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Facile eco-friendly synthesis of novel chromeno[4,3-b]pyridine-2,5-diones and evaluation of their antimicrobial and antioxidant properties

SATYANARAYANA REDDY JAGGAVARAPU[a], ANAND SOLOMON KAMALAKARAN[a], VENTKATA PRASAD JALLI[a], SRAVAN KUMAR GANGISETTY[a], MUNUSSWAMY RAMANUJAM GANESH[b] and GOPIKRISHNA GADDAMANUGU[a,*]

[a]Department of Chemistry, Sankar Foundation Research Institute, Naiduthota, Vishakapatnam 530 047, India
[b]ISISM, SRM university, Kattangulathur, 603 203, India
e-mail: gkrishnagps@gmail.com

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Abstract. Rapid and facileaccess to novel chromeno[4,3-b]pyridine-2,5-dione derivatives was achieved by a mild base catalysed reaction of 4-chloro-3-formylcoumarin and acetoacetamides in PEG-300 as recyclable solvent. The compounds were evaluated for their antimicrobial activities against 3 Gram-positive and 3 Gram-negative bacteria (*Staphylococcus epidermis*, *Vibrio parahaemolyticus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*) with Cefotaxime control. They were further subjected to antioxidant studies using DPPH test with ascorbic acid control. While compounds **5d** and **5k** showed promising broad spectrum antibacterial properties against all the evaluated bacteria, compound **5g** exhibited good antioxidant properties.

Keywords. Chromeno[4,3-b]pyridine-2,5-diones; acetoacetamide; PEG-300; antimicrobial; antioxidant.

1. Introduction

Coumarins and 2-pyridones are classic heterocyclic scaffolds which constitute vital substructures of several natural products and received enormous admiration for their wide range of applications. Secondary metabolites and synthetic intermediates of 2-pyridone scaffolds demonstrate broad spectrum of synthetic, material and biological applications.[1] Ricinine[2] with its remarkable CNS stimulant activity was the first isolated 2-pyridone natural product followed by the discovery of analogous antibiotic natural products such as elfamycin,[3] ilicolicin[4] and efratomycin.[5] With excellent vasodilating properties,[6] synthetic 2-pyridone analogues milrinone and amrinone are extensively used for the treatment of acute congestive heart failure. Similarly, L-697,661 was identified as a specific non-nucleoside reverse transcriptase inhibitor which demonstrates efficient anti-HIV properties.[7] 2-Pyridone is an integral core of several alkaloids[8] and is a key structural intermediate in the bacterial metabolism.[9] Krawczyk *et al*. have developed 2-pyridones as novel class of multi-drug resistant modulators.[10] On the other hand, coumarins[11] exhibit excellent antioxidant,[12] antibacterial,[13] antirhinovirus,[14] cytotoxic,[15] anticancer,[16] antimicrobial,[17] and antihypertensive properties.[18] Aminocoumarins such as novobiocin, clorobiocin and coumermycin A1 with nitrogen functional group attached to the coumarin ring are elegant class of antibiotics which inhibit DNA gyrase enzyme involved in cell division of bacteria.[19]

Fused heterocyclic scaffolds with nitrogen and oxygen atoms are fundamental to the medicinal chemistry for the development of several new drugs. To date, very few reports are available in the literature regarding the synthesis of fused chromeno[4,3-b]pyridine-2,5-dione scaffolds. One of the earliest documented report by Soliman *et al*. demonstrates synthesis of these scaffolds by the reaction of 4-hydroxycoumarin and aminocrotonitrile.[20] In a multistep protocol, Heber *et al*. reported the reaction of alkylaminocoumarin-3-carbaldehydes with Wittig ylides to achieve the required targets, albeit in poor yields.[21] Ivanov *et al*. reported Erlenmeyer–Ploechl reaction of alkylaminocoumarin-3-carbaldehydes with *N*-acetylgylcine derivatives to yield the chromeno[4,3-b]pyridine-2,5-dione derivatives.[22] However, the method suffers from the limitations of harsh reaction conditions of refluxing acetic acid and limited to the synthesis of only *N*-alkyl derivatives in poor yields. Similarly, alternative synthesis of these fused systems reported by Kafka *et al*. using camphoranils with

*For correspondence

excess of dimethylmalonate suffer from poor yields.[23] Owing to the necessity for an improved methodology for the synthesis of these fused scaffolds and prevalence of impressive biological properties of both pyridine-2-ones and coumarins, we were interested in developing a mild synthetic protocol for the synthesis of the fused chromeno[4,3-b]pyridine-2,5-dione scaffolds.

2. Experimental

The solvents, bases and other general reagents were of AR grade and purchased from Otto Chemie. ^{1}H and ^{13}C NMR spectra are recorded on 400 MHz Bruker Biospin FT-NMR spectrometer with CDCl3 as solvent and TMS as internal standard. Melting points were determined using NETZSCH DSC 200 instrument. IR spectra were recorded over Bruker a alpha-T spectrophotometer. Mass spectral analysis was carried out using EIMStechniques. Elemental analysis was performed on Elementar Vario EL III CHNS analyzer. Analytical thin-layer chromatography (TLC) was performed on 0.2 mm precoated plate Kieselgel 60 F254 (Merck).

2.1 *General procedure for synthesis of acetoacetamides derivatives (**3a–p**)*

To a stirred solution of amine (0.01 mol) in 10 ml of PEG-300 was added to ethylacetoacatate (0.03 mol) in 100 ml round bottom flask and refluxed at 120 °C for 1.5–2 h. After completion of the reaction (monitored by TLC) the reaction mixture was cooled and extracted with cold diethyl ether (3 × 10 mL) and purified by column chromatography (10–25% EtOAc in Hexane) gave the pure product **3a**–**p**. Final products were confirmed with the reported literature.[24]

2.2 *General method for the synthesis of 3-acetyl-1-phenyl-1H-chromeno[4,3-b]pyridine-2,5-dione **5a–p***

To a solution of 4-chloro-3-formylcoumarin **1a** (0.5 mmol) in PEG-300 (2 mL) was added acetoacetamides **3a** (0.5 mmol) and triethylamine (0.5 mmol) then stirred at 25°C for 15 min. The precipitate obtained was filtered by Whatman filter paper, washed with water and dried. Similar procedure was employed in case of substrates **5b**–**p**.

