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Research Paper

A Greener Synthesis of 2-Aminochromenes in Ionic Liquid and Evaluation of Their Anti-proliferative Activities

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Abstract: An improved simple and facile synthesis of chromene derivatives by employing three-component one-pot condensation reaction of β-naphthol, aromatic aldehydes, and malononitrile in ionic liquids is described. The ionic liquid can be reused and recycled. The chromene derivatives were evaluated for their anti-proliferative activity for human breast adenocarcinoma (MCF-7), ovarian adenocarcinoma (SK-OV-3), and acute lymphoblastic leukemia (CCRF-CEM) cell lines. Compounds **4b** inhibited the cell proliferation of all three cancer cells by 60-80% at 50 μM. Among all compounds, compound **4b** was the most potent against all three cancer cell lines whereas compound **4c, 4o** and **4p** exhibited more selectivity against CCRF-CEM.

Introduction

Chromenes and its fused derivatives are considered to be important biologically active heterocyclic compounds. They are main component of many naturally occurring products such as, alkaloids, flavonoids, tocopherols, and anthocyanins.[1] Among different types of chromene systems, 2-amino-4*H*-chromenes are of particular interest as they belong to privileged medicinal scaffold serving for generation of small-molecule ligands with highly pronounced spasmolitic, diuretic, anti-coagulant, or anti-anaphylactic activities.[1] They have also been shown to exhibit other biological activities such as anti-bacterial,[2,3] anti-viral,[4,5] anti-fungal,[6,7] anti-tumor,[8] hypotensive effects,[9] and central nervous system (CNS) activity.[10]

4*H*-Chromene-3-carboxylate derivatives have been known as antagonist of the anti-apoptotic proteins. A series of analogues with varied functional groups at the 6-position of the chromene ring has been evaluated for their binding interactions with three anti-apoptotic proteins: Bcl-2, Bcl-XL, and Bcl-w.[11] The 4-aryl-4*H*-chromenes

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inhibit tubulin polymerization and bind at or close to the binding site of colchicine. They are also active in the multidrug resistant MES-SA/DX5 tumor cells, and are highly active as single agent in combination with other anti-cancer agents in several tumor animal models. Several potent 7-substituted and 7,8-disubstituted analogues (Figure 1, **A-D**) have been identified as lead compounds for anti-tumor activities through SAR studies.

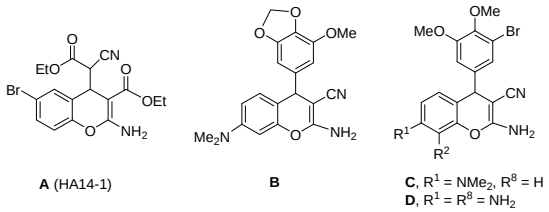
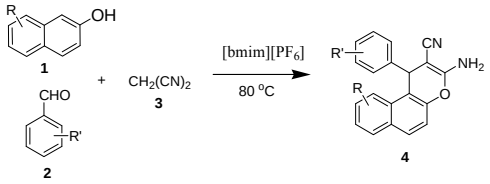


Figure 1: Structures of anti-tumor chromenes.

The most common method for the synthesis of chromenes is condensation of phenol, aldehyde, and malononitrile in the presence of an organic base, such as piperdine.[12] Several other reagents, such as CTACl,[13] Mg/Al hydrotalcite,[14] K_2CO_3 in water under microwave irradiation,[15] $InCl_3$,[16] KF or NaOAc[1], preyssler type heteropolyacid,[17] DBU[18], aqueous medium,[19] basic ionic liquid[20] and γ-alumina[21] have been reported to be effective catalysts for the one-pot synthesis of 2-aminochromenes. Most of these methods require longer reaction time, harsh reaction conditions, give low yields, and/or uses organic solvents. Recently, 4-unsubstituted 2-amino-4*H*-chromene-2-carbonitriles were synthesized in water using DBU as catalyst.[22] An organocatalytic asymmetric synthesis of chiral 2-amino-4*H*-chromene derivatives was reported by tandem Michael addition–cyclization of malononitrile to nitroalkenes.[23]

Ionic liquids are organic salts, which consist entirely of ions, and are liquid at or below 100 °C. They have been shown to have several advantages, such as low vapor pressure, high thermal stability and recyclability over volatile organic compounds.[7,8] Due to their unique chemical properties they have been extensively used as solvent, reagent and catalyst in organic synthesis. 1-Butyl-3-methylimidazolium-based ionic liquids are the most commonly used in organic synthesis among different ionic liquids. These ionic liquids are not only used as solvent but they also affect the rate of the reaction and thus act as catalyst in several organic reactions. The reactivity and selectivity for various organic reactions is different in ionic liquids compared to molecular organic solvents.[24-26] The rate of reaction and product selectivity are influenced by the nature of ionic liquid used.[27] Herein, we report an improved simple and facile synthesis of 2-aminochromene derivatives (**4**) by employing three-component one-pot condensation reaction of β-naphthol (**1**), aromatic aldehydes (**2**), and malononitrile (**3**) in 3-butyl-1-methylimidazolium hexafluorophosphate, [bmim][PF_6] ionic liquid (Scheme 1).



Scheme 1: Ionic liquid mediated synthesis of 2-aminochromenes.

Materials and methods:

Melting points were measured on a Micro scientific works apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR spectrometer. The ^{1}H NMR spectra were recorded on a Bruker Heaven 11400 (400 MHz) spectrometer using TMS as internal standard and DMSO-d_6 as solvent and the chemical shifts are expressed in ppm. Reactions were monitored by using thin layer chromatography (TLC) on 0.2 mm silica gel F_{254} plates (Merck).

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Malononitrile and naphthols were purchased from SD Fine and aldehydes were purchased from Sigma-Aldrich.

