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Synthesis and Characterization of Novel 2-Amino-Chromene-Nitriles that Target Bcl-2 in Acute Myeloid Leukemia Cell Lines

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Abstract
The anti-apoptotic protein Bcl-2 is a well-known and attractive therapeutic target for cancer. In the present study the solution-phase T3P-DMSO mediated efficient synthesis of 2-amino-chromene-3-carbonitriles from alcohols, malanonitrile and phenols is reported. These novel 2-amino-chromene-3-carbonitriles showed cytotoxicity in human acute myeloid leukemia (AML) cell lines. Compound 4g was found to be the most bioactive, decreasing growth and increasing apoptosis of AML cells. Moreover, compound 4g (at a concentration of 5 μM) increased the G2/M and sub-G1 (apoptosis) phases of AML cells. The AML cells treated with compound 4g exhibited decreased levels of Bcl-2 and increased levels of caspase-9. In silico molecular interaction analysis showed that compound 4g shared a similar global binding motif with navitoclax (another small molecule that binds Bcl-2); however compound 4g occupies a smaller volume within the P2 hot spot of Bcl-2. The intermolecular π-stacking interaction, direct electrostatic interactions, and docking energy predicted for 4g in complex with Bcl-2 suggest a strong affinity of the complex, rendering 4g as a promising Bcl-2 inhibitor for evaluation as a new anticancer agent.

Introduction
Programmed cell death, or apoptosis, is the primary mechanism for the removal of aged and damaged cells. Cancer cells can gain a growth advantage over their normal counterpart by either dividing more quickly, not undergoing terminal differentiation and thus remaining in the proliferative pool, or not undergoing apoptosis [1]. On the functional level, interactions between pro-apoptotic proteins such as Bax, Bak, Bad, Bim, Noxa, Puma, and pro-survival proteins such as Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and Bfl-1 control the regulation of programmed cell death. Cancer cells alter the balance among these opposing factions to undermine normal apoptosis, and thus gain a survival advantage [2,3]. The first identified apoptotic regulator, Bcl-2, was cloned from human follicular B cell lymphoma cells which nearly invariably have a chromosomal t(14;18) translocation, placing the Bcl-2 gene under the control of the powerful IgG heavy chain promoter [4,5] with the consequence of elevated levels of Bcl-2 promoting increased cell survival [6]. A common feature in many human tumors is overexpression of the pro-survival Bcl-2 family members Bcl-2 and Bcl-xL, which make tumor cells resistant to conventional cancer therapeutic agents.

Numerous synthetic small molecules targeting Bcl-2 protein have been studied extensively and few of them have advanced to clinical trials (Figure 1). Structure-based drug design approaches have previously yielded small molecules that bind to Bcl-2 such as navitoclax (ABT-263) [7]. This molecule binds to Bcl-2 and Bcl-xL; unfortunately in clinical trials it caused severe thrombocytopenia due to binding and inhibiting Bcl-xL [8]. Another structure-based synthesis has produced BM-957, a potent small-molecule inhibitor.
of Bcl-2 and Bcl-xL, which was capable of achieving complete tumor regression in a small lung cancer xenograft model [9]. Similarly, the co-crystal structure of Bcl-2 resulted in identification of a small molecule called ABT-199; a Bcl-2–selective inhibitor approved by the FDA for cancer therapy [10]. The above study strongly suggested that an indole based carbinol inhibited the growth of prostate cancer cells by arresting them in the G1 phase of the cell cycle, leading to apoptosis via down-regulation of Bcl-2.

![Figure 1. Known small molecules that target Bcl-2.](image1)

As can be seen, different bioactive scaffolds have been established, however both efficacy and avoiding off-target effects of this class of compounds still remains a challenge.

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![Figure 2. Molecular diversity of amino-nitrils.](image2)

Structural representation (ball and stick model) of the combinatorial libraries of Bcl-2 inhibitors that depicts top and bottom row side chains, which are incorporate to three amino nitrile scaffolds.