2.2a *3-Acetyl-1-phenyl-1H-chromeno[4,3-b]pyridine-2, 5-dione (**5a**)*: Whitesolid, mp 153–154°C; [Found: C, 72.42; H, 3.92; N, 4.22. $C_{20}H_{13}NO_4$ requires C, 72.50; H 3.95; N, 4.23%]; ν_{max}(KBr) 1719, 1702, 1679, 1618, 1583, 1479, 1418, 1240, 1041 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.73 (1H, s), 7.72 (1H, d, *J* 6.8 Hz), 7.56–7.50 (4H, m), 7.44–7.43 (2H, m), 7.30 (1H, d, *J* 6.8 Hz), 7.28–7.25 (1 H, m), 2.71 (3H, s); δ_C (100 MHz, $CDCl_3$) *δ*: 203.42, 159.84, 159.20,, 152.57, 145.68, 138.61, 137.14, 133.53, 129.78, 129.64,127.02, 126.07,125.98, 125.13, 118.77, 114.50, 103.35, 31.41, 8.57.; LCMS: MH^+, 332.

2.2b *3-Acetyl-1-(4-fluorophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5b**)*: White solid, mp 134–135°C; [Found: C, 68.70; H, 3.43; N, 4.00. $C_{20}H_{12}FNO_4$ requires C, 68.77; H, 3.46; N, 4.01%]; ν_{max}(KBr) 1721, 1705, 1680, 1621, 1579, 1469, 1421, 1245, 1048 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.69 (1H, s), 7.70 (1H, dd, *J* 7.6 Hz, *J* 1.2Hz), 7.58 (1H, td, *J* 7.6 Hz, *J* 1.2 Hz), 7.49–7.40 (2H, m), 7.32 (1H, dd, *J* 6.8 Hz, *J* 0.8 Hz), 7.30–7.20 (3H, m), 2.70 (3 H, s); δ_C (100 MHz $CDCl_3$) 203.25, 163.87, 161.87, 159.83, 159.10, 152.54, 145.52, 137.22, 134.49, 133.63, 128.17, 127.01, 125.97, 125.18, 118.80, 116.60, 114.41, 103.51, 31.39; LCMS: MH^+, 350.

2.2c *3-Acetyl-1-(3-fluorophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5c**)*: White solid, mp 138–139 °C; [Found: C, 68.70; H, 3.43; N, 4.00. $C_{20}H_{12}FNO_4$ requires C, 68.77; H, 3.46; N, 4.01%]; ν_{max}(KBr) 1721, 1705, 1683, 1621, 1587, 1488, 1421, 1243, 1021 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.70 (1H, s), 7.71 (1H, d, *J* 6.8 Hz), 7.59–7.51 (2H, m), 7.31 (1H, d, *J* 6.8 Hz), 7.30–7.26 (4H, m), 2.70 (3H, s); δ_C (100 MHz $CDCl_3$) 203.18,163.59, 161.60, 159.58, 159.03, 152.54, 145.28, 139.66, 139.58, 137.23, 133.69, 131.05, 130.98, m127.02, 126.07, 125.20, 121.91, 118.80, 117.13, 116.96, 114.35, 114.33, 114.13, 103.65, 31.39; LCMS: MH^+, 350.

2.2d *3-Acetyl-1-(4-bromophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5d**)*: White solid, mp 138–139°C; [Found: C, 55.80; H, 2.88. $C_{18}H_{11}BrO_5$ requires C, 55.84; H, 2.86%]; ν_{max}(KBr) 1721, 1705, 1682, 1621, 1577, 1472, 1410, 1233, 1033 cm^{-1}; δ_H (400 MHz $CDCl_3$) 7.83–7.77 (2H, m), 7.59–7.51(2H, m), 7.45 (1H, d, *J* 8.8 Hz), 7.32 (1H, t, *J* 7.6 Hz), 7.21 (1H, d, *J* 8.4 Hz), 6.80 (1H, d, *J* 8.8 Hz), 2.10 (3H, s); δ_C (100 MHz $CDCl_3$)203.14, 159.54, 158.96, 145.18, 137.42, 137.11, 133.60, 132.74, 127.71, 126.91, 125.88, 125.13, 123.83, 118.71, 114.29, 103.55, 45.79, 31.33, 8.56; LCMS: MH^+, 411.

2.2e *3-Acetyl-1-(3-bromophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5e**)*: White solid, mp 132–133°C; [Found: C, 58.56; H, 2.95, N, 3.41. $C_{20}H_{12}BrNO_4$ requires C, 58.50; H, 2.92, N, 3.41%]; ν_{max}(KBr) 1723, 1705, 1683, 1620, 1533, 1480, 1420, 1245, 1033 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.68 (1H, s), 7.76–7.70 (3H, m), 7.57 (1H, td, *J* 6.8 Hz, 0.8 Hz), 7.49–7.38 (3H, m), 7.33 (1H, d, *J* 6.8 Hz), 7.28 (1H, dd, *J* 6.8 Hz, 0.8 Hz), 2.70 (3H, s); δ_C (100 MHz $CDCl_3$) 203.09, 159.55, 158.58, 152.51, 145.20, 139.48, 137.22, 133.67, 132.95, 130.82, 129.37, 127.00, 126.01, 125.18, 124.90, 122.90, 118.77, 114.32, 103.64, 58.30, 31.37, 18.33; LCMS: MH^+, 411.

2.2f *3-Acetyl-1-(4-chlorophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5f**)*: White solid, mp 130–131°C; [Found: C, 65.61; H, 3.28, N, 3.82. $C_{19}H_{14}O_5$ requires C, 65.67; H, 3.31, N, 3.83%]; ν_{max}(KBr) 1723, 1706, 1683, 1630, 1579, 1481, 1420, 1238, 1033 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.67 (1H, s), 7.68 (1H, d, *J* 6.8 Hz), 7.61–7.46 (3H, m), 7.40 (1H, d, *J* 7.2 Hz), 7.30 (1H, d, *J* 6.4 Hz), 7.25 (1H, t, J 6.0 Hz), 2.68 (3H, s); δ_C (100 MHz $CDCl_3$) 203.18, 159.65, 159.02, 152.52, 145.28, 137.17, 136.95, 135.85, 133.65, 129.81, 127.48, 126.98, 125.96, 125.18, 118.77, 114.36, 103.59, 60.32, 31.38, 14.13; LCMS: MH^+, 366.

