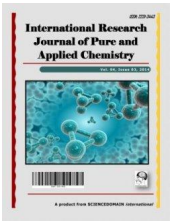

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Investigation of the H_3PO_4-Promoted Reaction of Benzaldehydes, Ethyl Acetoacetate or Ethyl Cyanoacetate or Malononitrile in the Presence of Urea as a Route to Pyrimidin-2-ones and 2-Iminochromenes

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Authors' contributions

This work was carried out in collaboration between all authors. Author IBM designed the study, participated in data analysis and wrote the first draft of the manuscript. Author NK preformed the laboratory experiments, acquired and analysed the spectral data and participated in the editing of the manuscript. Author RRM participated in spectral data analysis and editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The H_3PO_4-promoted reactions of benzaldehydes, active methylene compounds and urea are reported. Benzaldehydes, ethyl acetoacetate and urea react in the presence of H_3PO_4 to give substituted pyrimidin-2-ones in 49-84% yields. The three-component reaction failed when either ethyl cyanoacetate or malononitrile were used instead of ethyl acetoacetate and only the aldol products were formed. However, the reaction of salicylaldehydes and ethyl cyanoacetate or malononitrile gave 2-iminochromenes in moderate to high yields. Some of the prepared pyrimidin-2-ones exhibited weak antibacterial and antifungal activities. The 3-acetoxy-2-iminochromenes showed moderate antimicrobial activities whereas the 3-cyano-2-iminochromenes were inactive against all the test organisms.

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Keywords: Benzaldehyde; pyrimidin-2-one; Biginelli reaction; phosphoric acid; 2-iminochromene.

1. INTRODUCTION

The recent report of the creation of a living bacterium that can replicate unnatural DNA bases by scientists at the Scripts Research Institute [1] has ignited huge interest in the facile synthesis of nitrogen-containing heterocyclic compounds. Among these nitrogen heterocyclic compounds, substituted pyrimidin-2-ones are the most promising as their structure is close to that of natural base tyrosine. Classically, pyrimidin-2-ones are prepared by the HCl-catalysed three component reaction of aldehydes, ethyl acetoacetate and urea in ethanol [2-7]. Several modifications of this reaction known as the Biginelli reaction have been reported. Both Bronsted and Lewis acids have been used as catalysts in the Biginelli reaction [8-13]. Among the Bronsted acids, H3PO4 has featured very sparely in the catalysis of this reaction.

As part of our broad project of synthesis of heterocyclic compounds [14-17] and use of phosphoric acid in organic synthesis, [18,19] we report the H3PO4-promoted reaction of benzaldehydes, ethyl acetoacetate and urea as a facile route to pyrimidin-2-ones. Two other active methylene compounds, ethyl cyanoacetate and malononitrile were found to undergo a reaction with salicylaldehydes in the presence of H3PO4 to give 2-iminochromene derivatives.

2. EXPERIMENTAL

2.1 Materials and Methods

Laboratory grade chemicals and solvents were procured from Sigma-Aldrich and used without any further purification. Reactions were monitored by TLC using Merck's TLC Silica gel 60 F254 aluminium sheets. Melting point measurements were determined on a Stuart melting point apparatus and are uncorrected. Infrared spectra were recorded neat on a Perkins Elmer FT-IR spectrophotometer 1000. High resolution mass spectra were recorded on a GCT Premier mass spectrometer (Waters) with an ionization energy of 70 eV. NMR spectra were recorded on a Bruker Avance DPX 300 MHz NMR spectrometer with TMS as an internal standard. UV experiments were performed on a Shimadzu UV-2101 PC UV-Vis Scanning spectrophotometer.

General procedure for the synthesis of 4-arylpyrimidin-2-one derivatives 4a-l

A mixture of benzaldehyde (2.91 mmol), ethyl acetoacetate (3.11 mmol), urea (3.42 mmol) and phosphoric acid (1 mL) were taken into a round bottom flask (25 mL) with THF (4 mL) as a solvent and refluxed for 24 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured over about 25 mL of ice, stirred and the solid product was collected by filtration. The product was dried and recrystallised in ethanol to obtain the pure product.

Ethyl 6-methyl-4-phenyl-3,4-dihydropyrimidin-2-one-5-carboxylate (4a):

Yield 75 %, white crystal; Mp:206-207°C; IR (neat, v_{max}/cm^{-1}): 2900 (NH), 1698 (C=O), 1567(C=C). UV (λ_{max} / nm) 314; ^{1}H NMR (300MHz, DMSO) δ 1.10 (3H, *t*, *J* = 7.1 Hz, H-10), 2.25 (3H, *s* ,H-7), 3.99 (2H, *q*, *J* = 7.1 & 14.3 Hz, H-9), 5.15 (1H, *d*, *J* = 3.3 Hz, H-4), 7.23 (1H, *t*, *J* = 7.8 Hz, H-4´), 7.26 (2H, *m*, H-3´& 5´), 7.32 (2H, *m*, H-2´& 6´), 7.74 (1H, *d*, *J* = 2.6 Hz, NH), 9.18 (1H, *d*, *J* = 1.2 Hz, NH). ^{13}C NMR (DMSO) δ 14.0 (C-3´´), 17.1 (C-8), 53.9 (C-4), 59.2 (C-2´´), 99.3 (C-6), 126.2 (C-2´ & 6'), 127.3 (C-4´), 128.4 (C-3´ & 5'), 144.7 (C-1´), 148.3 (C-5), 152.1 (C-7), 165.3 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{16}N_2O_3$ (M^+) 260.4132; Found: 260.4129.