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Chromene-based natural and synthetic compounds have contributed substantially to the development of therapeutics as anti-neoplastic agents against various human malignancies [11,12]. Sesilin, tephrosin, calanone and acronycine are some of the naturally occurring chromene derivatives with a very good anti-cancer activity. An important breakthrough in the development of 4H-chromenes as anti-cancer agents was given by the discovery of HA 14-1, which targets the Bcl-2 protein. It is reported to inhibit Bcl-2 by abrogating the interaction of Bax/Bcl-2, and it has also shown synergistic effect when combined with flavopiridol [13–15]. Therefore, the design of new chromene derivatives against anti-apoptotic members of the Bcl-2 family provides a plausible target in cancer therapeutics [16]. We recently reported the synthesis and explored the biological property of chromene-containing benzoxazines and other biologically important heterocyclic libraries [17,18].

In order to improve the efficacy of chromene-based small molecules as selective inhibitors of Bcl-2, we have in the current work synthesized a library of 2-amino chromene-3-carbonitriles to quantify and compare the molecular diversity between different types of libraries that would interact with Bcl-2 [Figure 2]. We also outline the simple graphical approach for describing and comparing molecular diversity within the library of amino-nitriles.

Materials and Methods

Synthesis and characterization of various 2-amino-chromene-nitriles were provided as supplementary data [Data S1 and Table S1].

![Figure 3. Synthesis of a library of 2-amino-chromene-3-carbonitriles from alcohols.](https://doi.org/10.1371/journal.pone.0107118.g003)

![Figure 4. Plausible mechanism of the T3P-DMSO mediated synthesis of title compounds.](https://doi.org/10.1371/journal.pone.0107118.g004)
Table 1. Physical characteristics of the synthesized 2-amino-chromene-3-carbonitriles.

| Entry | 1 (Alcohol) | 3 (Phenols) | Title compounds (4a-t) | Time (Hr) | Yield (%) | MP (°C) |
|-------|-------------|-------------|------------------------|-----------|-----------|---------|
| 4a    | (3-nitrophenyl)methanol | naphthalen-2-ol | 2-amino-4-(3-nitrophenyl)-4H-benzo[g]chromene-3-carbonitrile | 3–4 | 95* | 179 – 182 |
| 4b    | (4-bromophenyl)methanol | naphthalen-2-ol | 2-amino-4-(4-bromophenyl)-4H-benzo[g]chromene-3-carbonitrile | 4.5 | 96* | 138–140 |
| 4c    | (1H-indol-3-yl)methanol | naphthalen-2-ol | 2-amino-4-(1H-indol-3-yl)-4H-benzo[g]chromene-3-carbonitrile | 5 | 92* | 198–200 |
| 4d    | 3-(hydroxymethyl)-4H-chromen-4-one | naphthalen-2-ol | 2-amino-4-(4-oxo-4H-chromen-3-yl)-4H-benzo[g]chromene-3-carbonitrile | 9 | 84 | - |
| 4e    | (2-butyl-4-chloro-1H-imidazol-5-yl)methanol | naphthalen-2-ol | 2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-4H-benzo[g]chromene-3-carbonitrile | 12 | 79 | - |
| 4f    | (2-nitrophenyl)methanol | naphthalen-2-ol | 2-amino-4-(2-nitrophenyl)-4H-benzo[g]chromene-3-carbonitrile | 4-5 | 89* | - |
| 4g    | (2,6-dichlorophenyl)methanol | naphthalen-2-ol | 2-amino-4-(2,6-dichlorophenyl)-4H-benzo[g]chromene-3-carbonitrile | 8 | 91 | - |
| 4h    | (4-fluorophenyl)methanol | naphthalen-2-ol | 2-amino-4-(4-fluorophenyl)-4H-benzo[g]chromene-3-carbonitrile | 4.5 | 93* | 187–189 |
| 4i    | (4-fluorophenyl)methanol | resorcinol | 2-amino-4-(4-fluorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile | 6 | 96* | 218–220 |
| 4j    | (1H-indol-3-yl)methanol | resorcinol | 2-amino-7-hydroxy-4-(1H-indol-3-yl)-4H-chromene-3-carbonitrile | 5 | 92* | - |
| 4k    | 3-(hydroxymethyl)-4H-chromen-4-one | resorcinol | 2’-amino-7’-hydroxy-4-oxo-4’, 4’-[3,4’-bichromene]-3’-carbonitrile | 9.5 | 82 | - |
| 4l    | (2-butyl-4-chloro-1H-imidazol-5-yl)methanol | resorcinol | 2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-7-hydroxy-4H-chromene-3-carbonitrile | 14 | 74 | - |
| 4m    | (4-bromophenyl)methanol | 4-hydroxy-2H-chromen-2-one | 2-amino-4-(4-bromophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 3.5 | 97* | 254–256 |
| 4n    | (1H-indol-3-yl)methanol | 4-hydroxy-2H-chromen-2-one | 2-amino-4-(1H-indol-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 5.5 | 86* | 215–217 |
| 4o    | 3-(hydroxymethyl)-4H-chromen-4-one | 4-hydroxy-2H-chromen-2-one | 2-amino-5-oxo-4-(4-oxo-4H-chromen-3-yl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 10 | 72 | - |
| 4p    | (2-butyl-4-chloro-1H-imidazol-5-yl)methanol | 4-hydroxy-2H-chromen-2-one | 2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 9 | 81 | - |
| 4q    | (3,4-dimethoxyphenyl)methanol | 4-hydroxy-2H-chromen-2-one | 2-amino-4-(3,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 2.5 | 82* | 228–230 |
| 4r    | (2-methyl-1H-indol-3-yl)methanol | 4-hydroxy-2H-chromen-2-one | 2-amino-4-(2-methyl-1H-indol-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 6 | 69 | - |
| 4s    | (3,4-dimethoxyphenyl)methanol | 4-hydroxy-6,7-dimethyl-2H-chromen-2-one | 2-amino-4-(3,4-dimethoxyphenyl)-8,9-dimethyl-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 3.5 | 84 | - |
| 4t    | (4-(trifluoromethyl)phenyl)methanol | 4-hydroxy-6,7-dimethyl-2H-chromen-2-one | 2-amino-8,9-dimethyl-5-oxo-4-(4-(trifluoromethyl)phenyl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile | 4 | 89 | - |