2.2g *3-Acetyl-1-p-tolyl-1H-chromeno[4,3-b]pyridine-2,5-dione (**5g**)*: White solid, mp 132–133°C; [Found: C, 73.03; H, 4.38, N, 4.06 $C_{20}H_{16}O_6$requires C, 72.96; H, 4.38, N, 4.05%]; ν_{max}(KBr) 1722, 1705, 1683, 1621, 1579, 1480, 1420, 1230, 1039 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.70 (1H, s), 7.70 (1H, d, *J* 6.4 Hz), 7.55 (1H, t, *J* 5.6 Hz), 7.33–7.23 (6H, m), 2.69 (3H, s), 2.42 (3H, s); δ_C (100 MHz $CDCl_3$) 203.45, 159.86, 159.15, 152.44, 145.75, 139.91, 136.92, 136.02, 133.37, 130.07, 126.90, 125.69, 125.00, 118.64, 114.44, 103.10, 31.33, 21.08, 18.26; LCMS: MH^+, 346.

2.2h *3-Acetyl-1-m-tolyl-1H-chromeno[4,3-b]pyridine-2,5-dione (**5h**)*: White solid, mp 138–139°C; [Found: C, 73.03; H, 4.38, N, 4.06 $C_{20}H_{16}O_6$requires C, 72.96; H, 4.38, N, 4.05%]; ν_{max}(KBr) 1721, 1705, 1682, 1623, 1579, 1482, 1420, 1241, 1039 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.70 (1H, s)), 7.70 (1H, s, J 6.4 Hz), 7.55 (1H, t, *J* 5.6 Hz), 7.33–7.23 (6H, m), 2.69 (3H, s), 2.42 (3H, s); δ_C (100 MHz $CDCl_3$) 203.51, 159.95, 159.30, 152.63, 145.79, 139.99, 138.58, 137.15, 133.53, 130.59, 129.48, 127.07, 126.62, 125.99, 125.16, 123.07, 118.82, 114.58, 103.27, 31.46, 21.30; LCMS: MH^+, 346.

2.2i *3-Acetyl-1-(4-methoxyphenyl)-1H-chromeno[4, 3-b]pyridine-2,5-dione (**5i**)*: White solid, mp 230–232°C; [Found: C, 58.22; H, 2.69; N, 3.75. $C_{21}H_{15}NO_5$ requires C, 58.23; H, 2.71, N, 3.77%]; ν_{max}(KBr) 1722, 1708, 1683, 1620, 1579, 1480, 1420, 1239, 1039 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.70 (1H, s), 7.70 (1H, d, *J* 6.8 Hz), 7.55 (1H, t, *J* 6.8 Hz), 7.35–7.22 (4H, m), 7.02 (1H, d, *J* 7.2 Hz), 3.86 (3H, s), 2.70 (3H, s); δ_C (100 MHz $CDCl_3$)) 203.50, 160.30, 160.02, 159.22, 152.51, 145.87, 136.97, 133.42, 131.29, 127.20, 125.06, 118.70, 114.70, 103.13, 55,59, 31.38; LCMS: MH^+, 362.

2.2j *3-Acetyl-1-(3-methoxyphenyl)-1H-chromeno [4,3-b]pyridine-2,5-dione (**5j**)*: White solid, mp 133–135°C; [Found: C, 69.73; H, 4.15; N, 3.87. $C_{21}H_{15}NO_5$ requires C, 69.80; H, 4.18, N, 3.88%]; ν_{max}(KBr) 1723, 1707, 1688, 1620, 1579, 1481, 1420, 1240, 1041 $cm^{-1;}$ δ_H (400 MHz $CDCl_3$) 8.71 (1H, s), 7.70 (1H, d, *J* 6.4 Hz), 7.56 (1H, t, *J* 6.4 Hz), 7.44 (1H, t, *J* 6.4 Hz), 7.31 (1H, d, *J* 6.4 Hz), 7.39–7.21 (2H, m), 7.04 (1H, d, *J* 6.8 Hz), 7.00–6.96 (2H, m), 3.85 (3H, s), 2.71 (3H, s); δ_C (100 MHz $CDCl_3$) 203.44, 160.33, 159.15, 152.53, 145.70, 139.57, 137.05, 133.50, 130.40, 126.98, 125.93, 125.10, 118.09, 115.76, 114.47, 111.87, 103.22, 55.58, 31.40; LCMS: MH^+, 362.

2.2k *3-Acetyl-1-(3-nitrophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5k**)*: White solid, mp 134–136°C; [Found: C, 63.77; H, 3.28; N, 7.44. $C_{20}H_{12}N_2O_6$ requires C, 63.83; H, 3.21, N, 7.44%]; ν_{max}(KBr) 1725, 1706, 1681, 1620, 1579, 1477, 1420, 1238, 1038 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.71 (1H, s), 8.42–8.31 (2H, m), 7.83 (1H, d, *J* 6.4 Hz), 7.77 (1H, t, *J* 6.4 Hz), 7.70 (1H, d, *J* 6.4 Hz,), 7.59 (1H, t, *J* 6.0 Hz), 7.33 (1H, d, *J* 6.8 Hz), 7.28 (1H, t, *J* 6.0 Hz), 2.71 (3H, s); δ_C (100 MHz $CDCl_3$) 202.81, 159.50, 158.86, 152.64, 148.75, 144.65, 139.29, 137.65, 134.02, 132.32, 130.69, 127.16, 126.30, 125.39, 124.65, 121.87, 118.96, 114.27, 104.34, 31.44; LCMS: MH^+, 377.

