into ice water. A gummy product was obtained which was dissolved in absolute alcohol and filtered. The volume of the filtrate was then reduced to one-forth by rotary vacuum evaporator and the sample was transferred in a conical flask. Then the wall of the conical flask was scratched in an ice bath and a solid product was obtained; it was filtered and washed with cold absolute alcohol. The dried product was recrystallized with absolute alcohol to obtain the pure product.



Scheme-1

6-Methyl-1-phenyl-2-thioxo-4-p-tolyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester, 4a :

Yield 87%; white crystalline solid; m.p.128º-130ºC; R_f value: 0.52 ($CHCl_3$); IR (KBr) (ν_{max}, cm^{-1}): 3180 (N-H) 3032 (aromatic C-H stretching), 2835 (C-H stretching for $-CH_3$), 1701 (C=O stretching), 1631 (>C=C< stretching in conjugation with >C=O), 1492 (C=S stretching), 1344 (-CH_3

bending), 825 (aromatic C-H bending); ^{1}H NMR δ (in ppm): 8.17 (s, 1H, NH, 3-H), 7.43-6.87 (m, 9H, arom), 5.40 (bs, 1H, 4-H), 4.12 (q, 2H, J=7.2, CH_3-C$\underline{H}_2$-COO-), 2.46 (s, 3H, C$\underline{H}_3$-C_4'), 2.15 (s, 3H, C$\underline{H}_3$-C_6=C_5-), 1.19 (t, 3H, J=7.2, C$\underline{H}_3$-CH_2-COO-); ^{13}C NMR δ (in ppm): 178.46 (2-C), 165.73 (CH_3-CH_2-$\underline{C}$OO-), 145.90 (6-C), 140.56 (C-1'), 139.31 (C-1"), 137.85 (C-4'), 129.51-126.31 ($9C_{arom}$), 107.02 (5-C), 60.58 (CH_3-$\underline{C}H_2$-COO-), 54.06 (4-C), 21.09 ($\underline{C}H_3$-C_6H_4-), 18.59 ($\underline{C}H_3$- C_6=C_5-), 14.02 ($\underline{C}H_3CH_2$-COO-); Anal. Found: C, 68.78; H, 5.98; N, 7.02; Calcd. for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.64%.

4-(4-Methoxy-phenyl)-6-methyl-1-phenyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester, 4b :

Yield 90%; white crystalline solid; m.p.134°-136°C; R_f value: 0.50 ($CHCl_3$); IR (KBr) (ν_{max}, cm^{-1}): 3398 (N-H) 3028 (aromatic C-H stretching), 2922 (C-H stretching for – CH_3), 1708 (C=O stretching), 1635 (>C=C< stretching in conjugation with >C=O), 1442 (C=S stretching), 1342 (-CH_3 bending), 837, 761 (aromatic C-H bending); ^{1}H NMR δ (in ppm): 8.12 (s, 1H, NH, 3-H), 7.43-7.15 (m, $9H_{arom}$) 5.42 (bs, 1H, 4-H), 4.13 (q, 2H, J=7.2, CH_3-C$\underline{H}_2$-COO-), 3.79 (s, 3H, C$\underline{H}_3$O-C_4'), 2.09 (s, 3H, C$\underline{H}_3$-C_6=C_5-), 1.20 (t, 3H, J=6.9, C$\underline{H}_3$-CH_2-COO-); ^{13}C NMR δ (in ppm): 178.46 (2-C), 165.74 (CH_3-CH_2-$\underline{C}$OO-), 159.38 (C-4'), 145.76 (6-C), 140.56 (C-1"), 134.52 (C-1'), 128.68-114.16 ($9C_{arom}$), 107.10 (5-C), 60.58 (CH_3-$\underline{C}H_2$-COO-), 55.25 (4-C), 53.80 ($\underline{C}H_3O$-C_4'), 18.58 ($\underline{C}H_3$- C_6=C_5-), 14.08 ($\underline{C}H_3$-CH_2-COO-); Anal. Found: C, 65.91; H, 5.29; N, 7.17; Calcd. for $C_{21}H_{22}N_2O_3S$: C, 65.95; H, 5.80; N, 7.32%.