* Isolated yield.
MP - Melting point.
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Cell Culture

Myeloid leukemia cell lines; MOLM13 and MV4-11 were kind gifts by Dr. Martin Grundy (Department of Academic Haematology, University of Nottingham) [28]. MOLM14 cells were generously provided by Dr. Didier Bouscary (Department of Hematology-Immunology, Institut Cochin, Paris, France) [29]. The HL-60 cell line was purchased from ATCC. Cell lines were cultured and maintained in RPMI medium containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere with 5% CO2. All cell lines were regularly screened for absence of mycoplasma. Bone marrow cells from C57BL/6 mice were cultured in IMDM supplemented with 20% FBS and cytokines (10 ng/ml recombinant mouse IL-3, 10 ng/ml recombinant mouse IL-6 and 50 ng/ml recombinant mouse Stem Cell Factor (SCF). Antibodies against Bcl-2, Bcl-xL, Cleaved caspase-9 and GAPDH were purchased from Cell Signalling. ABT-737 was used as a reference compound for all the experiments.

Cell proliferation assay

Cellular proliferation of AML cell lines was measured by using methylthiazolyl-diphenyl-tetrazolium bromide (MTT; sigma-aldrich). 8,000 cells per well were seeded in triplicate, 96-well plates (Corning, Lowell, MA, USA) in 100 μl of medium having 2-amino-chromene-nitriles derivatives. Cells were incubated overnight at 37°C in a CO2 incubator. At the end of the culture duration, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added into each well, and the final concentration of MTT in each well was 0.5 mg/ml. MTT plates were incubated at 37°C in a CO2 incubator for 3 hr. After incubation, Formazan crystals were dissolved in 100 μl of stop solution (SDS-HCl). Absorbance was measured at 570 nm using a Tecan Infinite 200 PRO spectrophotometer (Mannedorf, Switzerland).

Annexin V and propidium iodide (Annexin V–PI) apoptosis assays

HL-60, MOLM13, MOLM14 and MV4-11 (1 x 10^5) cells were seeded into each well of a 6-well plate and treated with 4g at the IC50 concentration for 48 and 72 hours. Staining was performed using FITC Annexin V Apoptosis Detection Kit II, BD Pharmingen (BD Biosciences, USA) according to the manufacturer protocol. Briefly, cells were washed with PBS at least three times, re-suspended in 1 x binding buffer, and FITC-conjugated annexin V and propidium iodide (PI) was added for 15 min in the dark. Samples were analyzed by a LSR-II flow cytometer (Becton-Dickinson, San Jose, CA, USA).