2.2l *3-Acetyl-1-(2-nitrophenyl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5l**)*: White solid, mp 138–140°C; [Found: C, 62.12; H, 3.54; N, 3.81. $C_{19}H_{13}NO_7$ requires C, 62.13; H, 3.57, N, 3.81%]; ν_{max}(KBr) 1723, 1704, 1682, 1620, 1579, 1482, 1420, 1218, 1045 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.69 (1H, s), 8.23 (1H, d, *J* 6.4 Hz), 8.12 (1H, d, *J* 6.8 Hz), 7.82–7.71 (2H, m), 7.77 (1H, t, *J* 6.4 Hz), 7.70 (1H, t, *J* 6.4 Hz,), 7.59 (1H, t, *J* 6.0 Hz), 7.33–722 (2H, m), 2.69 (3H, s); δ_C (100 MHz $CDCl_3$) 160.12, 158.50, 152.57, 151.22,

144.21, 137.57, 132.38, 125.30, 125.01, 124.01, 120.32, 119.18, 118.12, 117.57, 103.77, 98.56, 91.40, 23.56, 21.07; LCMS: MH^+, 377.

2.2m *3-Acetyl-1-(pyridin-2-yl)-1H-chromeno[4,3-b] pyridine-2,5-dione (**5m**)*: white solid, mp 132–135°C; [Found: C, 68.61; H, 3.61 N, 8.42. $C_{19}H_{12}N_2O_4$ requires 68.67; H, 3.64 N, 8.43%]; ν_{max}(KBr) 1724, 1707, 1682, 1621, 1581, 1481, 1421, 1239, 1040 cm^{-1}; δ_H (400 MHz $CDCl_3$) δ 9.04 (1H, s), 8.69 (1H, s), 8.08 (1H, *t*, *J* 6.0 Hz), 7.93 (1H, d, *J* 5.4 Hz), 7.69–7.59 (3H, m), 7.41–7.36 (2H, m), 2.64 (3H, s); δ_C (100 MHz $CDCl_3$) 188.30, 186.10, 165.55, 161.27, 159.09, 154.79, 135.93, 135.47, 129.64, 125.86, 125.72, 125.45, 124.67, 117.46, 116.49, 114.45, 110.17; LCMS: MH^+, 333.

2.2n *3-Acetyl-1-butyl-1H-chromeno[4,3-b]pyridine-2,5-dione (**5n**)*: White solid, mp 138–141°C; [Found: C, 69.37; H, 5.46, N, 4.49. $C_{24}H_{16}O_5$ requires C, 69.44; H, 5.50, N, 4.50%]; ν_{max}(KBr) 1721, 1707, 1681, 1620, 1579, 1460, 1421, 1219, 1039 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.62 (1H, s), 7.66 (1H, d, *J* 6.0 Hz), 7.52 (1H, t, *J* 6.8 Hz), 7.29 (1H, d, *J* 6.4 Hz), 7.26–7.21 (1H, m), 4.08 (1H, t, *J* 6.0 Hz), 2.67 (3H, s), 1.82 (2H, q,), 1.42 (m, 2H), 0.98 (3H, t); δ_C (100 MHz $CDCl_3$) 203.75, 159.98, 159.31, 152.43, 145.34, 136.63, 133.20, 126.81, 125.13, 124.97, 118.64, 114.58, 102.67, 50.73, 31.42, 31.03, 19.76, 13.48; LCMS: MH^+, 312.

2.2o *3-Acetyl-1-ethyl-1H-chromeno[4,3-b]pyridine-2,5-dione (**5o**)*: White solid, mp 132–134°C; [Found: C, 67.77; H, 4.58, N, 4.94, $C_{16}H_{13}NO_4$ requires C, 67.84; H, 4.63, 4.94%]; ν_{max}(KBr) 1725, 1708, 1679, 1621, 1592, 1479, 1456, 1246, 1050 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.67 (1H, s), 7.67 (1H, dd, *J* 6.4 Hz, 0.8 Hz), 7.54 (1H, td, *J* 6.8, *J* 1.2 Hz), 7.31 (1H, dd, *J* 6.8 Hz, *J* 0.8 Hz), 7.24 (1H, td, *J* 6.8, *J* 0.8 Hz), 4.16 (2H, q, *J* 5.6 Hz), 2.68 (3H, s), 1.47 (3H, t, *J* 5.6 Hz); δ_C (100 MHz $CDCl_3$) 203.77, 159.86, 159.29, 152.42, 144.97, 136.66, 133.21, 126.80, 125.13, 124.98, 118.64, 114.57, 102.85, 46.05, 31.45, 14.39, 8.54; LCMS: MH^+, 284.

2.2p *3-Acetyl-1-benzyl-1H-chromeno[4,3-b]pyridine-2,5-dione (**5p**)*: White solid, mp 135–137°C; [Found: C, 72.97; H, 4.34; N, 4.05 $C_{21}H_{15}NO_4$ requires C, 73.03; H, 4.38, N, 4.06%]; ν_{max}(KBr) 1723, 1705, 1681, 1621, 1592, 1489, 1458, 1247, 1053 cm^{-1}; δ_H (400 MHz $CDCl_3$) 8.63 (1H, s), 7.66 (1H, d, *J* 6.0 Hz), 7.52 (1H, t, *J* 6.0 Hz), 7.44–7.31 (5H, m), 7.30–7.19 (2H, m), 5.24 (2H, s), 2.68 (3H, s); δ_C (100 MHz $CDCl_3$) 203.62, 160.09, 159.15, 152.46, 145.08, 136.77, 134.13, 133.34, 129.27, 128.96, 128.73, 126.88, 125.33, 125.04, 118.67, 114.54, 103.13, 53.22, 31.47; LCMS: MH^+, 346.