6-Methyl-1-phenyl-2-thioxo-4-o-tolyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester, 4c :

Yield 92%; white crystalline solid; m.p. 138°-140°C; R_f value: 0.50 ($CHCl_3$); IR (KBr) (ν_{max}, cm^{-1}): 3188 (N-H) 3096 (aromatic C-H stretching), 2985 (C-H stretching for – CH_3), 1705 (C=O stretching), 1492 (C=S stretching), 1342 (-CH_3 bending), 825 (aromatic C-H bending); ^{1}H NMR δ (in ppm): 7.54 (s, 1H, NH, 3-H), 7.46 -7.16 (m, $9H_{arom}$), 5.68 (d, J=2.4,1H, 4-H), 4.02 (q, 2H, J=6.9, CH_3-C$\underline{H}_2$-COO-), 2.46 (s, 3H, C$\underline{H}_3$-C_6H_4-), 2.15 (s, 3H, C$\underline{H}_3$-C_6=C_5-), 1.05 (t, 3H, J=6.9, C$\underline{H}_3$-CH_2-COO-); ^{13}C NMR δ (in ppm): 177.70 (2-C), 165.60 (CH_3-CH_2-$\underline{C}$OO-), 146.05 (6-C), 140.74 (C-1'), 139.58 (C-1"), 135.06 (C-2'), 130.99 -126.86 ($9C_{arom}$), 105.81 (5-C), 60.48 (CH_3-$\underline{C}H_2$-COO-), 51.93 (4-C), 19.13 ($\underline{C}H_3$-C_6H_4-), 18.63 ($\underline{C}H_3$-C_6=C_5-), 13.87 ($\underline{C}H_3CH_2$-COO-); Anal. Found: C, 68.72; H, 6.23; N, 7.15; Calcd. for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.64%.

4-(2-Methoxy-phenyl)-6-methyl-1-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester, 4d :

Yield 82%; white crystalline solid; m.p. 148°-150°C; R_f value: 0.53 ($CHCl_3$); IR (KBr) (ν_{max}, cm^{-1}): 3219 (N-H) 3022 (aromatic C-H stretching), 2933 (C-H stretching for – CH_3), 1701 (C=O stretching), 1468 (C=S Stretching), 1340 (-CH_3 bending), 873 (aromatic C-H bending); ^{1}H NMR δ (in ppm): 7.63 (s, 1H, NH, 3-H), 7.41-6.91 (m, $9H_{arom}$), 5.78 (d, J=2.1, 1H, 4-H), 4.09 (q, 2H, J=7.2, CH_3-C$\underline{H}_2$-COO-), 3.9 (s, 3H, C_6H_4-OC$\underline{H}_3$), 2.20 (s, 3H, $\underline{H}_3$C-C_6=C_5-), 1.10 (t, 3H, J=7.2, C$\underline{H}_3$-CH_2-COO-); ^{13}C NMR δ (in ppm): 178.27 (2-C), 165.78 (C$\underline{H}_3$-CH_2-$\underline{C}$OO-), 156.89 (C_2'), 147.68 (6-C), 140.68 (C-1"), 129.56 (C-1'), 128.97-110.92 ($9C_{arom}$), 104.29 (5-C), 60.48 (CH_3-$\underline{C}H_2$-COO-), 55.56 (4-C), 49.36 ($\underline{C}H_3O$-C_4'), 18.57 ($\underline{C}H_3$-C_6=C_5-), 14.02 ($\underline{C}H_3$-CH_2-COO-); Anal. Found: C, 65.93; H, 5.39; N, 7.11; Calcd. for $C_{21}H_{22}N_2O_3S$: C, 65.95; H, 5.80; N, 7.32%.

4-(4-Chloro-phenyl)-6-methyl-1-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester, 4e:

Yield 80%; white crystalline solid; m.p. 124°-126°C; R_f value: 0.49 ($CHCl_3$); IR (KBr) (ν_{max}, cm^{-1}): 3176 (N-H), 3030 (aromatic C-H stretching), 1710 (C=O stretching), 1635 (>C=C< stretching in conjugation with >C=O), 1445 (C=S Stretching), 1382 (-CH_3 bending), 1082 (C-Cl stretching), 837, 690 (aromatic C-H bending); ^{1}H NMR δ (in ppm): 8.37 (s, 1H, NH, 3-H), 7.51–7.02 (m, $9H_{arom.}$), 5.43 (d, J=3.2, 1H, 4-H), 4.13 (q, 2H, J=7.2, CH_3-C$\underline{H}_2$-COO), 2.09 (s, 3H, C$\underline{H}_3$-C_6=C_5-), 1.19 (t, 3H, J=7.2, $\underline{C}H_3$-CH_2-COO-); ^{13}C NMR δ (in ppm): 178.46 (2-C), 165.52 (CH_3-CH_2-$\underline{C}$OO-), 146.49 (6-C), 140.71 (C-1'), 140.32 (C-1"), 133.98 (C-4'), 129.03-127.83 ($9C_{arom}$), 106.56 (5-C), 60.76 (CH_3-$\underline{C}H_2$-COO-), 53.61 (4-C), 18.66 ($\underline{C}H_3$- C_6=C_5-), 14.02 ($\underline{C}H_3$-CH_2-COO-); Anal. Found: C, 61.97; H, 4.26; N, 6.99; Calcd. for $C_{20}H_{19}ClN_2O_2S$: C, 62.09; H, 4.95; N, 7.24%.