Immunoblot analysis

Cell lysates were prepared using the ProteoJet Mammalian Cell lysis reagent (Fermentas) and the 1 x protease inhibitor mixture (Roche Molecular Biochemicals, Pleasanton, CA). Immunoblotting was performed as described earlier [30]. Monoclonal antibodies against Bcl-2, GAPDH and cleaved caspase-9 (Cell Signalling, Boston, MA, USA) were used.

Figure 5. Screening of active compounds affecting the proliferation of HL-60 AML cells from a library of 2-amino-chromene-nitriles derivatives. MTT assays were performed after incubation of HL-60 cells with indicated concentrations of chromene derivatives 4 (a–y) for 72 hr. For each concentration, percent inhibition values were calculated and data was normalized to diluent controls. The scale X-axis is non-linear and the data represent mean ± SD from three independent experiments done in quadruplicates. ** P<0.001 (Student’s t-test).

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In Vitro analysis of the effect of amino-nitriles against Bcl-2

Zymed Bcl-2 ELISA kit was used for the evaluation of the binding of small molecules to Bcl-2. Initially various concentrations of small molecules and the human Bcl-2 was incubated for 5 minutes and transferred the mixture to the mAb coated 96-well plate. The bound Bcl-2 was tagged with anti-Bcl-2 that conjugated with biotin. The biotin conjugate was bound with streptavidin-HRP. The Streptavidin-HRP was reacted with TM and the absorbance is measured at 450 nm. A standard curve is prepared to determine the Bcl-2 concentration and% inhibition of the Bcl-2 binding to its antibody was presented.

Molecular docking analysis

The molecular modeling was achieved with commercially available InsightII, Discovery Studio (DS) Version 2.5 software packages. Initially, the 3D structure of Bcl-2 was cleaned and the navitoclax binding site was considered for further analysis. All of the calculations were performed using the CHARMM force field as reported previously [31]. Each energy-minimized final docking position of the individual apigenin structural analogues was evaluated using the interaction score function in the CDOCKER module of DS version 2.5 as reported previously from our group [32]. Based on the low CDOCKER energy value, compound 4g was selected for further studies.

Results and Discussion

Synthesis of novel 2-amino chromene-3-carbonitriles derivatives

Naturally occurring 2-amino-carbonitriles containing pyrano[3,2-c]pyridine-based structural motifs are commonly found in alkaloids and exhibit diverse biological activities, including anticancer activity [19]. A series of pyrano[3,2-c]pyridine based flavone and coumarin isosteres containing a 2-amino carbonitrile moiety have also exhibited a broad spectrum of antitumor activity against breast cancer cells [20]. In continuation of our efforts to develop efficient solution phase synthesis of novel small molecules...
with anti-cancer agents, we reported before the synthesis of 2-amino-chromene-3-carbonitriles [21,22], where solution phase T3P-DMSO mediated the efficient synthesis using alcohols, malanonitrile and phenols (Figure 3). The possible mechanism involves the reaction of DMSO with T3P to give an electrophilic sulphur species (c), followed by a substitution reaction of alcohol to form an arylsulphonium salt. This is followed by a hydrogen abstraction of the arylsulphonium salt by hydrolyzed T3P (d) to create a carbonyl compound after elimination of dimethyl sulfide. Next, the phenolic hydroxyl group (2) attacks the carbonyl carbon, and the oxygen on the carbonyl group attacks one more T3P to form an intermediate (c), which in turn attacks malanonitrile (3) to give an anion. This anion attacks intermediate (d), which upon further cyclization converts to compound 4(a-t) (Figure 4). In addition, a variety of aromatic and heterocyclic alcohols, β-naphthol/resorcinol/4-hydroxycoumarin and malanonitrile were added to T3P (50% solution in ethylacetate), DMSO and ethyl acetate as a solvent medium, in order to obtain compounds 4a-t.