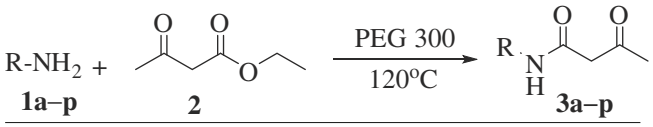
3. Results and discussions

Application of green and sustainable chemistry protocols has seen enormous surge in recent times for the development of novel and eco-friendly methodologies towards the synthesis of valuable synthetic scaffolds and drug intermediates. Polyethylene glycol (PEG) has gained wide popularity as alternative solvent in contributing to such green methodologies by successfully plummeting the generation of industrial waste.[25]

We have earlier demonstrated the utility of 4-chloro-3-formylcoumarin as a versatile starting material for the syntheses of various privileged heterocycles such as chromeno-quinolines,[26a] chromeno-benzazepines,[26b] chromeno-pyrimidine-N-oxides[26c] and chromeno-trioxabicylco[3,3,1]nonadienes.[26d] In continuation of our efforts to synthesize novel heterocyclic molecules of biological importance, we envisaged a mild base mediated synthesis of the target molecules from 4-chloro-3-formylcoumarin and acetoacetamides via simultaneous nucleophilic *N*-alkylation and Knoevenagel reactions using PEG-300 as a recyclable eco-friendly solvent.

The popular methodologies for the synthesis of acetoacetamides in general employ reaction of amines with excess of ethyl acetoacetate in solvents such as benzene, toluene or xylenes at reaction temperatures up to 140°C.[24] As an alternative eco-friendly procedure for the synthesis of starting materials **3a**–**p**, we explored the possibility of PEG-300 as a benign solvent for their reaction. We found that there are no reports utilizing PEG-300 as a solvent for the synthesis of the acetoacetamides to date. When the reaction of aniline **1a** was conducted in PEG-300 with 1 equivalent of ethyl acetoacetate, the product **3a** was isolated in moderate yields (60%) after 2 h of reaction at 120°C (table 1, entry 1). Increasing the quantity of ethyl acetoacetate to 2 equivalents resulted in good yields (81%) of the required acetoacetamide **3a** (table 1, entry 2). The present optimized reaction conditions successfully circumvented the utilization of large excess of ethyl acetoacetate unlike the earlier reported methodologies. As shown in table 1, the reaction conditions were further exploited for

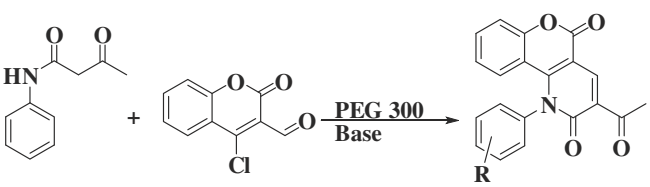
Table 1. Synthesis of acetoacetamides with diverse amines and ethyl acetoacetate[a].



Entry	R	Time (h)	Yield[b] (%)
3a	Ph	2.0	60[c]
3a	Ph	2.0	81
3b	*p*-F-Ph	2.5	63
3c	*m*-F-Ph	3.0	60
3d	*p*-Br-Ph	2.5	66
3e	*m*-Br-Ph	3.0	64
3f	*p*-Cl-Ph	2.5	68
3g	*p*-CH_3-Ph	2.0	69
3h	*m*-CH_3-Ph	2.0	70
3i	*p*-OCH_3-Ph	2.0	73
3j	*m*-OCH_3-Ph	2.0	58
3k	*m*-NO_2-Ph	3.0	56
3l	*o*-NO_2-Ph	3.0	66
3m	2-Aminopyridine	2.0	66
3n	*n*-butyl	1.5	76
3o	*n*-ethyl	1.5	74
3p	*n*-benzyl	1.5	75

[a]All the reactions were performed on 0.01 mol scale in 10 ml solvent at 120°C
[b]Isolated yields
[c]One equivalent of ethyl acetoacetate

Table 2. Screening studies of reaction of acetoacetamide (**3a**) and 4-chloro-3-formylcoumarin (**4**) with various bases in PEG-300[a].



Entry	Base	Time	Yield[c] (%)
1	Et_3N	15 min	83[b]
2	K_2CO_3	6 h	50
3	$NaHCO_3$	6 h	60
4	NaOAc	10 h	NR[d]
5	DMAP	10 h	NR
6	DIEA	15 min	78
7	DBU	15 min	70

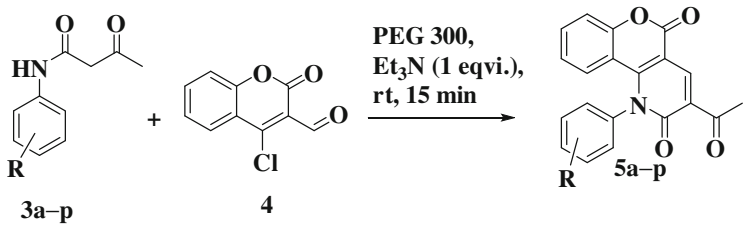
[a]All the reactions were performed on 0.5 mmol scale with 1 equiv of base in 2 ml solvent at room temperature
[b]Pure precipitated yield
[c]Isolated yields
[d]No reaction.

the synthesis of acetoacetamides **3b–p** (entries 2–16) successfully with a variety of functional groups in good yields (56–81%). The methodology worked satisfactorily for aryl, heteroaryl and aliphatic substrates.

To further optimize the reaction conditions for the synthesis of chromeno[4,3-b]pyridine-2,5-diones 5**a–p**, 4-choloro-3-formylcoumarin **4** and acetoacetanilide **3a** were chosen as model substrates. An initial reaction was attempted where **4** was reacted with acetoacetamide **3a** in PEG-300 employing triethylamine base. Completion of the reaction was indicated by the formation of a precipitate which could be easily filtered from the reaction mixture. To our satisfaction, we found that the product obtained was in good yield (83%) and of high purity (table 2, entry 1). To ascertain the efficiency of triethylamine, various bases as shown in table 2 were screened for the reaction. While bases such as K_2CO_3 and $NaHCO_3$ gave moderate yields (50–60%) of product **5a** after 6 h of reaction, surprisingly, sodium acetate and DMAP did not catalyse the reaction (table 2, entries 2–5). Competitive yields of **5a** were observed when DMAP (78%) and DBU (70%) were employed as bases (table 2, entries 6 and 7). Triethylamine was considered as the best base from the standardization of reaction conditions. PEG solvent could easily be recycled by washing with small quantities (5 ml) of diethyl ether. Our attempts to synthesize the target chromeno[4,3-b]pyridine-2,5-dione **3a** in one-pot procedure starting from aniline **1a** unfortunately did not yield satisfactory results.