Ethyl 6-methyl-4-(4-methoxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4b):

Yield 70 %, white solid; Mp: 201-203 °C; IR (neat, v_{max}/cm^{-1}): 1724 (C=O), 1650 (C=C). UV (λ_{max} / nm) 324; ^{1}H NMR (300MHz, DMSO) δ 1.11 (3H, *t*, *J* = 7.1 Hz, H-10), 2.25 (3H, *s* , 3H, H-7), 3.99 (2H, *q*, *J* = 7.1; 14.3 Hz, H-9), 5.10 (1H, *d*, *J* = 3.3 Hz, H-4), 6.88(2H, *dd*, *J* = 2.1 & 8.7 Hz, H-2´ & 6´), 7.15 (2H, *dd*, *J* = 2.1; 8.7 Hz, H-3´ & 5´), 7.67 (1H, *t*, *J* = 2.4 Hz, NH), 9.15 (1H, *d*, *J* = 1.2 Hz, NH).^{13}C NMR (DMSO) δ 14.6 (C-10), 18.2 (C-7), 53.8 (C-4), 59.6 (C-9), 100.1 (C-6), 114.2 (C-3´ & 5´), 127.9 (C-2´ & 6'), 137.5 (C-1´), 148.5 (C-5), 152.6 (C-8), 158.9 (C-4´), 165.9 (C-2). HRMS (EI): *m/z* calcd for $C_{15}H_{18}N_2O_4$ (M^+) 274.4401; Found: 274.4398.

Ethyl 6-methyl-4-(4-hydroxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4c):

Yield 69 %, white solid; Mp: 218-220 °C; IR (neat, v_{max}/cm^{-1}): 2991, (NH), 1680 (C=O), 1647 (C=C). UV (λ_{max} / nm) 314; ^{1}H NMR (300MHz, DMSO) δ 1.11 (3H, *t*, *J* = 7.1 Hz, H-10), 2.27 (3H, *s* ,7-H), 3.98 (2H, *q*, *J* = 7.1; 14.3 Hz, H-9), 5.05 (1H, *d*, *J* = 3.0 Hz, H-4), 6.72 (2H, *dd*, *J* = 1.8 & 6.9 Hz, H-2´ & 6´), 7.03 (2H, *dd*, *J* = 2.0 & 6.8 Hz, H-3´ & 5´), 7.62 (1H, *t*, *J* = 2.7 Hz, NH), 9.11 (1H, *d*, *J* = 1.2 Hz, NH).^{13}C NMR (DMSO) δ 14.6 (C-10), 18.2 (C-7), 53.9 (C-4), 59.6 (C-9), 100.2 (C-6), 115.5 (C-3´ & C-5´), 127.9 (C-2´ & 6´), 135.9 (C-1´), 148.2 (C-5), 152.6 (C-8), 157.0 (C-4´), 165.9 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{16}N_2O_4$ (M^+) 276.4126; Found: 276.4130.

Ethyl 6-methyl-4-(3-hydroxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4d):

Yield 63 %, white solid; Mp: 128-129 °C; IR (neat, v_{max}/cm^{-1}): 2991, 2895 (NH), 1680 (C=O), 1641 (C=C). UV (λ_{max} / nm) 324; ^{1}H NMR (300MHz, DMSO) δ 1.13 (3H, *t*, *J* = 7.1 Hz, H-10), 2.25 (3H, *s* ,H-7), 4.00 (2H, *q*, *J* = 7.2 & 14.1 Hz, H-9), 5.07 (1H, *d*, *J* = 3.3 Hz, H-4), 6.63 (1H, *dd*, *J* = 2.6; 9.0 Hz, H-4´), 6.66 (1H, *d*, *J* = 7.8 Hz, H-6´), 6.69 (1H, *d*, *J* = 2.1 Hz, H-2´), 7.10 (1H, *t*, *J* = 8.0 Hz, H-5´), 7.69 (1H, *d*, *J* = 2.1Hz, NH), 9.16 (1H, *d*, *J* = 1.2 Hz, NH). ^{13}C NMR (DMSO) δ 14.6 (C-10), 18.2 (C-7), 54.3 (C-4), 59.7 (C-9), 99.9 (C-6), 113.6 (C-4´), 114.6 (C-2´), 117.4 (C-6´), 129.7 (C-5´), 146.7 (C-1´), 148.5 (C-5), 152.7 (C-8), 157.8 (C-3´),165.9 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{16}N_2O_4$ (M^+) 267.4126; Found: 267.4124

Ethyl 6-methyl-4-(2-hydroxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4e):

