Brønsted acidic ionic liquid catalysis: An efficient and eco-friendly synthesis of novel fused pyrano pyrimidinones and their antimicrobial activity

JANARDHAN BANOTHU, RAJITHA GALI, RAVIBABU VELPULA
and RAJITHA BAVANTULA*

Department of Chemistry, National Institute of Technology, Warangal 506 004, India
e-mail: rajitha_nitw@yahoo.com; rajitabhargavi@yahoo.com

MS received 30 October 2012; revised 11 February 2013; accepted 5 April 2013

Abstract. A series of novel fused pyrano pyrimidinones were synthesized by the condensation of 2-amino-4-aryl-4H-benzo[h]chromene-3-carbonitriles with 2-oxo-2H-chromene-3-carboxylic acid under neat conditions employing an efficient, eco-friendly and reusable Brønsted acidic ionic liquid, (4-sulfobutyl)tris(4-sulfophenyl)phosphonium hydrogen sulphate as catalyst. All the synthesized compounds were characterized by their analytical and spectroscopic data and they were screened for *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* as Gram-positive, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as Gram-negative bacterial strains and antifungal activity against *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Candida rugosa* and *Candida albicans*. Compounds **2c** and **2d** have shown good antibacterial activity against *Escherichia coli* while **2g** has shown marked antifungal activity against *Candida rugosa*.

Keywords. Antibacterial activity; antifungal activity; Brønsted acidic ionic liquid; fused pyrano pyrimidinones; neat conditions.

1. Introduction

Recent studies reveal that bacterial infections have increased at an alarming rate causing deadly diseases and wide spread epidemics in humans. Pyran derivatives possess a wide range of medicinal applications such as antimicrobial,^{1–4} antitubercular,⁴ antioxidant,⁵ antigenotoxic,⁶ cytotoxic,⁷ ATP sensitive potassium channel openers⁸ and antiangiogenic activities.⁹ On the other hand, coumarins and pyrimidines were also found to possess several biological activities like antimicrobial,^{10–14} antiviral,¹⁵ anticonvulsant,¹⁶ anticoagulant,¹⁷ antioxidant,¹⁸ anti-HIV,¹⁹ antihistamine,²⁰ antitubercular,²¹ antitumour,²² antiinflammatory^{23,24} and antileishmanial activities.²⁵ Several methods have been reported for the synthesis of pyrimidinones using Yb(OTf)₃ in ionic liquids,²⁶ Zn(OAc)₂ under microwave irradiation,²⁷ cyanuric chloride²⁸ and acidic ionic liquid²⁹ as catalysts. Most of these reported methods are involving multistep process and suffering from one or several drawbacks such as low yield, long reaction time, tedious work-up, harsh reaction conditions and use of large quantity of expensive reagents. Moreover,

nowadays organic solvents were replaced by ionic liquids because of their interesting properties like high thermal stability, non-volatility, eco-friendly, high selectivity, high atom efficiency, easy separation from the reaction mixture and reusability.³⁰ Similarly, reactions under solvent-free conditions³¹ usually need shorter reaction times and simple work-up procedures.

In view of the high degree of bio-activity shown by pyrans, coumarins and pyrimidines, we have focused on the design of a novel structural entity that incorporates these three structural moieties into a single molecular scaffold to evaluate the potential additive effect on antimicrobial activity. Also, in continuation of our earlier studies on pyrimidinones,³² we now elicit the synthesis of fused pyrano pyrimidinones utilizing inexpensive, eco-friendly and reusable Brønsted acidic ionic liquid as a catalyst under solvent-free conditions as marked antimicrobial agents.

2. Experimental

2.1 Materials

All commercially available reagents and solvents were employed without further purification. Melting points

*For correspondence

were determined in open capillaries using Stuart SMP30 apparatus and are uncorrected. The progress of the reactions as well as purity of compounds was monitored by thin layer chromatography with F₂₅₄ silica-gel pre-coated sheets (Merck, Darmstadt, Germany) using hexane/ethyl acetate 8/2 as eluent; UV light was used for detection. IR spectra were recorded on Perkin-Elmer 237 spectrophotometer using KBr pellet, values are expressed in cm⁻¹. NMR spectra were recorded on Bruker 300 MHz spectrometer using DMSO as solvent and TMS as internal standard, chemical shifts are expressed in ppm. Elemental analysis was performed on a Carlo Erba modal EA1108 and the values are $\pm 0.4\%$ of the theoretical ones. Mass spectra were recorded on a Jeol JMSD-300 spectrometer.