4-(2-Chloro-phenyl)-6-methyl-1-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester, 4f :

Yield 86%; white crystalline solid; m.p. 142°-144°C; R_f value: 0.65 ($CHCl_3$); IR (KBr) (ν_{max}, cm^{-1}): 3486 (N-H), 3034 (arom. C-H stretching), 2927 (C-H stretching for – CH_3),1708 (C=O stretching), 1447 (C=S stretching), 1352 (-CH_3 bending), 1085 (C-Cl stretching), 823, 754 (arom. C-H bending); ^{1}H NMR δ (in ppm): 7.51 (s, 1H, NH, 3-H), 7.42–7.11 (m, $9H_{arom.}$), 5.91 (bs, 1H, 4-H), 4.04 (q, 2H, J=5.6, CH_3-C$\underline{H}_2$-COO-), 2.22 (bs, 3H, C$\underline{H}_3$-C_6=C_5-), 1.06 (t, 3H, J=5.8, C$\underline{H}_3$-CH_2-COO-); ^{13}C NMR δ (in ppm): 178.43 (2-H), 165.25 (CH_3-CH_2-$\underline{C}$OO-), 147.90 (6-C), 140.53 (C-1'), 138.01 (C-1"), 132.94 (C-2'),

130.20 -127.60 (9C_{arom}), 104.24 (5-C), 60.60 (CH_3-$\underline{C}H_2$-COO-), 51.57 (4-C), 18.55 ($\underline{C}H_3$- C_6=C_5-), 13.86 ($\underline{C}H_3$-CH_2-COO-); Anal. Found: C, 61.99; H, 4.77; N, 7.21; Calcd. for $C_{20}H_{19}ClN_2O_2S$: C, 62.09; H, 4.95; N, 7.24%.

In Vitro antimicrobial activity (Microorganisms and media) (CLSI 2012)

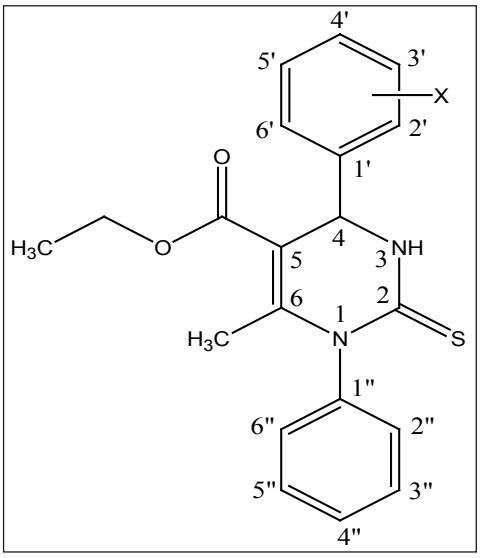
The test organisms used in this study were as follows: *Bacillus cereus* (ATCC 19637), *Staphylococcus aureus* (Coagulate), *Escherichia coli* (ATCC 20120829) and *Salmonella typhimerium* (JCM-1692). Routine culture was performed on Tryptone Soya Broth (pH 7.3±0.2 at 25°C) in a required number of inoculation tube. 5 falcon tubes containing 5 ml nutrient rich media each was inoculated through sterilized loop tip with each isolated bacteria strain. The incubation of the strain for the antimicrobial test was executed in an aerobic chamber at 37°C in an incubator for 24 hours. After that the growth was checked by a cloudy haze in the media. Test sample were prepared in DMSO with concentration 1 mg/ml.

Results and discussion

Initially we studied a model reaction employing benzaldehyde, ethyl acetoacetate and 1-phenyl thiourea in the presence of four different molar ratios of (2, 5, 10 and 15 mol%) in order to investigate the catalytic efficiency of $Ni(NO_3)_2.6H_2O$ to establish the optimum quantity of this catalyst and our study has revealed that 10 mol% of the catalyst as the optimum ratio. The structures of the compounds 4a-f were confirmed with the help of their IR, ^{1}H-NMR and ^{13}C-NMR spectral data and elemental analyses.