The reaction between 1, 2 and 3 in the presence of T3P (2.5 equiv) DMSO generally resulted in a product of appreciable yield (see Table 1) as well as being rather versatile with regard to the choice of substrates. It was observed during the reaction that an increase in temperature resulted in the gradual decrease in yield of the product.

2-amino chromene-3-carbonitrile derivatives elicit an anti-proliferative effect against AML cells

4H-chromenes are potent cytotoxic agents which have been tested against a panel of human cancer cell lines, and a chromene analog, Crolibulin (EPC2407) is currently in Phase I/II clinical trials for the treatment of advanced solid tumors [23]. We initially tested a series of 20 chromene derivatives 4(a-t) for their cytotoxic effects against a human acute myeloid leukemia (AML) cell line (HL-60), using three different concentrations (10, 50 and 100 μM) of 2-amino-chromene-carbonitriles. Among the tested compounds, 4g significantly (*P*≤0.001) decreased proliferation of the AML cells, even at 10 μM concentrations. Other derivatives of these compounds showed either no or very low effect at 10 μM concentrations (Figure 5).

Additional studies were performed for compound 4g, which was the most active among the structurally related compounds tested in culture. The anti-proliferative activity of compound 4g against three additional AML cell lines (MOLM13, MOLM14 and MV4-11) was examined. Again, compound 4g significantly decreased...
the proliferation of the AML cells with an ED50 of 5 to 10 μM after 48, 72 and 96 hours of culture (Figures 6). Compound 4g inhibited proliferation of the AML cells with an IC50 of 5 μM. Further, C57BL/6 mouse bone marrow cells were treated with 5, 10 μM compound 4g to assess the effect on normal hematopoietic cells. As shown in Figure 7A, compound 4g (5, 10 μM) treatment displayed minimal anti-proliferative response against the normal bone cells suggesting that compound 4g decreases proliferation of the AML cells, and has little effect on proliferation of normal bone marrow cells.

Compound 4g inhibits Bcl-2 protein and triggers cell cycle arrest at G2/M phase in AML cells

HA14-1 is a cell permeable Bcl-2 inhibitor, and acts by binding to the surface pocket of Bcl-2 [24] and disrupts the Bax/Bcl-2 interaction, resulting in induction of apoptosis of tumour cells. Interestingly, treatment of HL-60, MOLT-3, MOLM-14, MV4-11 cell lines with compound 4g (5 μM, 24 hr) decreased levels of Bcl-2 (Figure 7B). Moreover, we checked the effect of compound 4g on the expression of the Bcl-xL. Our western blotting results showed that compound 4g does not inhibit the expression of Bcl-xL protein (Figure 7C).

The effect of compound 4g (5 μM, 72 hr) on the cell cycle analysis of MOLM13, MOLM14, and MV4-11 AML cells using flow cytometry showed significant increase in the G2/M and sub-G1 phases (apoptosis), as compared to the diluents control cells (Figures 8A and 8B). The cell population in the G1 phase of the cell cycle decreased.

Compound 4g induces apoptosis of AML cells

Core scaffolds such as flavones or chromenes induce apoptosis in human myeloid tumor cells [25]. Therefore, the HL-60, MOLT-3, MOLM-14, and MV4-11 cells were treated with compound 4g (5 μM for 48 and 72 hr) and analysed for the induction of apoptosis using FITC-conjugated annexin V (AV) and propidium iodide (PI). Both early (AV+PI−), as well as late (AV+PI+) apoptosis occurred in AML cells (Figure 9A). Detailed percentage of apoptotic cells in cell lines are summarized in supplementary data (Table S1). This was associated with increased levels of cleaved caspase-9 in these cell lines (Figure 9B), which is an enzyme known to be activated during programmed cell death.
In Vitro analysis of the effect of amino-nitriles against Bcl-2

Bcl-2 ELISA kit from Zymed was used to determine the inhibitory activity of most active of small molecule towards Bcl-2. Among the four tested compounds, 4a, 4h, and 4f at 30 µM concentration, inhibited the Bcl-2 binding to its antibody by 37.8, 53.0, 61.9% respectively (Figure 10). The most active compound 4g inhibited the Bcl-2 binding to its antibody effectively with an IC₅₀ value of 17.8 µM, indicate its strong affinity towards Bcl-2 compared to structurally related chromenes. Among the tested small molecules, 2,6- dichlorophenyl, 2-nitrophenyl, 4-fluorophenyl and 3-nitrophenyl side chain containing amino-nitrils displayed significant binding affinity towards Bcl-2 indicating that electron withdrawing group favors the effective binding to Bcl-2.