General procedure for preparation of 2-aminochromenes: 2-Naphthol (1 eq), 4-chlorobenzaldehyde (1 eq), and malononitrile (1 eq) were taken in 10 mL round bottom flask containing 1.5 mL [bmim][PF_6] ionic liquid. The reaction mixture was heated to 80 °C for 2 h. The reaction was monitored by TLC. After completion of the reaction, ethanol was added to the crude product; the solid was collected by filtration and dried under vacuum. The compound was purified by recrystalization in ethanol. All the compounds were characterized by ^{1}H NMR, IR spectroscopy, and mass spectrometry.

3-Amino-1-(4-chlorophenyl)-1H-benzo[f]chromene-2-carbonitrile (4a): IR (KBr) 3415-3331, 2198, 1643, 1591, 1400, 1259, 1102, 1045, 808, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (t, *J* = 7.86 Hz, 2H, ArH), 7.59 (dd, *J* = 9.56 & 6.72 Hz, 1H, ArH), 7.39 (dd, *J* = 5.8 & 6.8 Hz, 2H, ArH), 7.25 (d, *J* =1.32 Hz , 1H, ArH), 7.22 (d, *J* = 8.94 Hz, 2H, ArH), 7.10 (d, *J* = 8.4 Hz, 2H, ArH), 5.2 (s, 1H, C*H*), 4.63 (bs, 2H, NH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.16, 147.25, 145.11, 131.62, 131.27, 130.49, 130.14, 129.21, 128.95, 127.61, 125.44, 123.98, 120.78, 117.24, 115.60, 57.88, 37.81; HRMS (ESI): *m/z* calcd. for $C_{20}H_{14}ClN_2O^+$ 333.0789; found 333.0391 $[M + H]^+$.

3-Amino-1-(4-nitrophenyl)-1H-benzo[f]chromene-2-carbonitrile (4b): IR (KBr) 3460-3325, 2185, 1665, 1600, 1570, 1540, 1500, 1325, 1270, 1190, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (dd, *J* = 6.96 & 6.92 Hz, 2H, ArH), 7.82-7.86 (m, 2H, ArH), 7.54-7.58 (m, 1H, ArH), 7.35-7.42 (m, 4H, ArH), 7.30 (d, *J* = 9.0 Hz, 1H, ArH), 6.05 (bs, 2H, NH_2), 5.35 (s, 1H, C*H*); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.77, 155.64, 151.13, 135.29, 134.18, 132.61, 131.90, 131.39, 129.19, 128.17, 127.01, 120.62, 117.45, 42.58; HRMS (ESI): *m/z* calcd. for $C_{20}H_{14}N_3O_3^+$ 344.1030; found 344.0623 $[M + H]^+$.

3-Amino-1-(3-nitrophenyl)-1H-benzo[f]chromene-2-carbonitrile (4c): IR (KBr) 3470-3350, 2185, 1660, 1600, 1580, 1540, 1500, 1335, 1270, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, *J* = 8.16 Hz, 1H, ArH), 7.97 (s, 1H, ArH), 7.82 – 7.87 (m, 2H, ArH), 7.61 (d, *J* = 8.28 Hz, 2H, ArH), 7.39-7.51 (m, 3H, ArH), 7.31 (d, *J* = 9.0 Hz, 1H, ArH), 6.00 (bs, 2H, NH_2), 5.36 (s, 1H, C*H*); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.17, 153.12, 152.17, 138.89, 136.05, 135.62, 135.18, 133.78, 132.58, 130.33, 128.66, 127.01, 126.51, 125.36, 122.05, 119.77, 62.20, 42.54. HRMS (ESI): *m/z* calcd. for $C_{20}H_{14}N_3O_3^+$: 344.1030; found: 344.0625 $[M + H]^+$.

3-Amino-1-p-tolyl-1H-benzo[f]chromene-2-carbonitrile (4d): IR (KBr) 3460-3350, 2975, 2170, 1665, 1600, 1540, 1500, 1270, 1190, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, *J* = 8.4 Hz, 2H, ArH), 7.70 (t, *J* = 6.87 Hz, 1H, ArH), 7.37 (dd, *J* = 5.2 & 4.6 Hz, 2H, ArH), 7.26 (d, *J* = 8.92 Hz, 1H, ArH), 7.02 – 7.07 (m, 4H, ArH), 5.62 (bs, 2H, NH_2), 5.16 (s, 1H, C*H*), 2.20 (s, 3H, Ar-CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.03, 147.20, 143.25, 136.11, 131.25, 130.63, 129.83, 129.67, 128.87, 127.39, 125.31, 124.09, 120.98, 117.21, 116.23, 58.51, 38.20, 20.99; HRMS (ESI): *m/z* calcd. for $C_{21}H_{17}N_2O^+$ 313.1335; found: 313.0973 $[M + H]^+$.

3-Amino-1-(4-methoxyphenyl)-1H-benzo[f]chromene-2-carbonitrile (4e): IR (KBr) 3460-3350, 2985, 2180, 1665, 1600, 1540, 1500, 1300, 1250, 1190, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (d, *J* = 8.88 Hz, 2H, ArH), 7.70 (t, *J* = 7.3 Hz, 1H, ArH), 7.35 – 7.40 (m, 2H, ArH),

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7.25 (d, *J* = 8.92 Hz , 1H, ArH), 7.09 (d, *J* = 8.5 Hz, 2H, ArH), 6.76 (d, *J* = 8.48 Hz, 2H, ArH), 5.63 (bs, 2H, NH_2), 5.16 (s, 1H, C*H*), 3.70 (s, 3H, Ar-OC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.74, 163.02, 151.88, 143.10, 136.01, 135.37, 134.54, 133.62, 133.23, 132.19, 130.05, 128.88, 125.76, 121.97, 121.15, 119.23, 63.41, 60.16, 42.49; HRMS (ESI): *m/z* calcd. for $C_{21}H_{17}N_2O_2^+$ 329.1285; found: 329.0898 $[M + H]^+$.