Yield 77 %, white crystal; Mp: 212-213 °C; IR (neat, v_{max}/cm^{-1}): 3352 (NH), 1670 (C=O), 1567(C=C). UV (λ_{max} / nm, DMSO) 307; ^{1}H NMR (300MHz, DMSO) δ 1.10 (3H, *t*, *J* = 8.0 Hz, H-10), 2.27 (3H, *s*, H-7), 3.95 (2H, *q*, *J* = 8.0 & 17.6 Hz, H-9), 5.46 (1H, *d*, *J* = 3.0 Hz, H-4), 6.72 (1H, *t*, *J* = 7.5 Hz, H-5´), 6.80 (1H, *d*, *J* = 7.8 Hz, H-3´), 6.97 (1H, *dd*, *J* = 3.0 & 7.5 Hz, H-6´), 7.05 (1H, *td*, *J* = 3.3; 7.6 Hz, H-4´), 9.08 (1H, *s*, NH), 9.62 (1H, *s*, NH). ^{13}C NMR (DMSO) δ 14.4 (C-10), 18.1 (C-7), 49.6 (C-4), 59.4 (C-9), 98.2 (C-6), 115.8 (C-3´), 119.2 (C-5´), 127.6 (C-6´), 128.7 (C-4´), 130.2 (C-1´), 149.0 (C-5), 152.7 (C-8), 155.1 (C-2´), 166.0 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{16}N_2O_4$ (M^+) 267.4126; Found: 267.4122

Ethyl 6-methyl-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4f):

Yield 56 %, white solid; Mp: 179-181 °C; IR (neat, v_{max}/cm^{-1}): 2991, 2895 (NH), 1697 (C=O). UV (λ_{max} / nm) 320; ^{1}H NMR (300MHz, DMSO) δ 1.11 (3H, *t*, *J* = 7.2; H-10), 2.25 (3H, *s*, H-7), 3.99 (2H, *q*, *J* = 7.2; 14.1 Hz,, H-9), 5.08 (1H, *d*, *J* = 3.0 Hz, H-4), 5.99 (2H, *d*, *J* = 0.9 Hz, H-2´´), 6.01 (2H, *d*, *J* = 0.9 Hz, H-1´´), 6.70 (1H, *dd*, *J* = 1.7; 8.0 Hz, H-6´), 6.75 (1H, *d*, *J* = 1.5 Hz, H-2´), 6.85 (1H, *d*, *J* = 7.8 Hz,, H-5´), 7.69 (1H, *d*, *J* = 2.6 Hz, NH), 9.18 (1H, *br s*, NH). ^{13}C NMR (DMSO) δ: 14.6 (C-10), 18.2 (C-7), 54.1 (C-4), 59.7 (C-9), 99.8 (C-6), 101.4 (C-1´´ & 2), 107.1 (C-5´), 108.5 (C-2´), 119.8 (C-6´), 139.3 (C-1´), 146.8 (C-3´), 147.7 (C-4´), 148.7 (C-5), 152.5 (C-8), 165.8 (C-2). HRMS (EI): *m/z* calcd for $C_{15}H_{16}N_2O_5$ (M^+) 304.2979; Found: 304.2983.

Ethyl 6-methyl-4-(2-hydroxy-5-methoxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4g):

Yield 84 %, white solid; Mp: 199-200 °C; IR (neat, v_{max}/cm^{-1}): 3233, 2991, 2895 (NH), 1703 (C=O), 1698 (C=C). UV (λ_{max} / nm) 310; ^{1}H NMR (300MHz, DMSO) δ 1.56 (3H, *t*, *J* = 6.3 Hz, H-10), 2.84 (3H, *s* ,H-7), 4.11 (3H, *br s*, 5´-OC<u>H</u>$_3$), 4.47 (2H, *br s*, H-9), 6.03 (1H, *br s*, H-4), 7.06 (1H, *br s*, H-6´), 7.17 (1H, *dd*, *J* = 2.1; 8.4 Hz, H-4´), 7.28 (1H, *d*, *J* = 6.0 Hz, H-3´), 9.53 (1H, *br s*, NH).^{13}C NMR (DMSO) δ: 14.4 (C-10), 18.1 (C-7), 50.3 (C-4), 59.7 (C-9), 98.6 (C-6), 113.1 (C-4´), 113.8 (C-3´), 116.8 (C-6´), 131.3 (C-1´), 149.3 (C-5), 149.6 (C-5´), 153.2 (C-8), 153.4 (C-2´), 166.3 (C-2). HRMS (EI): *m/z* calcd for $C_{15}H_{18}N_2O_5$ (M^+) 306.3138; Found: 306.3141.

Ethyl 6-methyl-4-(2-hydroxy-3-methoxyphenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4h):

Yield 80 %, white solid; Mp: 212-213 °C; IR (neat; v_{max}/cm^{-1}): 3240, 2998 (NH), 1682 (C=O), 1645 (C=C). UV (λ_{max} / nm) 315; ^{1}H NMR (300MHz, DMSO) δ 1.05 (3H, *t*, *J* = 7.1 Hz, H-10), 2.27 (3H, *s*, H-7), 3.79 (3H, *s*, 3´-O<u>C</u>H$_3$), 3.93 (2H, *q*, *J* = 2.0; 7.1 Hz, H-9), 5.52 (1H, *d*, *J* = 2.7 Hz, H-4), 6.62 (1H, *t*, *J* = 6.6 Hz, H-6´), 6.70 (1H, *t*, *J* = 8.0 Hz, H-5´), 6.85 (1H, *d*, *J* = 7.8 Hz, H-4´), 8.74 (1H, *s*, NH), 9.09 (1H, *s*, NH). ^{13}C NMR (DMSO) δ: 14.5 (C-10), 18.2 (C-7), 49.3 (C-4), 59.5 (C-29), 98.6 (C-6), 111.3 (C-4´), 119.0 (C-5´), 119.4 (C-6´), 131.0 (C-1´), 144.0 (C-3´),148.0 (C-2´), 148.9 (C-5), 152.7 (C-8),

165.9 (C-2). HRMS (EI): *m/z* calcd for $C_{15}H_{18}N_2O_5$ (M^+) 306.3138; Found: 306.3135.