In silico docking analysis of chromene based small molecule (4g) that targets Bcl-2

Bcl-2 appears to be a possible target for compound 4g as it induced the apoptosis by decreasing the level of anti-apoptotic protein Bcl-2 in AML cell lines. Hence, we performed in silico
molecular docking using the three-dimensional structures of Bcl-2, which consists of two central primarily hydrophobic $\alpha$-helices surrounded by amphipathic helices. Our study was aided by the recent definition of the X-ray crystal structure of Bcl-2–navitoclax (PDB ID: 4LVT) [10]. Navitoclax engages the two hydrophobic ‘hot spots’ regions termed P2 and P4 that are known to show a high-affinity binding by proapoptotic peptides [26,27]. All docking calculations were performed using Accelrys Discovery Studio Version 2.5 and the automated docking program CDOCKER, which utilises a molecular dynamics-based simulated annealing algorithm. The active site of the enzyme was defined, and CDOCKER docked the most active compound against the navitoclax binding hotspot of Bcl-2. Figure 10 shows the best-docked conformation of compound 4g and navitoclax in Bcl-2 obtained from the CDOCKER program. Docking energies of the best configuration of compound 4g and navitoclax against Bcl-2 were 4.55 and 5.45 kcal/mol, respectively. In addition, we also carried out the molecular docking analysis for the other bioactive amino nitriles with Bcl-2 and its CDOCKER energies are provided in the supplementary data (Table S2). The 3-dimensional illustrations of the interaction map of Bcl-2 with compound 4g and navitoclax were produced using the Accelrys visualization tool (Figure 11A), and an intermolecular $\pi$-stacking interaction of napthyl group with Phe101 and Tyr105 of P4 host spot of Bcl-2 was observed. The 2,6-dichlorophenyl group of compound 4g showed electrostatic interactions with the conserved arginine (Arg104) and in turn made a unique contact with the intra-molecular hydrogen bond formed by Ala97, Asp100, Phe101, and Tyr199 (Figure 11A). In addition to this, the amino-chromene scaffold was buried in the hydrophobic pocket of Gly142, Val145, Trp141, Asn140, Phe195, Leu198, and Tyr199. The analysis of compound 4g bound to the navitoclax binding site showed that it shares a similar global binding motif with navitoclax, but occupies a smaller volume within the P2 hot spot (Figure 11B).

Conclusions
We report for the first time the solution phase, T3P-DMSO mediated, efficient synthesis of 2-amino-chromene-3-carbonitriles from alcohols, malanonitrile and phenols. These 2-amino-chromene-3-carbonitriles were tested for their cytotoxicity using human AML cell lines. Compound 4g was the most bioactive among the structurally related compounds. It decreased proliferation, increased caspase-9 mediated apoptosis, and caused G2/M arrest of the MOLM13, MOLM14, and MV4-11 AML cells. Our docking analysis of 4g suggests Bcl-2 as a reasonable target, hence rendering compound 4g as a promising Bcl-2 inhibitor for future studies as a new anticancer agent.

Supporting Information
Table S1 Quantification of early apoptotic and late apoptotic cells (%). AML cells were cultured with 4g (5 $\mu$M) for either 48 or 72 hours and propidium iodide (PI) and FITC conjugated Annexin V positive cells were enumerated. Results are a representative experiment. The experiments were done three times in triplicates. (DOCX)

Table S2 Computational analysis of the binding of amino nitriles against Bcl-2. (DOCX)
Data S1  General procedure for one pot synthesis of 2-amino chromene-3-carbonitriles.

(DOCX)

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Author Contributions

Conceived and designed the experiments: MG B KSR HPK. Performed the experiments: HKK CDM MG VM DK DJM RS B. Analyzed the data: MG B KSR HPK. Wrote the paper: MG DJM B KSR HPK.