To validate general feasibility of the methodology, variety of aromatic and aliphatic acetoacetamides **3b–p** were utilized in the reaction with 4-chloro-3-formylcoumarin under the optimized reaction conditions. As demonstrated in table 3, halogen substituted acetoacetamides **3b–f** possessing *p*-fluoro, *m*-flouro, *p*-bromo, *m*-bromo and *p*-chloro substituents reacted smoothly to afford the corresponding benzopyrano-2-pyridoneproducts **5b–f** in 78–82% yields. While electron donating substrates **3g–j** possessing methyl and methoxy substituents afforded slightly higher yields (84–90%) of the corresponding products **5g–j**, the electron withdrawing substrates *p*-NO_2 and *m*-NO_2 groups afforded 72 and 75% yields of products **5k** and **5l**, respectively. Heteroaromatic and aliphatic substrates **3m–p** also furnished good yields (72–84%) of coumarino[4,3-b]pyrid-2-one products **5m–p** under the optimized reaction conditions. The present recyclable eco-friendly methodology offers advantages of the obtaining pure precipitates of **5a–p** in good to excellent yields without requirement of tedious purification protocols.

Table 3. Synthesis of chromeno [4, 3-b] pyridine-2, 5-diones.



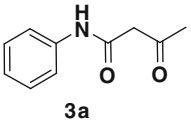
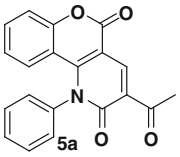
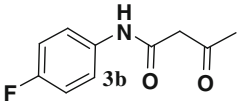
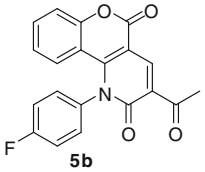
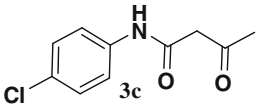
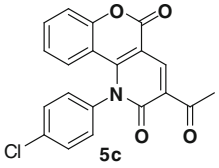
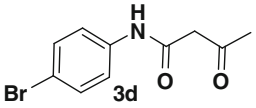
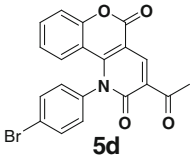
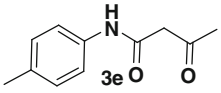
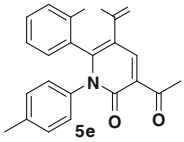
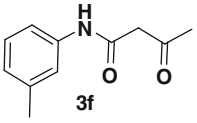
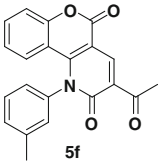
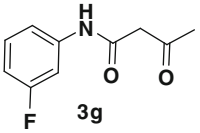
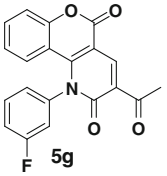
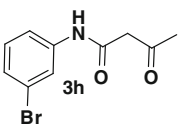
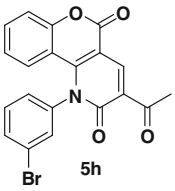
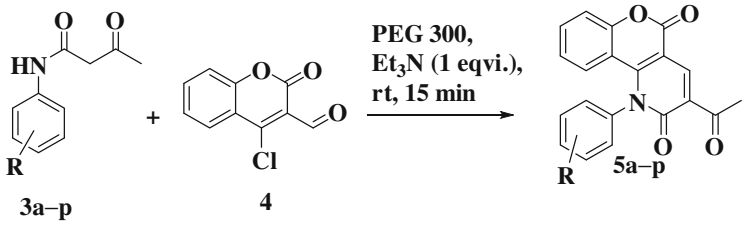
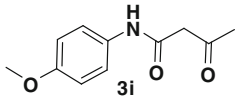
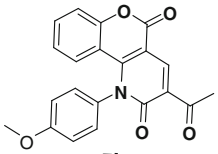
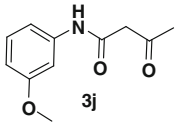
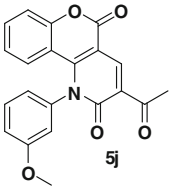
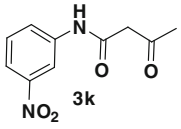
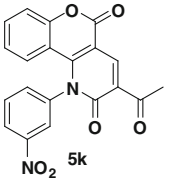
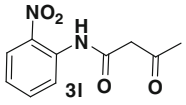
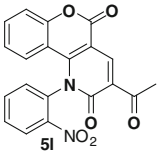
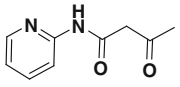
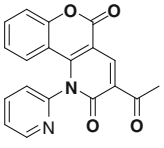
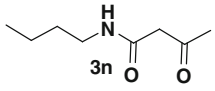
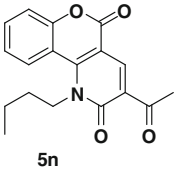
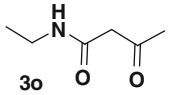
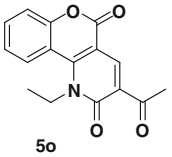
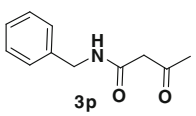
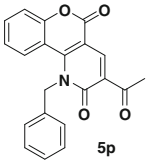
Entry	Acetoacetamide	Chromeno[4,3-b]pyridine-2,5-dione (**5a**–**n**)	Yield(%)[a]
1			83
2			79
3			80
4			82
5			78
6			80
7			82
8			87

Table 3. (continued)



Entry	Acetoacetamide	Chromeno[4,3-b]pyridine-2,5-dione (**5a–n**)	Yield(%)[a]
9	 **3i**	 **5i**	83
10	 **3j**	 **5j**	85
11	 **3k**	 **5k**	75
12	 **3l**	 **5l**	71
13	 **3m**	 **5m**	72
14	 **3n**	 **5n**	84
15	 **3o**	 **5o**	83
16	 **3p**	 **5p**	80

[a]Yields refer to the precipitated products

Table 4. Evaluation of antibacterial and antioxidant properties for **5a–p**.