Ethyl 6-methyl-4-(2-chlorophenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4i):

Yield 60 %, white solid; Mp: 219-220 °C; IR (neat, v_{max}/cm^{-1}): 3389, (NH), 1701 (C=O), 1644 (C=C). UV (λ_{max} / nm) 311; ^{1}H NMR (300MHz, DMSO) δ 1.00 (1H, *t*, *J* = 7.1 Hz, H-10), 2.31 (3H, *s*,H-7), 3.90 (2H δ 1.05 (3H, *t*, *J* = 7.1 Hz, H-10), 2.27 (3H, *s*, H-7), 3.79 (3H, *s*, 3´-O$\underline{C}H_3$), 3.93 (2H, *q*, *J* = 2.0; 7.1 Hz, H-9), 5.52 (1H, *d*, *J* = 2.7 Hz, H-4), 6.62 (1H, *t*, *J* = 6.6 Hz, H-6´), 6.70 (1H, *t*, *J* = 8.0 Hz, H-5´), 6.85 (1H, *d*, *J* = 7.8 Hz, H-4´), 8.74 (1H, *s*, NH), 9.09 (1H, *s*, NH). ^{13}C NMR (DMSO) δ: 14.5 (C-10), 18.2 (C-7), 49.3 (C-4), 59.5 (C-29), 98.6 (C-6), 111.3 (C-4´), 119.0 (C-5´), 119.4 (C-6´), 131.0 (C-1´), 144.0 (C-3´), 148.0 (C-2´), 148.9 (C-5), 152.7 (C-8), 165.9 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{15}ClN_2O_3$ (M^+) 294.7335; Found: 294.7339.

Ethyl 6-methyl-4-(4-chlorophenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4j):

Yield 58 %, white solid; Mp: 193-194 °C; IR (neat, v_{max}/cm^{-1}): 3390, 2991, (NH), 1700 (C=O), 1645 (C=C). UV (λ_{max} / nm) 320; ^{1}H NMR (300MHz, DMSO) δ 1.10 (3H, *t*, *J* = 7.1 Hz, H-10), 3.99 (2H, *q*, *J* = 7.2; 14.1 Hz, H-9), 2.26 (3H, *s*, 7-H), 5.15 (1H, *d*, *J* = 3.3 Hz, H-4), 7.26 (2H, *dd*, *J* = 1.8; 8.4 Hz, H-3´ & 5´), 7.40 (2H, *dd*, *J* = 2.0; 8.4 Hz, H-2´ & 6´), 7.71 (1H, *br s*, NH), 9.28 (1H, *br s*, NH). ^{13}C NMR (DMSO) δ: 14.5 (C-10), 18.3 (C-7), 53.9 (C-4), 59.7 (C-9), 99.3 (C-6), 128.7 (C-3´ & 5´), 128.9 (C-2´ & 6´), 132.3 (C-1´), 144.4 (C-4´), 149.2 (C-5), 152.4 (C-8), 165.7 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{15}ClN_2O_3$ (M^+) 294.7335; Found: 294.7338.

Ethyl 6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4k):

Yield 59 %, white solid; Mp: 228-229 °C; IR (neat, v_{max}/cm^{-1}): 3433, 2991 (NH), 1707 (C=O), 1687 (C=C). UV (λ_{max} / nm, DMSO) 336; ^{1}H NMR (300MHz, DMSO) δ 1.10 (3H, *t*, *J* = 7.1 Hz, H-10), 2.28 (3H, *s*, H-7), 4.00 (2H, *q*, *J* = 6.9; 14.1 Hz, H-9), 5.32 (1H, *d*, *J* = 3.6 Hz, H-4), 7.67 (2H, *m*, H-5´& 6´), 7.91 (1H, *t*, *J* = 2.6 Hz, NH), 8.09 (1H, *t*, *J* = 1.8 Hz, H-2´), 8.14 (1H, *dt*, *J* = 2.0; 7.8 Hz,, H-4´), 9.37 (1H, *d*, *J* = 1.5 Hz, NH).^{13}C NMR (DMSO) δ: 14.5 (C-10), 18.3 (C-7), 54.0 (C-4), 59.9 (C-9), 98.9 (C-6), 121.5 (C-4´), 122.8 (C-6´), 130.7 (C-5´), 133.5 (C-2´), 148.2 (C-3´), 147.4 (C-1´), 149.9 (C-5), 152.3 (C-8), 165.6 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{15}N_3O_5$ (M^+) 305.2860; Found 305.2863.