3-Amino-1-(3-chlorophenyl)-1H-benzo[f]chromene-2-carbonitrile (4f): IR (KBr) 3400-3270, 2180, 1670, 1400, 1102, 1045, 808, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, *J* = 7.9 Hz, 2H, ArH), 7.65 (d, *J* = 6.7, 1H, ArH), 7.41(dd, *J* = 5.3 & 3.3 Hz, 2H, ArH), 7.28 (d, *J* = 9.0 Hz, 1H, ArH), 7.21 (t, *J* = 7.4 Hz, 1H, ArH), 7.13 (d, *J* = 8.2 Hz, 2H, ArH), 7.09 (s, 1H, ArH), 5.88 (bs, 2H, N*H*$_2$), 5.19 (s, 1H, C*H*); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.28, 148.59, 147.32, 133.65, 131.21, 130.47, 130.24, 128.98, 127.71, 127.13, 126.16, 125.51, 123.96, 120.71, 117.26, 115.40, 57.75, 37.97; HRMS (ESI): *m/z* calcd. for $C_{20}H_{14}ClN_2O^+$ 333.0789; found: 333. 0389 $[M + H]^+$.

3-Amino-1-(3-methoxyphenyl)-1H-benzo[f]chromene-2-carbonitrile (4g): IR (KBr) 3460-3350, 2985, 2180, 1665, 1600, 1540, 1500, 1300, 1250, 1190, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, *J* = 9.1 Hz, 2H, ArH), 7.69 – 7.73 (m, 2H, ArH), 7.36 – 7. 42 (m, 1H, ArH), 7.27 (d, *J* = 8.9 Hz, 1H, ArH), 7.14 – 7.18 (m, 1H, ArH), 6.77 (d, *J* = 7.7 Hz, 1H, ArH), 6.67 – 6.69 (m, 2H, ArH), 6.05 (bs, 2H, N*H*$_2$), 5.16 (s, 1H, C*H*), 3.73 (s, 3H, Ar-OC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.20, 159.78, 147.68, 147.26, 131.23, 130.66, 130.29, 129.93, 128.88, 127.53, 125.38, 124.08, 120.93, 119.61, 117.18, 116.03, 113.87, 111.62, 58.22, 55.36, 38.41; HRMS (ESI): *m/z* calcd. for $C_{21}H_{17}N_2O_2^+$ 329.1285; found: 329.0898 $[M + H]^+$.

3-Amino-1-phenyl-1H-benzo[f]chromene-2-carbonitrile (4h): IR (KBr) 3460-3350, 2180, 1665, 1600, 1540, 1500, 1190, 1080 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (dd, *J* = 9 & 9.3 Hz, 2H, ArH), 7.75 (d, *J* = 6.8 Hz, 1H, ArH), 7.35 – 7.41 (m, 2H, ArH), 7.12 – 7.30 (m, 6H, ArH), 6.60 (bs, 2H, N*H*$_2$), 5.20 (s, 1H, C*H*); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.14, 147.27, 146.15, 131.26, 130.61, 129.93, 129.13, 128.90, 127.47, 127.03, 125.35, 124.06, 120.95, 117.22, 116.12, 58.37, 38.54; HRMS (ESI): *m/z* calcd. for $C_{20}H_{15}N_2O^+$ 299.1179; found: 299.048 $[M + H]^+$.

3-Amino-1-(4-chlorophenyl)-9-methoxy-1H-benzo[f]chromene-2-carbonitrile (4i): IR (KBr) 3460-3350, 2985, 2180, 1665, 1600, 1540, 1500, 1300, 1250, 1190, 1080, 808, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 – 7.60 (m, 2H, ArH), 7.22 (dd, *J* = 6.5 & 6.59 Hz, 2H, ArH), 7.15 (dd, *J* = 6.6 & 6.4 Hz, 2H, ArH), 7.10 (d, *J* = 8.8 Hz, 1H, ArH), 7.02 (dd, *J* = 8.88 & 8.92 Hz, 1H, ArH), 6.86 (d, *J* = 2.3 Hz, 1H, ArH), 5.86 (bs, 2H, N*H*$_2$), 5.10 (s, 1H, C*H*), 3.71 (s, 3H, Ar(naphthyl)-OC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.13, 158.49, 147.70, 145.18, 132.02, 131.56, 130.49, 129.70, 129.48, 129.07, 126.43, 120.83, 117.33, 114.88, 114.49, 103.56, 57.87, 55.61, 37.76; HRMS (ESI): *m/z* calcd. for $C_{21}H_{16}ClN_2O_2^+$ 363.0895; found: 363.0478 $[M + H]^+$.

3-Amino-9-methoxy-1-(3-nitrophenyl)-1H-benzo[f]chromene-2-carbonitrile (4j): IR (KBr) 3470-3350, 2960, 2185, 1660, 1600, 1580, 1540, 1500, 1335, 1270, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 – 8.04 (m, 2H, ArH), 7.64 – 7.77 (m, 3H, ArH), 7.48 – 7.52 (m, 1H, ArH), 7.15 (d, *J* = 8.9 Hz, 1H, ArH), 7.02 (dd, *J* = 8.88 & 8.92 Hz, 1H, ArH), 6.89 (d, *J* = 2.2 Hz, 1H, ArH), 6.22 (bs, 2H, N*H*$_2$), 5.31 (s, 1H, C*H*), 3.72 (s, 3H, Ar(Naphthyl)-OC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.46, 158.68, 148.36, 147.80, 134.37, 131.89, 130.84,

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130.59, 130.03, 126.45, 122.27, 121.97, 120.69, 117.59, 114.50, 103.35, 57.36, 55.64, 37.66; HRMS (ESI): *m/z* calcd. for $C_{21}H_{16}N_3O_4^+$ 374.1135; found: 374.0721 [M + H]$^+$.