The IR spectral data of the compounds 4a-f shows the wave number. The functional groups of the compounds have been confirmed by the peaks. For example, peaks for N-H stretching were found in between 3180-3486 cm^{-1}, C=O stretching of esters were found in between 1701-1710 cm^{-1}, C=C in conjugation with C=O were found in between 1631-1635 cm^{-1}, C=S stretching were found in between 1442-1492 cm^{-1} and C-Cl were found in 1082-1085 cm^{-1}.

The ^{1}H-NMR spectral data shows the presence of all the protons attached to carbons in the compounds, 4a-f. A slight variation in the positions of N-H protons were observed at δ 7.51-8.37 ppm may be due the change in the substituents on the benzene ring from *para* position to *ortho* position. The methoxy groups, in which the methyl group being attached to an electronegative oxygen atom were slightly deshielded with a chemical shift δ 3.79-3.90 ppm than the methyl group (δ 2.46 ppm). Two other methyl groups were distinguished from

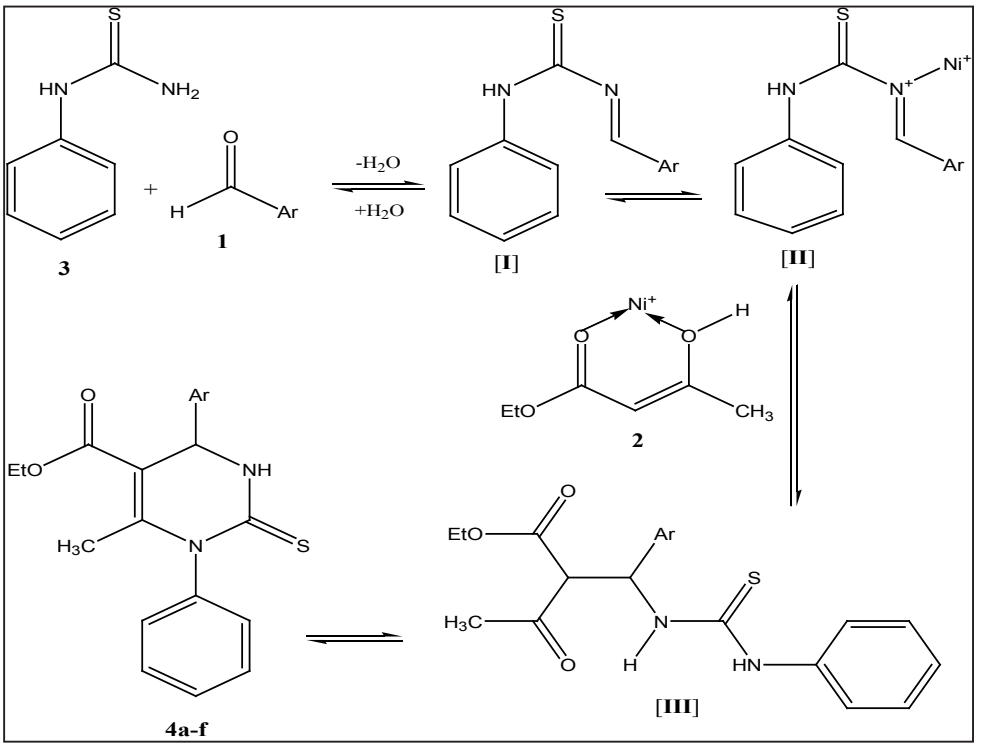


4a-f

each other by their attachment to neighbouring atoms. Methyl protons attached to an alkene carbons gave peaks at δ2.09-2.22 ppm and another methyl protons attached to an ester and a methylene group gave peaks in a shielded region at δ1.05-1.20 ppm. The methylene protons being attached to a methyl in one side and an ester on the other side further deshielded due to direct link with one oxygen of ester group and gave peaks at δ 4.02-4.13 ppm. The proton at position 4 appeared as a doublet (or broad singlet) due to the vicinal coupling with the proton at position 3. The chemical shifts were observed at δ 5.40-5.91 ppm. All aromatic protons have shown peaks in their expected positions.