2.2 General procedure for the synthesis of fused pyrano pyrimidinones (2a–j)

To a mixture of 2-amino-4-aryl-4*H*-benzo[*h*]chromene-3-carbonitrile (**1a–j**, 1 mmol) and 2-oxo-2*H*-chromene-3-carboxylic acid (1 mmol); ionic liquid (10 mol%) was added and heated at 120°C for about 8–12 h without any solvent. After completion of the reaction shown by TLC, 2 mL of water was added and stirred at RT for additional 5 min. The solid separated out was filtered and purified over column chromatography using silica gel (230–400 mesh) with *n*-hexane and ethyl acetate (8:2) as eluent. The aqueous layer containing catalyst was recovered, washed with acetone, dried and reused for subsequent reactions without loss in its activity and product yield.

2.3 Characterization of compounds (2a–j)

2.3a 10-(2-Oxo-2*H*-chromen-3-yl)-7-phenyl-9*H*-naphtho[3',4':5,6]pyrano[2,3-*d*]pyrimidin-8-one (**2a**): Pale yellow solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3289 (NH), 1772 (CO of lactone), 1723 (CO of lactam), 1599 (C=N), 1160 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 5.29 (s, 1H), 7.15–7.26 (m, 5H), 7.34 (d, *J* = 6.9 Hz, 1H), 7.41–7.45 (m, 4H), 7.71–7.75 (m, 1H), 7.83 (d, *J* = 6.0 Hz, 1H), 7.90–7.93 (m, 3H), 8.75 (s, 1H), 10.82 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.6, 164.8, 163.9, 159.6, 154.4, 146.8, 145.6, 134.2, 130.7, 130.1, 129.4, 128.6, 128.4, 127.0, 126.9, 126.5, 124.8, 124.7, 123.5, 120.4, 118.3, 117.9, 116.7, 116.0, 106.5, 38.0; MS: *m/z* = 509 (M+K); Anal. Calcd. For C₃₀H₁₈N₂O₄: C, 76.59; H, 3.86; N, 5.95; Found: C, 76.64; H, 3.72; N, 5.98.

2.3b 7-(2-Chlorophenyl)-10-(2-oxo-2*H*-chromen-3-yl)-9*H*-naphtho[3',4':5,6]pyrano[2,3-*d*]pyrimidin-8-one (**2b**): Colourless solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3275 (NH), 1768 (CO of lactone), 1715 (CO of lactam), 1585 (C=N), 1172 (C–O–C), 762 (C–Cl); ¹H NMR (300 MHz, DMSO-*d*₆): δ 5.42 (s, 1H), 7.01 (d, *J* = 6.3 Hz, 1H), 7.22–7.46 (m, 6H), 7.59–7.65 (m, 3H), 7.72 (d, *J* = 5.7 Hz, 1H), 7.88–7.92 (m, 2H), 8.24 (d, *J* = 6.0 Hz, 1H), 8.75 (s, 1H), 10.86 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.0, 164.7, 163.9, 160.3, 154.4, 142.9, 142.1, 134.1, 132.7, 131.9, 131.1, 130.1, 129.7, 128.8, 127.8, 127.6, 126.8, 126.6, 125.3, 124.7, 124.0, 122.6, 120.7, 120.0, 118.3, 117.9, 105.9, 38.1; MS: *m/z* = 505 (M+1); Anal. Calcd. For C₃₀H₁₇ClN₂O₄: C, 71.36; H, 3.39; N, 5.55; Found: C, 71.62; H, 3.17; N, 5.84.