Ethyl 6-methyl-4-(4-nitrophenyl)-3,4-dihydropyrimidin-2-one-5-carboxylate (4l):

Yield 49 %, white solid; Mp: 211-212 °C; IR (neat, v_{max}/cm^{-1}): 3300, 2993 (NH), 1726 (C=O), 1646 (C=C). UV (λ_{max} / nm, DMSO) 325; ^{1}H NMR (300MHz, DMSO) δ 1.10 (3H, *t*, *J* = 7.1 Hz, H-10), 2.28 (3H, *s*, 7-CH_3), 4.00 (2H, *q*, *J* = 7.2; 14.1 Hz, H-9), 5.29 (1H, *d*, *J* = 3.3 Hz, H-4), 7.52 (2H, *dd*, *J* = 1.8; 6.9 Hz, H-3´ & 5´), 7.90 (1H, *br s*, NH), 8.23 (2H, *dd*, *J* = 1.8; 6.9 Hz, H-2´ & 6´), 9.36 (1H, *br s*, NH). ^{13}C NMR (DMSO) δ: 14.5 (C-10), 18.3 (C-7), 54.2 (C-4), 59.9 (C-9), 98.7 (C-6), 124.3 (C-3´ & 5´), 128.1 (C-2´), 128.2 (C-6´), 147.2 (C-1´), 149.8 (C-5), 152.2 (C-8), 152.5 (C-4´), 165.5 (C-2). HRMS (EI): *m/z* calcd for $C_{14}H_{15}N_3O_5$ (M^+) 305.2860; Found 305.2867.

General procedure for the synthesis of 3-acetoxy-2-iminochromene derivatives 7

A mixture of benzaldehyde (2.3 mmol), ethyl cyanoacetate (2.3 mmol) and phosphoric acid (1 mL) were taken into a round bottom flask (25 mL) with THF (4 mL) as a solvent and refluxed for 24 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured over about 25 mL of ice, stirred and the solid product was collected by filtration. The crude product was subjected to preparative TLC eluting with PE/EtOAc (8:2) to obtain 3-acetoxy-2-iminochromene **7**.

3-Acetoxy-2-iminochromene (7e):

Yield 44 %, yellow powder; Mp: 179-180 °C; IR (neat, v_{max}/cm^{-1}): 2918 (NH), 1724 (C=O), 1603 (C=C). UV (λ_{max} / nm, $CHCl_3$) 334; ^{1}H NMR (300MHz, DMSO) δ 1.32 (3H, *t* ,*J* = 7.2 Hz, H-11), 4.30 (2H, *q*,*J* = 7.2; 14.1 Hz, H-10), 7.43 (2H, *dd*, *J* = 3.0; 7.8 Hz, H-6&8), 7.74 (1H, *td*, *J* = 1.7; 8.7 Hz,, H-7), 7.92 (1H, *dd*, *J* = 1.4; 7.8 Hz, H-5), 8.75 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 14.5 (C-11), 61.7 (C-10), 118.2 (C-3), 116.6 (C-8), 118.3 (C-4a), 125.3 (C-6), 135.0 (C-7), 130.7 (C-5), 149.1 (C-4), 155.0 (C-8a), 156.5 (C-2), 163.1 (C-9). HRMS (EI): *m/z* calcd for $C_{12}H_{11}NO_3$ (M^+) 217.2206; Found: 217.2211.

3-Acetoxy-6-methoxy-2-iminochromene (7g):

Yield 51 %, yellow powder; Mp: 130-131 °C; IR (neat, v_{max}/cm^{-1}): 1753 (C=O), 1606 (C=C). UV

(λ_{max} / nm, $CHCl_3$) 364; ^{1}H NMR (300MHz, DMSO) δ 1.46 (3H, *t* , *J* = 7.1 Hz, H-11), 4.20 (2H, *q*, *J* = 7.2; 14.1 Hz, H-10), 7.05 (1H, *d*, *J* = 2.7 Hz, H-7), 7.33 (1H, *br s*, H-5), 7.92 (1H, *d*, *J* = 2.7 Hz, H-8), 8.53 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 14.5 (C-11), 61.7 (C-10), 116.6 (C-8), 118.2 (C-3), 118.3 (C-4a), 125.3 (C-6), 130.7 (C-5), 135.0 (C-7), 149.1 (C-4), 155.0 (C-8a), 156.5 (C-2), 163.1 (C-9). HRMS (EI): *m/z* calcd for $C_{13}H_{13}NO_4$ (M^+) 247.2466; Found: 247.2470.

3-Acetoxy-8-methoxy-2-iminochromene (7h):

Yield 53 %, yellow powder; Mp: 74-76 °C; IR (neat, v_{max}/cm^{-1}): 3352 (NH), 1670 (C=O), 1567(C=C). UV (λ_{max} / nm, $CHCl_3$) 306; ^{1}H NMR (300MHz, DMSO) δ 1.41 (1H, *t* , *J* = 7.1 Hz, H-11), 4.41 (*q*, *J* = 7.2; 14.4 Hz, 2H, H-10), 7.17 (1H, *d*, *J* = 1.8 Hz, H-7), 7.20 (1H, *br s*, H-5), 7.29 (1H, *dd*, *J* = 3.0; 10.1 Hz,, H-6), 8.51 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 14.5 (C-11), 56.8 (8-O<u>C</u>H$_3$), 61.7 (C-10), 116.6 (C-8), 118.2 (C-3), 149.1 (C-4), 118.3 (C-4a), 125.3 (C-6), 130.7 (C-5), 135.0 (C-7), 155.0 (C-8a), 156.5 (C-2), 163.1 (C-9). HRMS (EI): *m/z* calcd for $C_{13}H_{13}NO_4$ (M^+) 247.2466; Found: 247.2468.

General procedure for the synthesis of 3-cyano-2-iminochromene derivatives 10

A mixture of benzaldehyde (2.4 mmol), malononitrile (2.6 mmol) and phosphoric acid (1 mL) in THF (4 mL) were refluxed for 24 hours in a round bottom flask (25 mL). The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured over 25 g of ice, stirred and the solid product was collected by filtration. The product was dried and recrystallized in ethanol/ acetone (1:1) to obtain pure product.