3-Amino-1-(3-chlorophenyl)-9-methoxy-1H-benzo[f]chromene-2-carbonitrile (4k): IR (KBr) 3460-3350, 2985, 2180, 1665, 1600, 1540, 1500, 1300, 1250, 1190, 1080, 808, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 – 7.68 (m, 2H, ArH), 7.11 – 7.26 (m, 5H, ArH), 7.01 (dd, *J* = 8.88 & 8.84 Hz, 1H), 6.94 (d, *J* =2.3 Hz, 1H, ArH), 5.14 (s, 1H, C*H*), 6.40 (bs, 2H, N*H*$_2$), 3.73 (s, 3H, Ar(naphthyl)-OCH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.25, 158.53, 148.66, 147.71, 133.57, 132.00, 131.08, 130.52, 129.79, 127.44, 127.08, 126.42, 120.81, 117.40, 114.68, 114.51, 103.54, 57.71, 55.58, 38.00; HRMS (ESI): *m/z* calcd. for $C_{21}H_{16}ClN_2O_2^+$ 363.0895; found: 363.0477 [M + H]$^+$.

3-Amino-9-methoxy-1-(3-methoxyphenyl)-1H-benzo[f]chromene-2-carbonitrile (4l): IR (KBr) 3460-3350, 2985, 2180, 1665, 1600, 1540, 1500, 1300, 1250, 1190, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (t, *J* = 7.89 Hz, 2H, ArH), 7.20 – 7.17 (m, 1H, ArH), 7.10 (d, *J* = 8.8 Hz, 1H, ArH), 7.00 (dd, *J* = 8.88 & 8.84 Hz, 1H, ArH), 6.96 (d, *J* = 2.2 Hz, 1H, ArH), 6.84 (d, *J* = 7.7 Hz, 1H, ArH), 6.70 (dd, *J* = 9.0 & 7.9 Hz, 2H, ArH), 5.06 (s, 1H, C*H*), 5.82 (bs, 2H, NH_2), 3.71 (s, 6H, Ar-OCH$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.15, 159.76, 158.39, 147.73, 132.18, 130.41, 130.20, 129.49, 126.40, 120.97, 119.91, 117.28, 115.32, 114.46, 113.95, 111.77, 103.68, 58.21, 55.47, 38.43; HRMS (ESI): *m/z* calcd. for $C_{22}H_{19}N_2O_3^+$ 359.1390; found: 359.0991 [M + H]$^+$.

3-Amino-9-methoxy-1-(4-nitrophenyl)-1H-benzo[f]chromene-2-carbonitrile (4m): IR (KBr) 3470-3350, 2960, 2185, 1660, 1600, 1580, 1540, 1500, 1335, 1270, 1080, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (dd, *J* = 8.7 & 9.2 Hz, 2H, ArH), 7.76 (d, *J* = 8.9 Hz, 1H, ArH), 7.71 (d, *J* = 8.9 Hz, 1H, ArH), 7.44 (d, *J* = 8.7 Hz, 2H, ArH), 7.13 (d, *J* = 8.8 Hz, 1H, ArH), 7.00 (dd, *J* = 8.88 & 8.92 Hz, 1H, ArH), 6.88 (d, *J* = 2.2 Hz, 1H, ArH), 5.33 (s, 1H, C*H*), 6.62 (bs, 2H, NH_2), 3.71 (s, 3H, Ar(naphthyl)-OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.36, 158.65, 153.53, 147.81, 146.62, 131.97, 130.57, 130.03, 128.84, 126.44, 124.51, 120.63, 117.46, 114.54, 114.19, 103.41, 57.05, 55.65, 38.04; HRMS (ESI): *m/z* calcd. for $C_{21}H_{16}N_3O_4^+$ 374.1135; found: 374.0692 [M + H]$^+$.

3-Amino-1-(pyridin-4-yl)-1H-benzo[f]chromene-2-carbonitrile (4n): IR (KBr) 3470-3350, 2960, 2185, 1660, 1600, 1540, 1500, 1270, 1080 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.4 (s, 2H, ArH), 7.84 (t, *J* = 7.9 Hz, 2H, ArH), 7.58 (t, *J* = 7.4 Hz, 1H, ArH), 7.41 (dd, *J* = 6.16 & 6.24 Hz, 2H, ArH), 7.29 (d, *J* = 9.0 Hz, 1H, ArH), 7.15 – 7.13 (m, 2H, ArH), 6.09 (bs, 2H, NH_2), 5.22 (s, 1H, C*H*); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.47, 154.17, 150.52, 147.44, 131.25, 130.42, 129.01, 127.76, 125.55, 123.82, 122.63, 120.58, 117.25, 114.64, 56.79, 37.72; HRMS (ESI): *m/z* calcd. for $C_{19}H_{14}N_3O^+$ 300.1131; found: 300.0666 [M + H]$^+$.

2-Amino-4-(4-chlorophenyl)-4H-benzo[h]chromene-3-carbonitrile (4o): IR (KBr) 3470-3350, 2185, 1660, 1600, 1540, 1500, 1270, 1080, 800, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.85 (s, 1H, CH), 6.00 (bs, 2H, NH2), 6.99 (d, *J* = 8.44 Hz, 1H, ArH) 7.19 (d, *J* = 8.44 Hz, 2H, ArH), 7.27 (d, *J* = 8.4, 2H, ArH), 7.49 – 7.58 (m, 3H, ArH), 7.83 (d, *J* = 7.28 Hz 1H, ArH), 8.25 (d, *J* = 7.92 Hz, 1H, ArH); ^{13}C NMR (101 MHz, DMSO-d6) δ 157.15, 150.28, 141.32, 135.34, 131.21, 129.12, 129.14, 128.34, 127.23, 127.03, 126.45, 125.38, 125.18, 124.24, 122.14, 111.14, 57.19,

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41.18.; HRMS (ESI): *m/z* calcd. for $C_{20}H_{14}ClN_2O^+$ 333.0789; found: 333. 0389 $[M + H]^+$.