2.3c 7-(4-Chlorophenyl)-10-(2-oxo-2*H*-chromen-3-yl)-9*H*-naphtho[3',4':5,6]pyrano[2,3-*d*]pyrimidin-8-one (**2c**): Colourless solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3251 (NH), 1763 (CO of lactone), 1718 (CO of lactam), 1598 (C=N), 1184 (C–O–C), 752 (C–Cl); ¹H NMR (300 MHz, DMSO-*d*₆): δ 4.95 (s, 1H), 7.10 (d, *J* = 6.6 Hz, 1H), 7.28 (d, *J* = 6.3 Hz, 2H), 7.37–7.45 (m, 4H), 7.58–7.64 (m, 2H), 7.71–7.92 (m, 4H), 8.24 (d, *J* = 6.0 Hz, 1H), 8.75 (s, 1H), 10.96 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 172.9, 164.8, 163.9, 160.1, 154.4, 144.5, 142.7, 134.1, 132.7, 131.5, 130.1, 129.5, 128.6, 127.6, 126.8, 126.6, 126.0, 124.7, 123.9, 122.7, 120.6, 120.2, 118.3, 117.9, 117.3, 106.3, 38.0; MS: *m/z* = 505 (M+1); Anal. Calcd. For C₃₀H₁₇ClN₂O₄: C, 71.36; H, 3.39; N, 5.55; Found: C, 71.58; H, 3.21; N, 5.73.

2.3d 7-(3-Nitrophenyl)-10-(2-oxo-2*H*-chromen-3-yl)-9*H*-naphtho[3',4':5,6]pyrano[2,3-*d*]pyrimidin-8-one (**2d**): Colourless solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3302 (NH), 1758 (CO of lactone), 1709 (CO of lactam), 1604 (C=N), 1526 (NO₂), 1169 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 5.21 (s, 1H), 7.15 (d, *J* = 6.3 Hz, 1H), 7.32–7.45 (m, 4H), 7.60–7.66 (m, 2H), 7.71–7.76 (m, 2H), 7.91 (d, *J* = 6.0 Hz, 2H), 8.12 (d, *J* = 6.6 Hz, 2H), 8.26 (d, *J* = 6.0 Hz, 1H), 8.75 (s, 1H), 10.84 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 172.9, 164.3, 163.9, 160.0, 154.4, 147.9, 147.8, 142.9, 134.5, 134.2, 132.8, 130.4, 130.1, 127.6, 126.9, 126.7, 125.8, 124.7, 124.2, 122.7, 122.1, 122.0, 120.7, 120.1, 118.3, 117.9, 116.7, 106.2, 40.5; MS: *m/z* = 516 (M+1); Anal. Calcd. For C₃₀H₁₇N₃O₆: C, 69.90; H, 3.32; N, 8.15. Found: C, 69.95; H, 3.23; N, 8.30.

2.3e 10-(2-Oxo-2H-chromen-3-yl)-7-p-tolyl-9H-naphtho[3',4':5,6]pyrano[2,3-d]pyrimidin-8-one (**2e**): Pale yellow solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3298 (NH), 1752 (CO of lactone), 1706 (CO of lactam), 1594 (C=N), 1178 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.25 (s, 3H), 4.84 (s, 1H), 7.08–7.12 (m, 3H), 7.39–7.45 (m, 3H), 7.57–7.63 (m, 3H), 7.71–7.92 (m, 4H), 8.23 (d, *J* = 6.3 Hz, 1H), 8.75 (s, 1H), 10.86 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 174.2, 164.7, 163.9, 160.0, 154.4, 142.7, 142.6, 136.0, 134.2, 132.6, 130.1, 129.2, 127.6, 127.5, 126.6, 126.5, 126.2, 124.7, 123.7, 122.7, 120.6, 120.4, 118.3, 118.0, 117.9, 106.4, 40.5, 20.5; MS: *m/z* = 485 (M+1); Anal. Calcd. For C₃₁H₂₀N₂O₄: C, 76.85; H, 4.16; N, 5.78. Found: C, 76.92; H, 4.01; N, 5.89.

2.3f 7-(4-Methoxyphenyl)-10-(2-oxo-2H-chromen-3-yl)-9H-naphtho[3',4':5,6]pyrano[2,3-d]pyrimidin-8-one (**2f**): Pale yellow solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3273 (NH), 1763 (CO of lactone), 1721 (CO of lactam), 1608 (C=N), 1161, 1134 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.71 (s, 3H), 4.84 (s, 1H), 6.88 (d, *J* = 6.6 Hz, 1H), 7.09 (d, *J* = 6.3 Hz, 2H), 7.16 (d, *J* = 6.3 Hz, 2H), 7.39–7.45 (m, 2H), 7.55–7.65 (m, 3H), 7.71–7.92 (m, 3H), 8.24 (d, *J* = 6.0 Hz, 1H), 8.75 (s, 1H), 10.82 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.0, 164.6, 163.8, 160.0, 157.8, 154.3, 144.2, 142.7, 132.9, 132.7, 129.5, 128.6, 127.6, 126.7, 126.5, 126.1, 124.9, 123.9, 122.7, 120.6, 120.3, 118.5, 117.9, 117.4, 115.7, 106.9, 55.4, 38.1; MS: *m/z* = 501 (M+1); Anal. Calcd. For C₃₁H₂₀N₂O₅: C, 74.39; H, 4.03; N, 5.60. Found: C, 74.62; H, 4.38; N, 5.27.