3-Cyano-2-iminochromene (10e):

Yield 94 %, yellow powder; Mp:156-158 °C; IR (neat, v_{max}/cm^{-1}): 1724 (C=O), 1603 (C=C). UV (λ_{max} / nm, $CHCl_3$) 320; ^{1}H NMR (300MHz, DMSO) δ 7.47 (1H, *d*, *J* = 7.5 Hz, H-6), 7.52 (1H, *d*, *J* = 7.5 Hz, H-8), 7.80 (1H, *dd*, *J* = 1.5; 7.5 Hz, H-7), 7.83 (1H, *d*, *J* = 7.5 Hz, H-5), 8.96 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 102.6 (C-3), 115.0 (C-9), 117.3 (C-8), 118.0 (C-4a), 125.9 (C-6), 130.5 (C-5), 135.9 (C-7), 153.9 (C-4), 154.5 (C-8a), 157.3 (C-2). HRMS (EI): *m/z* calcd for $C_{10}H_6N_2O$ (M^+) 170.1674; Found: 170.1669.

3-Cyano-6-methoxy-2-iminochromene (10g):

Yield 82 %, yellow powder; Mp:229-231 °C; IR (neat, v_{max}/cm^{-1}): 1724 (C=O), 1604 (C=C). UV (λ_{max} / nm, $CHCl_3$) 358; ^{1}H NMR (300MHz, DMSO) δ 3.83 (3H, *s*, 6-OC<u>H</u>$_3$), 7.34 (1H, *d*, *J* = 3.0 Hz, H-5), 7.40 (1H, *dd*, *J* = 3.0; 9.0 Hz, H-7), 7.46 (1H, *d*, *J* = 9.3 Hz, H-8), 8.85 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 56.4 (6-O<u>C</u>H$_3$), 102.8 (C-3), 111.9 (C-7), 115.1 (C-9), 118.3 (C-4a), 118.5 (C-8), 123.7 (C-5), 149.0 (C-6), 153.6 (C-4), 156.5 (C-8a), 157.3 (C-2). HRMS (EI): *m/z* calcd for $C_{11}H_8N_2O_2$ (M^+) 200.1934; Found: 200.1941.

3-Cyano-8-methoxy-2-iminochromene (10h):

Yield 83 %, yellow powder; Mp:232-235 °C; IR (neat, v_{max}/cm^{-1}): 1723 (C=O), 1604 (C=C). UV (λ_{max} / nm, $CHCl_3$) 308; ^{1}H NMR (300MHz, DMSO) δ 3.94 (3H, *s*, 8-OC<u>H</u>$_3$), 7.35 (1H, *dd*, *J* = 1.5; 7.8 Hz, H-5), 7.41 (1H, *t*, *J* = 8.1 Hz, H-6), 7.50 (1H, *dd*, *J* = 1.7; 8.0 Hz, H-7), 8.94 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 56.8 (8-O<u>C</u>H$_3$), 102.8 (C-3), 115.0 (C-9), 118.0 (C-7), 118.5 (C-4a), 121.3 (C-6), 125.9 (C-5), 143.8 (C-8), 147.0 (C-8a), 154.2 (C-4), 157.1 (C-2). HRMS (EI): *m/z* calcd for $C_{11}H_8N_2O_2$ (M^+) 200.1934; Found: 200.1929.

3-Cyano-7-hydroxy-2-iminochromene (10m):

Yield 80 %, yellow powder; Mp:246-248 °C; IR (neat, v_{max}/cm^{-1}): 3000 (NH), 1723 (C=O),1608 (C=C). UV (λ_{max} / nm, $CHCl_3$) 378; ^{1}H NMR (300MHz, DMSO) δ 6.71 (1H, *dd*, *J* = 2.3; 8.6 Hz, H-6), 6.80 (1H, *d*, *J* = 2.1 Hz, H-8), 7.66 (1H, *d*, *J* = 8.7 Hz, H-5), 8.79 (1H, *s*, H-4). ^{13}C NMR (DMSO) δ: 96.5 (C-3), 103.0 (C-8), 110.8 (C-4a), 115.1 (C-6), 115.7 (C-9), 132.3 (C-5), 153.8 (C-4), 157.2 (C-2), 158.1 (C-8a), 165.4 (C-7). HRMS (ES): *m/z* calcd for $C_{10}H_6N_2O_2$ (M^+) 186.1668; Found: 186.1674.

2.2 General Procedure for Biological Tests

The micro-organisms were maintained on nutrient agar. Fresh broth media were prepared and were inoculated with the micro-organisms using sterilized nichrone wire, and incubated for 24 hours. The test organisms` cultures were transferred (2 mL) into fresh agar media and thoroughly mixed. The TLC alumunium sheets, pre-spotted with 10 µg of each compound in dimethylsulfoxide (DMSO) at concentrations of 50, 10, 0.5, 0.1 and 0.01 µg/mL, were then overlaid with the agar media inoculated with different microorganism's culture and incubated