2-Amino-4-(3-methoxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (4p): IR (KBr) 3460-3350, 2985, 2180, 1665, 1600, 1540, 1500, 1300, 1250, 1190, 1080, 760 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.70 (s, 1H, OCH_3), 4.75 (s, 1H, CH), 6.01 (bs, 2H, NH_2), 6.90 (d, *J* = 8.44 Hz, 1H, ArH) 7.15 (d, *J* = 8.44 Hz, 2H, ArH), 7.20 (d, *J* = 8.4, 2H, ArH), 7.41 – 7.58 (m, 3H, ArH), 7.75 (d, *J* = 7.28 Hz 1H, ArH), 8.19 (d, *J* = 7.92 Hz, 1H, ArH); ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.92, 157.15, 150.39, 143.21, 134.56, 130.12, 129.13, 127.25, 126.65, 126.24, 126.13, 125.26, 124.22, 124.04, 122.12, 116.34, 115.14, 111.28, 59.28, 57.25, 40.6; HRMS (ESI): *m/z* calcd. for $C_{21}H_{17}N_2O_2^+$ 329.1285; found: 329.0898 $[M + H]^+$.

Results and Discussion

Initially, we reacted β-naphthol, 4-chlorobenzaldehyde, and malononitrile in [bmim][PF_6] ionic liquid at 80 °C for 2 h. The reaction gave an excellent yield (86 %) for **4a.** We also tried different ionic liquids, such as [bmim][Br], [bmim][BF_4], *n*-butylpyridinium bromide, cetylpyridinium chloride, and *n*-butylpyridinium dodecylsulfate for the synthesis of **4a**. Among all these ionic liquids, [bmim][PF_6] gave the highest yield of **4a**. The yields of **4a** under different conditions are given in Table 1. The higher reactivity of [bmim][PF_6], may be due to the fact that it has higher Bronsted acidity and it is hydrophobic in nature, whereas other ionic liquids used are miscible with water. The immiscibility of [bmim][PF_6] ionic liquid and water may result in biphasic environment in the reaction. We also studied the model reaction with different catalyst such as 1,8-diazabicyclo[5.4.0]undec-7-en (DBU), L-proline, benzyltrimethyl-ammonium chloride and tetrabutyl-ammonium fluoride (TBAF) in aqueous medium. The yield of **4a** was moderate (Table 1) and required longer reaction time.

The structure of **4a** was confirmed by ^1H, ^{13}C NMR, and mass analysis. In ^1H NMR spectrum of **4a** a signal appeared as a singlet at δ 5.71 for CH proton and NH_2 protons resonated as a broad peak at δ 4.63. All the other protons were in good agreement with the proposed structure. In ^{13}C NMR spectrum of **4a** CH carbon appeared at δ 37.81 and CH-carbon adjacent to cyano group resonated at δ 57.88. All the remaining carbons were in agreement with required structure. In ESI-MS spectrum of **4a**, a peak appeared at *m/z* 333.0391 for $[M + H]^+$ ion.

Table 1 Synthesis of **4a** under different reaction conditions.

Entry	Catalyst	Time (h)	Yield (%)[a]
1	[bmim][BF_4]	2	71
2	[bmim][Br]	2	50
3	[bmim][PF_6]	2	86
4	*n*-Butylpyridinium bromide	2	50
5	*n*-Butyl pyridinium dodecylsulfate	12	-[b]
6	Cetylpyridinium chloride	3	70
7	DBU	4	60[c]
8	Ferric ammonium citrate	12	8[c]
9	L-Prolein	3	70[c]
10	TBAF	4	50[c]

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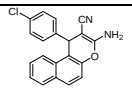
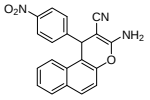
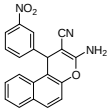
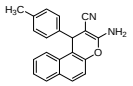
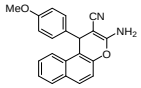
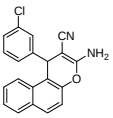
11	Citric acid	4	-[b,c]
12	Amberlyst A26 (OH)	4	-[b,c]
13	SDS	4	-[b,c]
14	Boric acid	4	-[b,c]
15	$Yb(OTf)_3$	4	-[b,c]
16	Benzyltrimethylammonium chloride	4	68[b]

[a]Yields refer to pure isolated compounds, [b]No desired compound, [c]Water is the reaction media.

After optimizing the reaction condition, we examined generality of the reaction by varying aldehydes and naphthols. All the substrate gave excellent yield of the corresponding substituted 2-amino-chromenes (**4**). The yields of different substituted 2-aminochromenes (**4a-p**) are given in Table 2. It is worth to mention that aldehydes with both electron-donating and electron-withdrawing groups reacted smoothly and gave corresponding product in excellent yields. The method also works well with α-naphthol and substituted β-naphthol. Simple reaction condition, easy work-up, and high yield under environmental friendly condition are advantages of the method.

The recovery and reuse of [bmim][PF_6] was also examined, and model reaction was performed using starting materials (**1a, 2a, 3**). After completion of reaction, ethanol was added to the reaction mixture and the precipitate was filtered. The filtrate was concentrated on rotatory evaporator to recover ionic liquid, which was dried under vacuum at 60 ^oC for 30 min. The recovered [bmim][PF_6] was again used for the synthesis of **4a** following the standardized reaction condition. This was repeated five times. The recovered ionic liquid could be used efficiently for the synthesis of **4a** and there was no appreciable decrease in yield of **4a** (Table 3).