2.3g 7-(3,4-Dimethoxyphenyl)-10-(2-oxo-2H-chromen-3-yl)-9H-naphtho[3',4':5,6]pyrano[2,3-d]pyrimidin-8-one (**2g**): Pale yellow solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3281 (NH), 1751 (CO of lactone), 1737 (CO of lactam), 1601 (C=N), 1153, 1127 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.70 (s, 6H), 4.84 (s, 1H), 6.72–6.75 (m, 1H), 6.89 (d, *J* = 6.0 Hz, 2H), 7.14 (d, *J* = 6.3 Hz, 1H), 7.39–7.45 (m, 3H), 7.56–7.63 (m, 2H), 7.71–7.92 (m, 3H), 8.22 (d, *J* = 6.3 Hz, 1H), 8.75 (s, 1H), 10.84 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.0, 164.3, 163.9, 160.0, 156.6, 154.4, 147.7, 142.2, 138.1, 134.2, 132.6, 130.1, 127.6, 126.6, 126.5, 126.2, 124.7, 123.6, 122.7, 120.6, 120.4, 119.8, 118.3, 118.0, 117.9, 116.0, 112.1, 105.9, 55.4, 40.4; MS: *m/z* = 531 (M+1); Anal. Calcd. For C₃₂H₂₂N₂O₆: C, 72.45; H, 4.18; N, 5.28. Found: C, 72.62; H, 4.38; N, 5.03.

2.3h 7-(4-Hydroxyphenyl)-10-(2-oxo-2H-chromen-3-yl)-9H-naphtho[3',4':5,6]pyrano[2,3-d]pyrimidin-8-one (**2h**): Pale yellow solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3429 (OH), 3294 (NH), 1770 (CO of lactone), 1719 (CO of lactam), 1603 (C=N), 1171 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 4.76 (s, 1H), 6.69 (d, *J* = 6.3 Hz, 2H), 7.02–7.10 (m, 3H), 7.39–7.45 (m, 2H), 7.56–7.64 (m, 3H), 7.71–7.92 (m, 3H), 8.22 (d, *J* = 6.0 Hz, 1H), 8.75 (s, 1H), 9.30 (s, 1H), 10.75 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.2, 164.3, 163.5, 160.0, 157.1, 154.7, 144.5, 142.8, 134.2, 132.6, 129.8, 128.6, 127.6, 126.8, 126.7, 126.2, 124.9, 123.6, 122.7, 120.5, 120.4, 118.6, 117.9, 117.4, 116.1, 105.9, 38.1; MS: *m/z* = 487 (M+1); Anal. Calcd. For C₃₀H₁₈N₂O₅: C, 74.07; H, 3.73; N, 5.76; Found: C, 74.21; H, 3.48; N, 5.88.

2.3i 7-(4-Hydroxy-3-methoxyphenyl)-10-(2-oxo-2H-chromen-3-yl)-9H-naphtho[3',4':5,6]pyrano[2,3-d]pyrimidin-8-one (**2i**): Pale yellow solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3417 (OH), 3280 (NH), 1762 (CO of lactone), 1724 (CO of lactam), 1610 (C=N), 1182, 1138 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.72 (s, 3H), 4.78 (s, 1H), 6.60 (d, *J* = 5.7 Hz, 1H), 6.70 (d, *J* = 6.3 Hz, 1H), 6.85 (s, 1H), 7.14 (d, *J* = 6.3 Hz, 1H), 7.39–7.45 (m, 3H), 7.56–7.62 (m, 2H), 7.71–7.92 (m, 3H), 8.22 (d, *J* = 6.0 Hz, 1H), 8.75 (s, 1H), 8.88 (s, 1H), 10.64 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.6, 164.4, 163.9, 159.9, 156.6, 154.4, 145.5, 142.4, 136.6, 134.2, 132.5, 130.1, 127.6, 126.5, 126.2, 124.7, 123.6, 122.7, 120.6, 120.0, 118.3, 117.9, 116.0, 115.6, 105.9, 55.6, 40.4; MS: *m/z* = 517 (M+1); Anal. Calcd. For C₃₁H₂₀N₂O₆: C, 72.09; H, 3.90; N, 5.42; Found: C, 72.26; H, 3.74; N, 5.62.