The structures of the compounds, 4a-f were further confirmed with their ^{13}C-NMR spectra. The 2-C carbons being attached to an electronegative sulphur atom on one side and nitrogen atom on other side gave peaks at a highly deshielded region (δ 177.70-178.46 ppm). The ester carbons attached to ethyl group were deshielded and gave peaks at δ165.25-165.78 ppm. The methyne carbons at 4-C gave a peak at δ51.57-55.56 ppm. One of the alkene carbons (5-C) gave peak at δ104.24-107.10 ppm and the other one (6-C) at δ145.76-147.90 ppm. The substituents in the benzene ring (-OCH_3 and -CH_3) showed slight variations in their peaks due to their attachment with an electronegative oxygen atom, resulting the methoxy carbon (δ 49.36-53.80 ppm) more deshielded than the methyl carbon (δ 19.13-21.09 ppm). The methyl and methylene carbons attached to ester gave peaks at δ13.86-14.08 ppm and δ 60.60-60.48 ppm respectively. The methyl carbons attached to 6-C gave peaks at 18.55-18.66 ppm. All aromatic carbons have shown peaks in their expected positions.

Although different mechanistic pathways have been proposed previously (Tamaddon *et al*., 2010; Murata *et al*., 2010; Nagarajaiah *et al*., 2016; Murthy *et al*., 2016), we believe that the reaction may proceed through an initially formed imine intermediate [I] from the reaction of the aldehyde, 1 and 1-phenyl thiourea, 3 (Scheme-2). The co-ordination of the lone pair of the nitrogen atom with the Lewis acid could lead to the *in situ* formation of iminum ion [II] which is sufficient electrophile to react with the enol form of ethyl acetoacetate, 2 affording the open chain intermediate [III]. Finally, intramolecular cyclization with loss of H_2O molecule, producing the 3, 4-dihydropyrimidin-2(*1H*) – thiones, 4a-f.



4a-f

Ar: 4-$CH_3C_6H_4$-, 4-$OCH_3C_6H_4$-, 2-$CH_3C_6H_4$-, 2-$OCH_3C_6H_4$-, 4-ClC_6H_4- & 2-ClC_6H_4-

Scheme-2: The probable reaction pathway of the compounds, 4a-f

Microbial test for the compounds, 4a-f

Susceptibility tests were performed by the disk diffusion method of Bauer *et. al.* Zone of inhibition were measured after 24 h of incubation. Commercial antibiotic (Nalidixic acid) was used as controls. Bacteria were transferred directly to the prepared petry plate (containing 20 ml Tryptone Soya Agar, pH 7.4 in each plate) with a wet swab containing the bacterial broth culture. The synthetic compounds impregnated disks were placed on the surface of the agar, using forceps. In each 100 mm plate 5 disks were placed for individual kind of bacteria. All plates then incubated at 37°C overnight. Zone of inhibition were measured on the underside of the plates with a metric ruler. Five zone diameters for particular compounds were records on different plates. The disk potency and their zone of inhibition were summarized in the following table (Table-I):

Compounds 4a, 4c-d and 4f showed strong (18 mm Z.D.) inhibitory activity for *Bacillus cereus* compared with Nalidixic acid (15 mm). Compounds 4b and 4e showed intermediate inhibitory activity for *Bacillus cereus*. Compounds 4a-f were found to be resistant for strain of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimerium*.

Table I. Antimicrobial Test Data For Compounds, 4a-f

Compound	Disk potency µg/mL	Zone of inhibition in mm			
		S. aureus	*E. coli*	*S. typhimerium*	*B. cereus*
4a	30	6	6	6	18
4b	30	6	6	6	17
4c	30	6	6	6	18
4d	30	6	6	6	18
4e	30	6	6	6	17
4f	30	6	6	6	18
Standard control	30	19	19	20	15

Table-II. Summerized Data of Antimicrobial Properties of Compounds, 4a-f

Compound	*S. aureus*	*E. coli*	*S. typhimerium*	*B. cereus*
4a	Resistant	Resistant	Resistant	Susceptible
4b	Resistant	Resistant	Resistant	Intermediate
4c	Resistant	Resistant	Resistant	Susceptible
4d	Resistant	Resistant	Resistant	Susceptible
4e	Resistant	Resistant	Resistant	Intermediate
4f	Resistant	Resistant	Resistant	Susceptible

Conclusion

With the aim for the development of environmentally friendly new technique, we have successfully developed an easy, high yielding and versatile protocol for the synthesis of 1-phenyl-3, 4-dihydropyrimidin-2(1*H*)-thiones from the reaction of aryl aldehydes, ethyl acetoacetate, 1-phenyl thiourea catalyzed by Nickel nitrate hexahydrate. As per literature review, this is a new and simple technique of high atom economy with good yields. Most of the synthesized compounds showed good to moderate antimictrobial activity against *Bacillus cereus*. Kirby Bauer disc-diffusion test demonstrated the result for the 30 µg concentration of particular compound, 4a-f are summarized in (Table-II).

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