Table 2 Synthesis of chromene derivatives (**4a-p**) in [bmim][PF_6] ionic liquid.

Sr. No.	R	R′	Product		Time (h)	Yield (%)[a]	M.p (Lit. M.p) °C
1	H	4-Cl		**4a**	2	86	210-212 (208)[28]
2	H	4-NO_2		**4b**	2	70	168-170 (186)[29]
3	H	3-NO_2		**4c**	2	86	233-235 (239)[30]
4	H	4-Me		**4d**	2	76	268-270 (253)[29]
5	H	4-OMe		**4e**	2	74	199-201 (194)[29]
6	H	3-Cl		**4f**	2	82	236-238 (240)[29]

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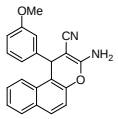
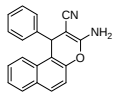
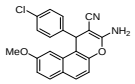
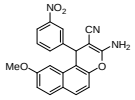
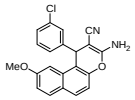
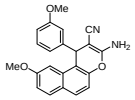
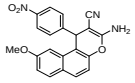
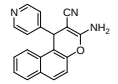
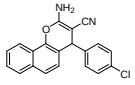
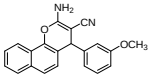
7	H	3-OMe		**4g**	2	81	256-258 (263)[29]
8	H	H		**4h**	2	86	286-288 (279)[29]
9	7-OMe	4-Cl		**4i**	2	79	257-259
10	7-OMe	3-NO_2		**4j**	2	90	248-250
11	7-OMe	3-Cl		**4k**	2	81	260-262
12	7-OMe	3-OMe		**4l**	2	82	205-207
13	7-OMe	4-NO_2		**4m**	2	86	239-241
14	H	4-pyridyl		**4n**	2	89	229-231
15	1-OH	4-Cl		**4o**	2	81	235-237 (232)[31]
16	1-OH	3-OMe		**4p**	2	78	231-233

[a]Yields refer to pure and isolated products.

Table 3: Recycling of ionic liquid for synthesis of **4a**

Run	1	2	3	4	5
Yield (%)[a]	86	83	82	80	76

[a]Isolated yield.

The role of ionic liquid in the reaction is not clear, but it is believed that it activates the aldehydes and malonitrile by coordinating through C-2 proton of imidazolium ion. Such activation of reactions due to acidic C-2 proton of imidazolium ionic liquids have been previously reported in literature.[32] It is proposed that the reaction either proceeds through *o*-quinone methide intermediate (**B**) mechanism (path a) or *via* Knoevenagel condensation followed by Friedel-Craft alkylation and cyclization (path b) (Scheme 2). In path a, *o*-QM is formed by the reaction of aldehyde and β-naphthol, which then reacts with active methylene group of malononitrile to form dicyano compound (**C**). In path b, first malonitrile reacts with aldehyde to give α,β-unsaturated nitrile (**E**) which then reacts with naphthol to give dicyano compound (**C**). The dicyano compound cyclizes to 2-aminochromenes (**4**).

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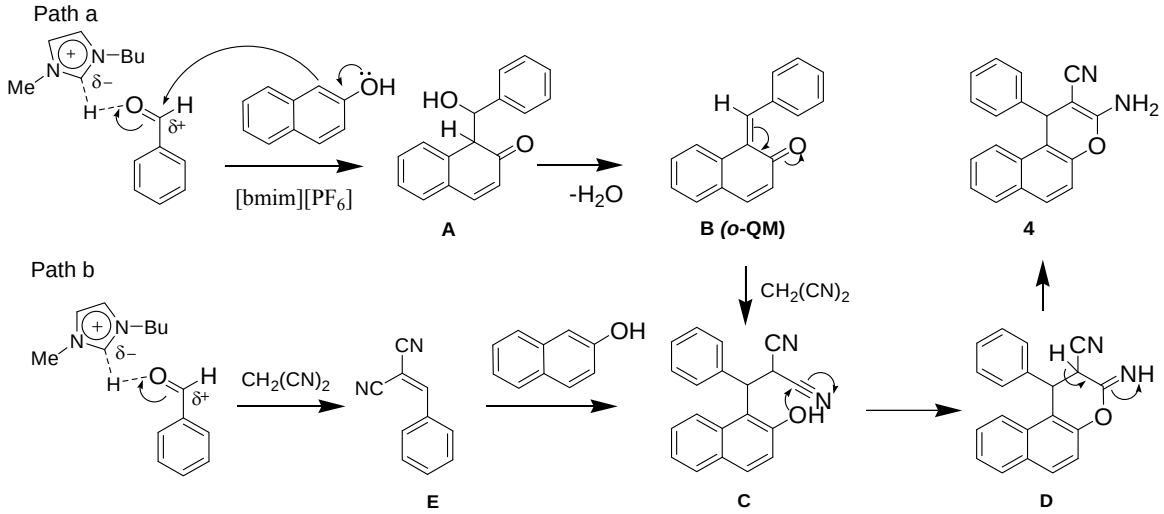

Scheme 2 Proposed mechanism for the formation of **4**.

Mutations and/or overexpression of Src kinase (Src), a prototype member of the Src family of kinases (SFKs) have been implicated in the development of several types of human tumors including breast, ovary, colon, pancreas, prostate, and lung.[33] Increased Src activity is observed in metastatic tumors and it also regulates specific angiogenic factors that promote tumor progression.[34] Thus, designing potent and selective Src kinase inhibitors as anti-cancer agents is a subject of major interest.[35,36] 4-Aryl substituted derivatives of 2-amino-7-dimethylamino-4H-chromene-3-carbonitrile have been evaluated for inhibition of Src kinase and anticancer activity.[37-39] Therefore, the synthesized chromene derivatives **4a-p** were studied for c-Src kinase inhibition (Table 4). Among the all the compounds, **4b**, **4c**, **4j**, **4k**, and **4i** showed modest inhibition of c-Src kinase with IC_{50} values below 100 µM, whereas other compounds were either poor Src kinase inhibitory activity or did not show any inhibition. Among all the compounds, 4-nitro derivative exhibited the highest Src kinase inhibitory activity with an IC_{50} value of 40 µM.