2.3j 7-(4-Dimethylamino-phenyl)-10-(2-oxo-2H-chromen-3-yl)-9H-naphtho[3',4':5,6]pyrano[2,3-d]pyrimidin-8-one (**2j**): Colourless solid; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3274 (NH), 1767 (CO of lactone), 1725 (CO of lactam), 1605 (C=N), 1182 (C–O–C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.11 (s, 6H), 4.93 (s, 1H), 6.85 (d, *J* = 6.6 Hz, 2H), 7.39–7.45 (m, 5H), 7.71–7.75 (m, 3H), 7.83 (d, *J* = 6.9 Hz, 2H), 7.91 (d, *J* = 5.7 Hz, 2H), 8.75 (s, 1H), 10.92 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): 173.6, 164.4, 163.5, 160.1, 154.7, 148.2, 144.6, 142.3, 132.9, 130.1, 129.7, 128.7, 127.6, 126.8, 126.5, 126.1, 124.9, 123.8, 122.7, 120.7, 120.3, 118.3, 117.9, 117.3, 115.3, 106.7, 40.7, 38.0; MS: *m/z* = 514 (M+1); Anal. Calcd. For C₃₂H₂₃N₃O₄: C, 74.84; H, 4.51; N, 8.18; Found: C, 74.66; H, 4.72; N, 8.04.

3. Results and discussions

Fused pyrano pyrimidinones (**2a–j**) were synthesized by the condensation of 2-amino-4-aryl-4*H*-benzo[*h*]chromene-3-carbonitriles (**1a–j**) with 2-oxo-2*H*-chromene-3-carboxylic acid under solvent-free conditions at 120°C utilizing Brønsted acidic ionic liquid, (4-sulfobutyl)tris(4-sulphophenyl)phosphonium hydrogen sulphate as green catalyst (scheme 1). The ionic liquid (IL) has been prepared according to the literature procedure.³³

Reaction between 2-amino-4-phenyl-4*H*-benzo[*h*]chromene-3-carbonitrile (**1a**) and 2-oxo-2*H*-chromene-3-carboxylic acid was carried out as a model reaction at different temperatures (Rt, 80, 120 and 150°C) by varying the amount of ionic liquid (5, 10 and 15 mol%)

under solvent-free conditions. The maximum yield of the product **2a** was observed at 120°C with 10 mol% of ionic liquid. The efficiency of the ionic liquid was also compared with different acid catalysts (sulphuric acid, silica sulphuric acid and cellulose sulphuric acid) and the results were presented in table 1.

At these optimizing conditions (solvent-free, 120°C, 10 mol% of IL), we performed the reaction using different 2-amino-4-aryl-4*H*-benzo[*h*]chromene-3-carbonitrile (**1a–j**) and synthesized the titled compounds (**2a–j**) in good yields (table 2). After completion of the reaction indicated by TLC, the catalyst was recovered by evaporating the aqueous layer, washed with acetone, dried and reused for subsequent reactions without significant loss in its activity and product yield. For example, the reaction of 2-amino-4-phenyl-4*H*-benzo[*h*]chromene-

Reaction conditions: (a) Ethanol, Et₃N, Reflux, 1–6 h; (b) 2-oxo-2*H*-chromene-3-carboxylic acid, Ionic liquid (10 mol%), Neat, 120°C, 8–12 h.

Scheme 1. Synthetic pathway of the fused pyrano pyrimidinones.

Table 1. Optimization of the reaction conditions.^a

S.No.	Catalyst (mol%)	Temp. (°C)	Time (h)	Yield ^b (%)
1	Ionic liquid (5 mol%)	RT	24	–
2	Ionic liquid (5 mol%)	80	12	28
3	Ionic liquid (5 mol%)	120	12	36
4	Ionic liquid (5 mol%)	150	12	37
5	Ionic liquid (10 mol%)	80	12	57
6	Ionic liquid (10 mol%)	120	10	72
7	Ionic liquid (10 mol%)	150	10	72
8	Ionic liquid (15 mol%)	120	10	72
9	Ionic liquid (15 mol%)	150	10	72
10	Sulphuric acid (10 mol%)	120	10	19
11	Silica sulphuric acid (10 mol%)	120	10	36
12	Cellulose sulphuric acid (10 mol%)	120	10	48

^aReaction conditions: 2-amino-4-phenyl-4*H*-benzo[*h*]chromene-3-carbonitrile (1 mmol), 2-oxo-2*H*-chromene-3-carboxylic acid (1 mmol), solvent-free conditions.