Entry	*E. coli* (-ve)	*V. parahaemolyticus* (-ve)	*K. pneumoniae* (-ve)	*S. epidermidis* (+ve)	*B. subtilis* (+ve)	*S. aureus* (+ve)	DPPH inhibition (%)
5a	>200	>200	>200	>200	>200	>200	80.21
5b	>200	>200	>200	>200	>200	>200	26.23
5c	>200	>200	>200	>200	>200	>200	**42.41**
5d	**6.25**	**12.5**	**12.5**	**25**	**12.5**	**3.13**	39.50
5e	>200	>200	>200	>200	>200	>200	**55.48**
5f	>200	>200	>200	>200	>200	>200	NA
5g	>200	>200	>200	>200	>200	>200	**62.13**
5h	>200	>200	>200	>200	>200	>200	NA
5i	>200	>200	>200	>200	>200	>200	36.27
5j	**12.5**	**50**	**50**	**50**	**25**	**50**	16.39
5k	**12.5**	**6.25**	**12.5**	**3.13**	**6.25**	**3.13**	**49.34**
5l	>200	>200	>200	>200	>200	>200	NA
5m	>200	>200	>200	>200	>200	>200	29.44

For evaluation of antibacterial properties of the synthesized compounds **5a–p** (table 4), clinically active Gram-negative *Escherichia coli*, *Vibrio parahaemolyticus*, *Klebsiella pneumonia* and Gram-positive *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Staphylococcus aureus* bacteria were selected. Dimethyl sulfoxide was chosen as negative control while Cefotoxime was chosen as positive control due to its broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The concentration at which the compounds inhibited the visible growth of the microbial cultures was taken as MIC for that compound by using the standard protocol of NCCLS Broth Microdilution MIC method.[27] The MIC values listed in table 4 suggest that compounds **5d**, **5j**, **5k**, **5n** and **5o** showed promising broad spectrum antibacterial activities. More specifically, compounds **5d** and **5k** demonstrated maximum activity, where **5d** was more selective in inhibiting *Staphylococcus aureus* (3.13 μg/mL) and **5k** exhibits maximum activity against both *Staphylococcus aureus* and *Staphylococcus epidermidis* (3.13 μg/mL).

The evaluation of total antioxidant activities of the compounds **5a–p** were evaluated using free radical scavenging activity determined by 1,1-diphenyl picryl hydrzyl (DPPH) procedure.[28] The inhibition percentage equation of the radical scavenging the radical scavenging activity was calculated by using the equation

$$\text{Inhibition}\,(\%) = [(A_0 - A_S)/A_0]\,\text{X}100,$$

where A_0 is absorbance of the blank and A_S is absorbance of the sample at 515 nm. All assays were conducted in triplicate. Testing was performed with 180 μL of 0.004% methanolic solution of DPPH pipetted into each well of a 96-well plate followed by 20 μL (20 mg/ml) of sample or solvent for the blank. Ascorbic acid was used as the positive control. The mixture was incubated at 30°C for 1 h, and the absorbance at 515 nm was measured with a microplate reader. From the results of the screening studies displayed in table 4, it can be suggested that all the compounds except **5f**, **5h**, **5l** and **5p** exhibit moderate to good antioxidant properties. Among the analogs, compound **5g** displayed best antioxidant property with 62.13% inhibition followed by **5e**, **5k** and **5c** with 55.48%, 49.34% and 42.41% activities, respectively.

In summary, a series of novel chromeno[4,3] pyridine-2,5-diones **5a–p** were synthesized by a mild eco-friendly high yielding methodology using PEG as a recyclable solvent. The compounds were further subjected to antimicrobial and antioxidant screening where **5d** and **5k** displayed potent antimicrobial activities and compound **5g** displayed promising antioxidant properties (table 4).

Acknowledgements

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References

1. (a) Jones G 1996 In *Comprehensive heterocyclic chemistry II*: A Mckillop (ed.) Pergamon Press, Oxford, 167; (b) Torres M, Gil S and Para M 2005 *Curr. Org. Chem.* **9** 1757; (c) Cocco M T, Congiu C and Onnis V 2000 *Eur. J. Med. Chem.* **35** 545; (d) Litvinov V P 2003 *Russ. Chem. Rev.* **72** 69; (e) Murray T and Zimmerman S 1995 *Tetrahedron Lett.* **36** 7627; (f) Guarna A, Belle C, Machetti F, Occhiato E G and Payne A H 1997 *J. Med. Chem.* **40** 1112; (g) Li Q, Mitscher L A and Shen L L 2000 *Med. Res. Rev.* **20** 231; (h) Stončius S, Orentas E,

Butkus E, Öhrström L, Wendt O F and Wärnmark K 2006 *J. Am. Chem. Soc.* **128** 8272; (i) Sasaki T, Guerrero J M, Leonard A D and Tour J M 2008 *Nano Res.* **1** 412; (j) Mohler M L, Bohl C E, Jones A, Coss C C, Narayanan R, He Y, Hwang D J, Dalton J T and Miller D D 2009 *J. Med. Chem.* **52** 3597