Table 4 Src-kinase inhibitory activity of **4a-p**.

Sr. No.	Compd.	IC_{50} (µM)[a]	Sr. No.	Compd.	IC_{50} (µM)[a]
1	**4a**	150	10	**4i**	>200
2	**4b**	40	11	**4j**	80
3	**4c**	80	12	**4k**	50
4	**4d**	100	13	**4l**	65
5	**4e**	150	14	**4m**	100
6	**4f**	150	15	**4n**	150
7	**4g**	150	16	**4o**	>200
8	**4h**	>200	17	**4p**	150
9	**PPI**	0.6			

[a]The concentration of the compound that inhibited enzyme activity by 50%.

The effect of compounds **4a-p** (50 µM) on the proliferation of selected cancer cell lines such as human breast adenocarcinoma (MCF-7), ovarian adenocarcinoma (SK-OV-3) and acute lymphoblastic leukemia (CCRF-CEM) was evaluated (Figure 2). Compound **4b** inhibited the cell proliferation of all three cancer cells by 60-80% at 50 µM. Compound **4b** was the most potent compound against all three cell lines whereas **4c, 4o** and **4p** showed higher selectivity against CCRF-CEM among all the screened compounds. Interestingly, compound **4b** was also the most potent Src

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kinase inhibitor in this class, suggesting that Src kinase inhibition could have contributed to anti-proliferative activity of this compound.

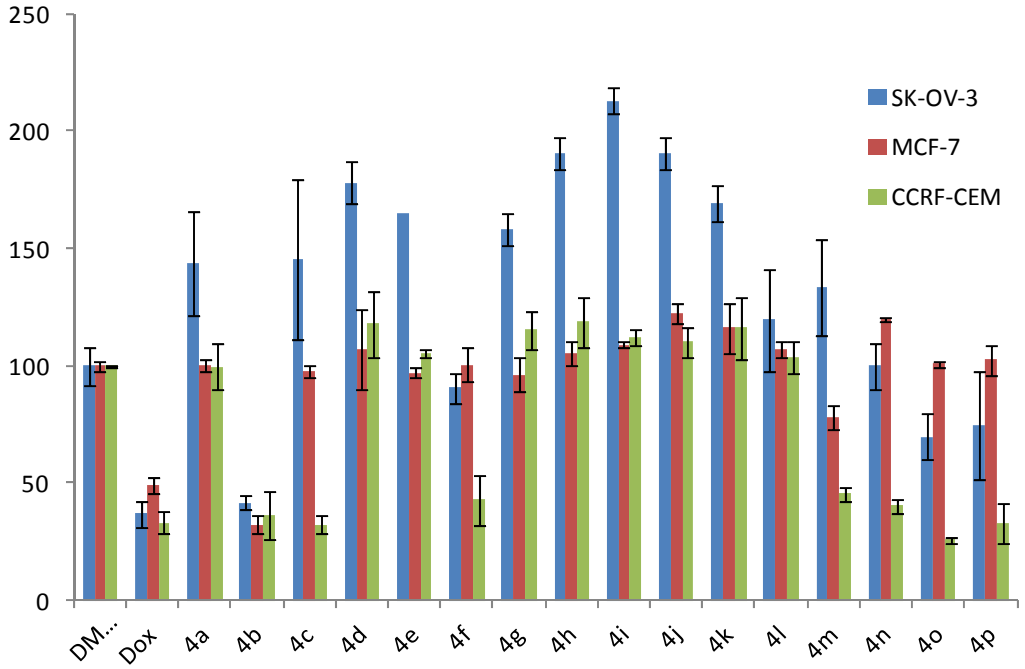

Figure 2. Anti-proliferative activity of chromene derivatives **4a-p**.

Conclusion

In conclusion, we have described an efficient, simple and eco-friendly method for the synthesis of 2-aminochromene derivatives by employing three-component one-pot condensation reaction using [bmim][PF_6] ionic liquid as solvent and catalyst. This method has advantage of simple reaction work up, easy isolation of product, recycle and reuse of catalyst, and high yields of product. All the synthesized chromene derivatives were evaluated for inhibition of Src kinase activity and anti-proliferative activities. Compound **4b** was most potent compound for all three cell lines whereas compound **4c, 4o** and **4p** was most selective for (CCRF-CEM). Compounds **4b** and **4k** showed modest inhibition of c-Src kinase with IC_{50} values below 50 µM. These data provide an efficient strategy for the synthesis of 2-aminochromene derivatives. Potential compounds can be optimized and investigated further as lead anti-proliferative agents.

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References

[1] M. N. Elinson, A. I. Ilovaisky, V. M. Merkulova, P. A. Belyakov, A. O. Chizhov, G. I. Nikishin, Tetrahedron, **2010**, 66, 4043-4048.

[2] A. Hiramoto, J. Nasuhara, K. Michiloshi, T. Kato, K. Kikugawa, Mutation Res., **1997**, 47, 395.

[3] A. M. El-Agrody, M. H. El-Hakim, M. S. Abd El-Latif, A. H. Fakery, E. S. M. El-Sayed, K. A. El-Ghareab, Acta. Pharm., **2000**, 50, 111D120.