^bYields refer to isolated pure product (**2a**)

3-carbonitrile (**1a**) with 2-oxo-2*H*-chromene-3-carboxylic acid gave the corresponding fused pyrano pyrimidinone (**2a**) in 72, 70, 70, 69 and 68% yields over additional five cycles. All the compounds were characterized by IR, NMR and Mass spectral data.

4. Biological activities

All the synthesized compounds (**2a–j**) were screened for *in vitro* antibacterial activity against Gram-positive bacterial strains: *Bacillus subtilis* (Bs), *Staphylococcus aureus* (Sa) and *Staphylococcus epidermidis* (Se), and Gram-negative bacterial strains: *Escherichia coli* (Ec),

Pseudomonas aeruginosa (Pa) and *Klebsiella pneumonia* (Kp) with respect to Penicillin and Streptomycin as the positive control drugs. Antifungal screening was carried out against *Aspergillus flavus* (Af), *Saccharomyces cerevisiae* (Sc), *Candida rugosa* (Cr) and *Candida albicans* (Ca) fungal strains using Amphotericin-B as the positive control drug.

4.1 Antibacterial activity

The minimum inhibitory concentration (MIC) values for analogs and the positive control drugs Penicillin and Streptomycin were determined against the six

Table 2. Synthesis of fused pyrano pyrimidinones **2(a–j)** using ionic liquid (IL).^a

Analog	R	Time (h)	Yield ^b (%)	M.p (°C)
2a	C ₆ H ₅	10	72	277–279
2b	2-ClC ₆ H ₄	12	68	290–292
2c	4-ClC ₆ H ₄	8	76	222–224
2d	3-NO ₂ C ₆ H ₄	12	71	231–233
2e	4-CH ₃ C ₆ H ₄	9	67	244–246
2f	4-OCH ₃ C ₆ H ₄	10	73	267–270
2g	3,4-(OCH ₃) ₂ C ₆ H ₃	10	74	255–257
2h	4-OHC ₆ H ₄	12	69	242–244
2i	4-OH-3-OCH ₃ C ₆ H ₃	8	77	249–251
2j	4-N(CH ₃) ₂ C ₆ H ₄	12	72	270–272

^aReaction conditions: 2-amino-4-aryl-4*H*-benzo[*h*]chromene-3-carbonitrile (**1a–j**, 1 mmol), 2-oxo-2*H*-chromene-3-carboxylic acid (1 mmol), Ionic liquid (10 mol%), Neat, 120°C.

^bYields refer to the pure isolated products

Table 3. Minimum inhibitory concentration (MIC) values for fused pyrano pyrimidinones (**2a–j**) and positive control drugs against different bacterial strains.

Analog	Minimum inhibitory concentration (μg/mL)					
	Gram-positive bacteria			Gram-negative bacteria		
	Bs	Sa	Se	Ec	Pa	Kp
2a	150	300	150	75	150	150
2b	150	300	150	75	150	300
2c	150	150	150	37.5	150	150
2d	150	300	150	37.5	150	150
2e	150	300	150	75	150	150
2f	300	450	300	75	150	150
2g	150	300	300	75	150	150
2h	450	300	150	75	150	300
2i	450	450	150	75	150	150
2j	150	150	150	75	150	150
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

Gram-positive bacteria: Bs: *Bacillus subtilis*; Sa: *Staphylococcus aureus*; Se: *Staphylococcus epidermidis*.

Gram-negative bacteria: Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Kp: *Klebsiella pneumonia*

Table 4. Zone of inhibition data for fused pyrano pyrimidinones **2(a–j)** against different fungal strains at 100 and 150 µg/mL concentrations.