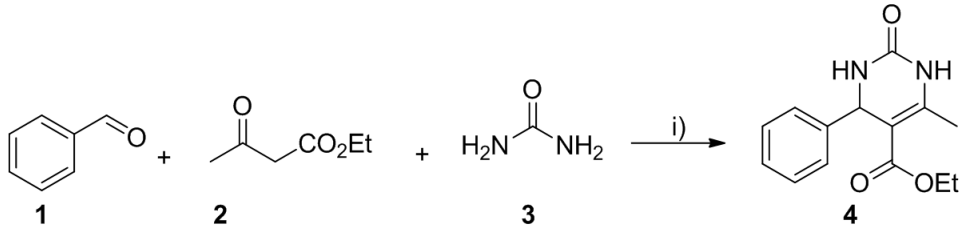
for 24 hours at 37°C. The TLC bioautograms were sprayed with an aqueous solution of thiazoyl blue (methylthiazolyltetrazolium bromide (MTTB); 200 mg in 100 mL distilled water) and further incubated for 4 hours after which results were scored. The inhibition zones were observed as white spots against a purple background and the lowest loading quantity to exhibit an inhibition zone was taken as the Minimum Inhibitory Quantity (MIQ).

3. RESULTS AND DISCUSSION

On the basis of some precedents, [20,21] the three component reaction was initial attempted in ethanol as the solvent. Thus, a solution of benzaldehyde **1a**, ethyl acetoacetate **2** and urea **3** in ethanol was treated with H_3PO_4 and refluxed to give a complex mixture. After successive purification by flash column chromatography, pyrimidinone **4a** was isolated in 31% yield, Scheme 1.

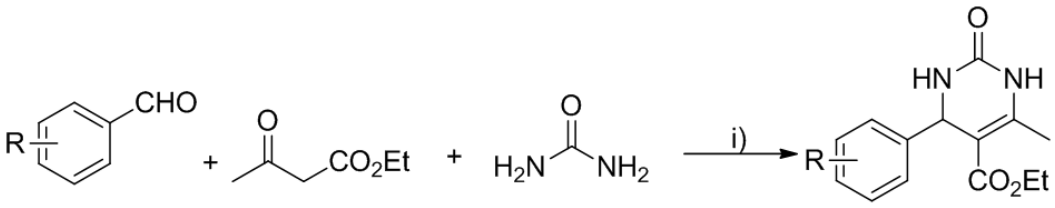
Suspecting that the protic and nucleophilic ethanol was responsible for the side products, an aprotic solvent THF was used instead and pyrimidinone **4a** was isolated in 75% yield (Scheme 2, entry 1). Next, the substrate scope of the H_3PO_4-promoted three component reaction was investigated by using benzaldehyde derivatives with electron-donating groups. Thus, replacing benzaldehyde **1a** with its derivatives **1b**-**1h** gave the corresponding pyrimidinones **4b**-**4h** in yields of 56-84% (Scheme 2, entries 2-8). The lowest yields were achieved when the benzaldehydes had electron-donating substituents at C-3 and C-4.

Further substrate scope investigations for the three component reaction involving benzaldehyde derivatives, **1i**-**1l**, ethyl acetoacetate **2** and urea **3** in THF in the presence of H_3PO_4 furnished pyrimidinones **4i**-**4l** in 49-60% yields, Scheme 3. It is important to note that the starting benzaldehydes in this case



1 **2** **3** **4**

Scheme 1: Reagents and conditions: i) H_3PO_4, EtOH, reflux, 24 h



1a-h **2** **3** **4a-h**

Entry	Aldehyde	R	Yield of **4** (%)
1	**1a**	H	75
2	**1b**	4-OCH_3	70
3	**1c**	4-OH	69
4	**1d**	3-OH	63
5	**1e**	2-OH	77
6	**1f**	3,4-$O_2C(CH_3)_2$	56
7	**1g**	2-OH, 5-OCH_3	84
8	**1h**	2-OH, 3-OCH_3	80

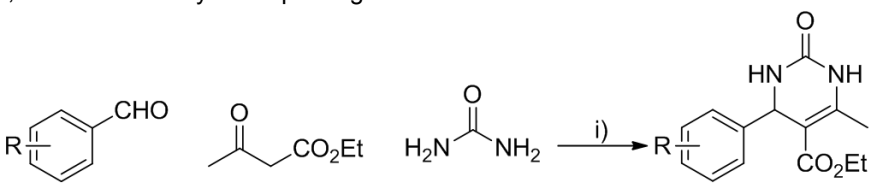
Scheme 2. Reagents and conditions: i) H_3PO_4, THF, reflux, 24 h

had electron-withdrawing chloro and nitro groups. These substrates were tolerated by the procedure but with reduced yields when compared to those with electron donating groups in Scheme 2.

In a parallel sequence of reactions the H_3PO_4-promoted three-component reaction was attempted using either ethyl cyanoacetate **5** or malononitrile **8** instead of **2**. Thus, when ethyl cyanoacetate **5** was used instead of **2**, the three component reaction failed and aldol product **6** was isolated in 95% yield, Scheme 4.

However, the reaction of salicylaldehyde **1e** and ethyl cyanoacetate **5** in the presence of H_3PO_4 gave 3-acetoxy-2-iminochromene **7e** in a low yield of 44%, Scheme 4 entry 1. Repeating this reaction in the presence of urea **3** still gave **7e** in comparable yield. Salicylaldehyde derivatives, **1g** and **1h** also reacted with ethyl cyanoacetate **5** to give 3-acetoxy-2-iminochromenes, **7g** and **7h** respectively in moderate yields, Scheme 5, entries 2 and 3. The low yields are attributed to the cyclisation reaction involving the ester functionality instead of the cyano group to give coumarins of type **8** which do not crystallize under the work-up conditions.