2. Richard T V 1864 *J. Chem. Soc.* **17** 195
3. (a) Elbein A D and Molyneux R J 1981 In *Alkaloids Chemical Biological Perspectives*, S Williams Pelletier (ed.) (John Wiley) **5** 1; (b) Li Q, Mitscher L A and Shen L L 2000 *Med. Res. Rev.* **20** 231; (c) Nagarajan M, Xiao X S, Antony S, Kohlhagen G, Pommier Y and Cushman M 2003 *J. Med. Chem* **46** 5712
4. (a) Dolle, R E and Nicolaou K C 1985 *J. Am. Chem. Soc.* **107** 1691; (b) Cox R J and Hagan D 1991 *J. Chem. Soc. Perkin Trans.* **1** 2537
5. Gary D and Byron A K 1991 *J. Ind. Microbiol.* **8** 265
6. Presti E L, Boggia R, Feltrin A, Menozzi G, Dorigo P and Mosti L 1999 *II Farmaco* **54** 465
7. (a) Clercq, E D 1999 *II Farmaco* **54** 26; (b) Parreira R L T, Abrahão O and Galembeck S E 2001 *Tetrahedron* **57** 3243
8. Jessen H J and Gademann K 2010 *Nat. Prod. Rep.* **27** 1168
9. Kaiser J P, Feng Y and Bollag J M 1996 *Microbiol. Rev.* **60** 483
10. Krawczyk S, Otto M, Otto A, Coburger C, Krug M, Seifert M, Tell V, Molnár J and Hilgeroth A 2011 *Bioorg. Med. Chem.* **19** 6309
11. (a) Jang K H, Lee B H, Choi B W, Lee H S and Shin J 2005 *J. Nat. Prod.* **68** 716; (b) Malquichagua S K J, Delgado P G E, Lluncor L R, Young M C M and Kato M J 2005 *Phytochemistry* **66** 573; (c) Burkhardt G, Becker H, Grubert M T J and Eicher T 1994 *Phytochemistry* **37** 1593; (d) Numata A, Kanbara S, Takahashi C, Fujiki R, Yoneda M, Usami Y and Fujita E 1992 *Phytochemistry* **31** 1209; (e) Kulkarni M M, Nagasampagi B A, Deshpande S G and Sharma R N 1987 *Phytochemistry* **26** 2969; (f) Isman M B and Proksch P 1985 *Phytochemistry* **24** 1949; (g) Kitamura R O S, Romoff P, Young M C M, Kato M J and Lago J H G 2006 *Phytochemistry* **67** 2398
12. (a) Shanthi G, Perumal P T, Rao U and Sehgal P K 2009 *Indian J. Chem. Sec. B* **48** 1319; (b) Foroumadi A, Dehghan G, Samzadeh K A, Arabsorkhi F, Sorkhi M, Shafiee A and Abdollahi M 2007 *Asian J. Chem.* **19** 1391
13. (a) Kumar D, Reddy V B, Sharad S, Dube U and Kapur S 2009 *Eur. J. Med. Chem.* **44** 3805; (b) El-Saghier A M M, Naili M B, Rammash B K, Saleh N A and Kreddan K M 2007 *Arkivoc.* **16** 83
14. Conti C and Desideri N 2009 *Bioorg. Med. Chem.* **17** 3720
15. Alizadeh B H, Ostad S N, Foroumadi A, Amini M, Dowlatabadi R, Navidpour L and Shafiee A 2008 *Arkivoc* **13** 45
16. (a) Gourdeau H, Leblond L, Hamelin B, Desputeau C, Dong K, Kianicka I, Custeau D, Boudreau C, Geerts L, Cai S X, Drewe J, Labrecque D, Kasibhatla S and Tseng B 2004 *Mol. Cancer Ther.* **3** 1375; (b) Reddy P. N, Reddy Y T, Rao M K and Rajitha B 2003 *Heterocycl. Commun.* 9 647
17. Babu K S, Raju B C Praveen B, Kishore K H, Murty U S and Rao J M 2003 *Heterocycl. Commun.* **9** 519; (b) El-Gaby M S A, Zahran M A, Ismail M M F and Ammar Y A 2000 *Farmaco* **55** 227
18. Cassidy F, Evans J M, Hadley M S, Haladij A H, Leach P E and Stemp G 1992 *J. Med. Chem.* **35** 1623
19. Maxwell A 1993 *Mol. Microbiol.* **9** 681
20. Soliman S G F and Kappe T Z 1976 *Z. Naturforsch.* **B31** 495
21. Heber D, Ivanov I C and Karagiosov S K 1995 *J. Heterocyclic. Chem.* **32** 505
22. Ivanov I C, Glasnov T N and Heber D 2005 *J. Heterocycl. Chem.* **42** 857
23. Kafka S, Aigner R and Kappe T 2006 *J. Heterocycl. Chem.* **43** 1105
24. (a) Suri O P, Satti, N K and Suri A 2000 *Syn. commun.* **30** 3709; (b) Sirisha K, Bikshapathi D, Achaiah G and Reddy V M 2011 *Eur. J. Med. Chem.* **46** 1564; (c) Virsodia V, Pissurlenkar R R, Manvar D, Dholakia C, Adlakha P, Shah A and Coutinho E C 2008 *Eur. J. Med. Chem.* **43** 2103; (d) García M J, Rebolledo F and Gotor V 1993 *Tertrahedron Lett.* **34** 6141; (e) Tennant G 1993 *J. Chem. Soc.* 2428; (f) García M J and Francisca R F 1994 *Tetrahedron* **50** 6935
25. (a) Chandrasekhar S, Narsihmulu Ch, Sultana S S and Reddy N R 2002 *Org. Lett.* **4** 4399; (b) Li J H, Liu W J and Xie Y X 2005 *J. Org. Chem* **70** 5409; (c) Carlos K Z A and Luana M A 2005 *Curr. Org. Chem.* **9** 195; (d) Ji C, Spear S K, Huddleston J G and Rogers R D 2005 *Green Chem.* **7** 64; (e) Aires da Conceição S, Jaqueline D S, Aguiar L C S, Alessandro B C S, Andréa de Souza L F, Luiz F B M and Antunes O A C 2010 *Tetrahedron Lett.* **51** 3883; (f) Gelson P, Elton L B, Paloma C R, Carvalho P N and Lenardão E J 2013 *Tetrahedron Lett.* **54** 1718
26. (a) Prasad J V, Prabhakar M, Manjulatha K, Rambabu D, Solomon K A, Krishna G G and Kumar K A 2010 *Tetrahedron Lett.* **51** 3109; (b) Prasad J V, Reddy J S, Kumar N R, Solomon K A and Gopikrishna G 2011 *J. Chem. Sci.* **123** 673; (c) Jalli V P, Jaggavarapu S R, Kamalakaran A S, Gangisetty S K, Nanubolu, J B and Gopikrishna G 2013 *Tetrahedron Lett.* **54** 1491; (d) Jaggavarapu S R, Kamalakaran A S, Gayatri, G, Shukla M, Dorai K and Gopikrishna G 2013 *Tetrahedron* **69** 2142
27. O Halloran N, James J P, Downey C A, O' Malley P, Duff T and Bertrand S 2008 *Heterocycles* **75** 2681
28. Kim K S, Lee S, Lee Y S, Jung S H, Park Y, Shin K H and Kim B K 2003 *J. Ethnolpharmacol.* **85** 69