Analog	Zone of inhibition (mm)							
	Af		Sc		Cr		Ca	
	100 µg	150 µg	100 µg	150 µg	100 µg	150 µg	100 µg	150 µg
2a	11	14	–	–	–	–	14	18
2b	12	16	–	–	11	15	–	–
2c	11	15	–	–	09	14	–	–
2d	09	12	–	–	–	–	09	14
2e	08	11	–	–	11	16	–	–
2f	–	–	–	–	16	20	12	16
2g	15	21	–	–	18	22	14	18
2h	–	–	–	–	11	15	12	15
2i	7	11	–	–	12	18	–	–
2j	13	16	–	–	12	16	11	15
AMP-B	25		25		22		23.5	

Fungal strains: Af: *Aspergillus flavus*; Sc: *Saccharomyces cerevisiae*; Cr: *Candida rugosa*; Ca: *Candida albicans*.

AMP-B: Amphotericin-B; '–' not active

bacterial strains by the liquid dilution method.^{34,35} Different concentrations of analogs and positive control drugs were prepared in DMSO. Inoculums of the bacterial cultures were also prepared. Inoculum (0.2 mL) and sterile water (3.8 mL) were added to a series of test tubes each containing 1 mL of test solution at different concentrations.

The tubes were incubated for 24 h at 37°C and carefully observed for the presence of turbidity. The minimum concentration at which no growth was observed was taken as the MIC value (table 3).

From the activity report (table 3) it was notified that, the compounds **2c** and **2d** have shown good activity against *Escherichia coli* (Gram-negative bacteria) with MIC 37.5 µg/mL compare to the standard drug Penicillin, remaining all the compounds have shown moderate activity against all the tested bacterial strains.

4.2 Antifungal activity

Zone of inhibition (in mm) values for analogs and positive control drug Amphotericin-B were determined against four different fungal strains by Agar disc-diffusion method.^{36,37} All the compounds were dissolved in DMSO. The fungal strains were grown and maintained on Sabouraud glucose agar plates. The plates were incubated at 26°C for 48 h, and resulting ZOI were measured. Antifungal screening for analogs and positive control was performed at a concentration of 100 and 150 µg/mL and the results are illustrated in table 4.

The zone of inhibition data indicating that the compound **2g** has shown good activity against *Candida*

rugosa (ZOI = 22 mm) at 150 µg/mL when compared to the standard drug Amphotericin-B, remaining compounds have shown moderate activity and all the compounds were inactive against *Saccharomyces cerevisiae*.

5. Conclusion

Various fused pyrano pyrimidinones (**2a–j**) were synthesized using an efficient, eco-friendly and reusable Brønsted acidic ionic liquid, (4-sulfobutyl)tris(4-sulfophenyl)phosphonium hydrogen sulphate as catalyst under neat conditions. All the synthesized compounds were screened for *in vitro* antibacterial and antifungal activity against different bacterial and fungal strains. The antimicrobial screening data revealed that the compounds **2c** and **2d** have shown good activity against Gram-negative bacteria *Escherichia coli*, where as compound **2g** has shown superior antifungal activity against *Candida rugosa* at 150 µg/mL compare to the standard antifungal drug Amphotericin-B.

Acknowledgements

The authors thank the Department of Biotechnology, Kakatiya University, India for screening the antimicrobial activity of the synthesized compounds. B J thanks Ministry of Human Resources Department (MHRD) and R G and R V thank the University Grants Commission (UGC) for providing research fellowship.