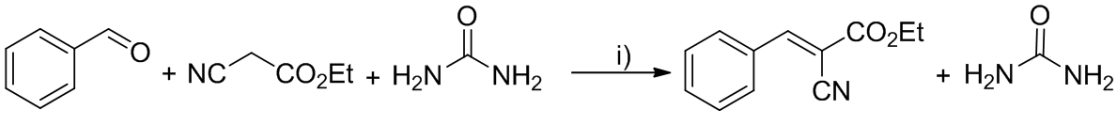
Gratifyingly, when malononitrile **9** was refluxed with salicylaldehyde derivatives **1e**, **1g**, **1h** and **1m**, the corresponding 3-cyano-2-iminochromenes of type **10** were prepared in acceptable yields of 80-83%, Scheme 6.



1i-K **2** **3** **4i-K**

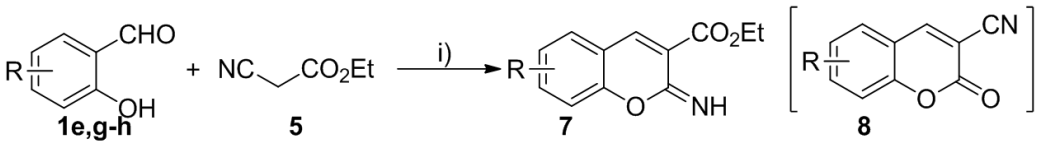
Entry	Aldehyde	R	Yield of 4 (%)
1	**1i**	2-Cl	60
2	**1j**	4-Cl	58
3	**1k**	3-NO_2	59
4.	**1l**	4-NO_2	49

Scheme 3. Reagents and conditions: i) H_3PO_4, THF, reflux, 24 h.



1a **5** **3** **6** **3**

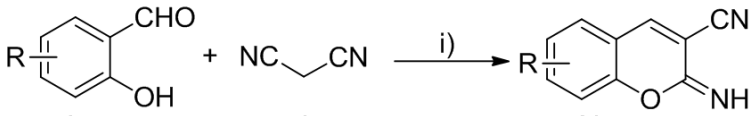
Scheme 4. Reagents and conditions: i) H_3PO_4, THF, reflux, 24 h



1e,g-h **5** **7** **8**

Entry	Aldehyde	R	Yield of 7 (%)
1	**1e**	H	44
2	**1g**	5-OCH_3	51
3	**1h**	3-OCH_3	53

Scheme 5: Reagents and conditions: i) H_3PO_4, THF, reflux, 24 h



1 **9** **10**

Entry	Aldehyde	R	Yield of 10
1	**1e**	H	94
2	**1g**	5-OCH_3	82
3	**1h**	3-OCH_3	83
4	**1m**	4-OH	80

Scheme 6: Reagents and conditions: i) H_3PO_4, THF, reflux, 24 h

Table 1. Antimicrobial activities of pyrimidin-2-ones and 2-iminochromenes

Compound no.	Minimum Inhibitory Quantity (MIQ, µg)				
	P. aeruginosa	***E. coli***	***S. aureus***	***B. subtilis***	***C. albican***
4a	10	na	50	na	10
4f	10	na	na	na	na
4i	na	na	50	na	50
4j	na	na	50	na	50
4k	na	na	50	na	na
4l	na	na	50	na	na
7e	10	10	10	50	na
7g	10	10	10	50	na
7h	10	10	10	50	na
Chloramphenicol	0.01	0.01	0.01	0.01	-
Miconazole	-	-	-	-	0.01

Note: na = no activity up to 100 µg

The synthesized compounds were screened *in vitro* for their antibacterial activities against Gram positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis*, Gram negative bacteria; *Psydomonas aeruginosa* and *Escherichia coli* and for their antifungal activities against *Candida albicans* using the agar overlay technique [22]. The data obtained is summarized in Table 1. Pyrimidin-2-ones **4i-k** exhibited weak activities against *S. aureus* and *C. albicans* with MIQ values of 50 µg, compounds **4a** and **4f** were active against *P. aeruginosa* with MIQ values of 10 µg. The 3-acetoxy-2-iminochromenes **7e,g-h** showed weak activities against all test organisms except for *C. albicans* whereas the 3-cyano-2-iminochromens **10e,g-h** and **10m** did not show any activity against all test organisms. While the antibacterial and antifungal activities of the prepared compounds are not at the levels of the positive standards chloramphenicol for bacteria and miconazole for fungi, their specificities for particular organisms make them interesting. Further work in collaboration with biologists will definitely involve the determination of the mechanism of action for the active compounds. All the other prepared compounds that are not listed in Table 1 did not show any activities at 100 µg against any of the test organisms.

4. CONCLUSION

The H_3PO_4-mediated reactions of benzaldehydes and active methylene compounds have proved to be reliable routes to the synthesis of heterocyclic compounds. While the H_3PO_4-promoted three-component reaction of benzaldehyde, ethyl acetoacetate and urea gave substituted pyrimidin-2-ones, the reactions of salicylaldehydes and ethyl cyanoacetate or malononitrile gave 2-iminochromenes in moderate to high yields. The prepared ethyl 2-iminochromene-3-carboxylates showed moderate antibacterial activities against both gram negative and gram positive bacteria but were inactive against the fungus. Pyrimidin-2-one **4a** showed promising antifungal activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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