[4] P. W. Smith, S. L. Sollis, P. D. Howes, P. C. Cherry, I. D. Starkey, K. N. Cobley, H. Weston, J. Scicinski, A. Merritt, A. Whittington, P. Wyatt, N. Taylor, D. Green, R. Bethell, S. Madar, R. J. Fenton, P. J. Morley, T. Pateman, A. Beresford, J. Med. Chem., **1998**, 41, 787-797.

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Chemistry & Biology Interface, 2012, 2, 6, 362-372

[5] A. Martínez-Grau, J. Marco, Bioorg. Med. Chem. Lett., **1997**, 7, 3165-3170.
[6] T. Ohira, J. Yatagai, Journal Wood Sci., **1993**, 39, 237.
[7] Chem. Abstr., **1993**, 119, 19585d.
[8] G. Bianchi, A. Tava, Agric. Biol. Chem., **1987**, 51, 2001.
[9] V. K. Tandon, M. Vaish, S. Jain, D. S. Bhakuni, R. C. Srimal, Indian J. Pharm. Chem., **1991**, 53, 22.
[10] F. Eiden, F. Denk, Arch. Pharm., **1991**, 324, 875D877.
[11] J. M. Doshi, D. Tian, C. Xing, J. Med. Chem., **2006**, 49, 7731-7739.
[12] A. G. A. Elagamey, F. M. A.-A. El-Taweel, M. N. M. Khodeir, M. H. Elnagdi, Bull. Chem. Soc. Jpn., **1993**, 66, 464-468.
[13] R. Ballini, G. Bosica, M. L. Conforti, R. Maggi, A. Mazzacani, P. Righi, G. Sartori, Tetrahedron, **2001**, 57, 1395-1398.
[14] M. P. Surpur, S. Kshirsagar, S. D. Samant, Tetrahedron Lett., **2009**, 50, 719-722.
[15] M. Kidwai, S. Saxena, M. K. Rahman Khan, S. S. Thukral, Bioorg. Med. Chem. Lett., **2005**, 15, 4295-4298.
[16] G. Shanthi, P. T. Perumal, Tetrahedron Lett., **2007**, 48, 6785-6789.
[17] M. M. Heravi, K. Bakhtiari, V. Zadsirjan, F. F. Bamoharram, O. M. Heravi, Bioorg. Med. Chem. Lett., **2007**, 17, 4262-4265.
[18] J. M. Khurana, B. Nand, P. Saluja, Tetrahedron, **2010**, 66, 5637-5641.
[19] K. Kumaravel, G. Vasuki, Green Chem., **2009**, 11, 1945-1947.
[20] K. Gong, H.-L. Wang, D. Fang, Z.-L. Liu, Catal. Commun., **2008**, 9, 650-653.
[21] R. Maggi, R. Ballini, G. Sartori, R. Sartorio, Tetrahedron Lett., **2004**, 45, 2297-2299.
[22] V. A. Osyanin, D. V. Osipov, Y. N. Klimochkin, Tetrahedron, **2012**, 68, 5612-5618.
[23] Y. Gao, W. Yang, D.-M. Du, Tetrahedron Asymm., **2012**, 23, 339-344.
[24] M. Haumann, A. Riisager, Chem. Rev., **2008**, 108, 1474-1497.
[25] J.-C. Plaquevent, J. Levillain, F. d. r. Guillen, C. Malhiac, A.-C. Gaumont, Chem. Rev., **2008**, 108, 5035-5060.
[26] M. A. P. Martins, C. P. Frizzo, D. N. Moreira, N. Zanatta, H. G. Bonacorso, Chem. Rev., **2008**, 108, 2015-2050.
[27] N. D. Khupse, A. Kumar, Indian J. Chem., **2010**, 49A, 635-648.
[28] A. G. A. Elagamay, F. M. A. El-Taweel, Indian J. Chem., **1990**, 29, 885.
[29] X.-S. Wang, G.-S. Yang, G. Zhao, Tetrahedron Asymm., **2008**, 19, 709-714.
[30] S. Balalaie, S. Ramezanpour, M. Bararjanian, J. H. Gross, Synth. Commun., **2008**, 38, 1078-1089.
[31] L. Chen, X.-J. Huang, Y.-Q. Li, M.-Y. Zhou, W.-J. Zheng, Monatch. Chem, **2009**, 140, 45-47.
[32] R. P. Swatloski, J. D. Holbrey, R. D. Rogers, Green Chem., **2003**, 5, 361-363.
[33] R. B. Irby, T. J. Yeatman, Oncogene, **2000**, 19, 5636-5642.
[34] J. M. Summy, G. E. Gallick, Cancer Metastasis Rev., **2003**, 22, 337-358.
[35] N. Rucci, M. Susa, A. Teti, Anticancer Agents Med. Chem., **2008**, 8, 342-349.
[36] A. Kathuria, S. Jalal, R. Tiwari, A. N. Shirazi, S. Gupta, S. Kumar, K. Parang, S. K. Sharma, Chem. Biol. Interface, **2011**, 1, 279-296.
[37] A. Fallah-Tafti, R. Tiwari, A. Nasrolahi Shirazi, T. Akbarzadeh, D. Mandal, A. Shafiee, K. Parang, A. Foroumadi, Med. Chem., **2011**, 7, 466-472.
[38] W. Kemnitzer, J. Drewe, S. Jiang, H. Zhang, Y. Wang, J. Zhao, S. Jia, J. Herich, D. Labreque, R. Storer, K. Meerovitch, D. Bouffard, R. Rej, R. Denis, C. Blais, S. Lamothe, G. Attardo, H. Gourdeau, B. Tseng, S. Kasibhatla, S. X. Cai, J. Med. Chem., **2004**, 47, 6299-6310.
[39] K. Mansouri, R. Khodarahmi, A. Foroumadi, A. Mostafaie, H. Mohammadi Motlagh, Med Chem Res, **2011**, 20, 920-929.