References

1. Zhang G, Zhang Y, Yan J, Chen R, Wang S, Ma Y and Wang R 2012 *J. Org. Chem.* **77** 878
2. Chetan B S, Divyesh C M, Manish P P and Ranjan G P 2012 *Chin. Chem. Lett.* **23** 57
3. El-Gaby M S A, Zahran M A, Ismail M M F and Ammar Y A 2000 *Il Farmaco* **55** 227
4. Divyesh C M, Manish P P, Dhanji P R and Ranjan G P 2011 *Eur. J. Med. Chem.* **46** 4192
5. Hwan K J, Eun K H, Kyung J J, Hwajung K, Jungsook C and Heesoon L 2006 *Arch. Pharm. Res.* **29** 728
6. Fakher C, Mehdi M, Hedi B M, Leila C and Mansour S 2007 *Eur. J. Med. Chem.* **42** 715
7. Ren S C, Sheau F Y, Chih M L, Amooru G, Damu T K, Cheng C P, Kenneth F B, Hsiung L K and Shung W T 2009 *Bioorg. Med. Chem.* **17** 6137
8. Cheng C Y, Chiu H, Chang M J, Lin Y C, Tsai M C and Yu H C 1998 *Bioorg. Med. Chem. Lett.* **8** 463
9. Abdou O A, Ahmed H E, Sayed A A and Yasser H Z 2005 *J. Sulf. Chem.* **26** 405
10. Modrana J N, Nawrot E and Graczyk J 2006 *Eur. J. Med. Chem.* **41** 1301
11. Sardari S, Mori Y, Horita K, Micetich R G, Nishibe S and Daneshtalab M 1999 *Bioorg. Med. Chem.* **7** 1933
12. Nimesh R K, Dhaval D H, Prashant T M and Saurabh K P 2010 *Eur. J. Med. Chem.* **45** 5056
13. Rai U S, Arun M I, Prakash S, Vijesh A M, Nithin P, Shrikrishna I, Thiageeswaran M and Hoong-Kun F 2010 *Eur. J. Med. Chem.* **45** 2695
14. Cecile G, Douguet D, Huteau V, Gilles M, Helene M L and Sylvie P 2008 *Bioorg. Med. Chem.* **16** 6075
15. Massimo C, Francesco E, Federica M, Carla M M, Prieto G S and Carlos R J 2003 *Aust. J. Chem.* **56** 59
16. Bhat M A, Siddiqui N and Khan S A 2006 *Indian J. Pharm. Sci.* **68** 120
17. Ruszat R, Wyler S, Forster T, Reich O, Christian G S, Thomas C G, Sulser T and Bachmann A 2006 *Eur. Assoc. Urol.* **50** 675
18. Tyagi A K, Raj H G, Vohra P, Gupta G, Kumari R, Kumar P and Gupta R K 2003 *Eur. J. Med. Chem.* **40** 413
19. Huang L, Yuon X, Yu D, Lee K H and Chin H C 2005 *Virology* **332** 623
20. Mohanty N, Rath P C and Rout M K 1967 *J. Indian Chem. Soc.* **44** 1001
21. Deshmukh M B, Salunkhe S M, Patil D R and Anbhule P V 2009 *Eur. J. Med. Chem.* **44** 2651
22. Lin R, Sigmond G, Johnson P J, Connolly S K, Wetter E, Binnun T V, Hughes W V, Murray N B, Pandey S J, Mazza M M, Adams A R, Pesquera F and Steven A M 2009 *Bioorg. Med. Chem. Lett.* **19** 2333
23. Lin C M, Huang S T, Lee F W, Sawkuo H and Lin M H 2006 *Bioorg. Med. Chem.* **14** 4402
24. Falcao E P, Da S, Melo S J, Srivastava R M, Catanho M T and Nascimento S C 2006 *Eur. J. Med. Chem.* **41** 276
25. Agarwal A, Ashutosh R, Goyal N, Chauhana M S and Gupta S 2005 *Bioorg. Med. Chem.* **13** 6678
26. Shu-Liang W, Ke Y and Xiang-Shan W 2011 *Chin. J. Org. Chem.* **31** 1235
27. Jing X, Lib Z, Wu L and Yan C 2011 *J. Iran. Chem. Soc.* **8** 107
28. Moni S, Shashi P, Kuldeep C, Deepty S, Brijesh K and Prem M S C 2012 *J. Org. Chem.* **77** 929
29. Heravi M M, Tavakoli-Hoseini N and Bamoharram F 2011 *Synth. Commun.* **41** 707
30. Martyn J E and Kenneth R S 2000 *Pure Appl. Chem.* **72** 1391
31. Sheldon R A 1996 *J. Mol. Catal. A: Chem.* **107** 75
32. Janardhan B and Rajitha B 2012 *Chin. Chem. Lett.* **23** 1015
33. Hamid R S, Mohammad R and Kobra A 2011 *J. Mol. Liq.* **162** 95
34. Omrum U, Arikan S, Kocago S, Semeak B and Unala D 2000 *Microbiol. Infect. Dis.* **38** 101
35. Malue M, Bastide J M and Biancard A 2005 *Int. J. Antimicrob. Agents* **25** 321
36. Alam S 2004 *Chem. Sci.* **166** 325
37. Reddy P M, Ho Y P, Shanker K, Rohini R and Ravinder V 2009 *Eur. J. Med. Chem.* **44** 2621