Oncology Research, Vol. 27, pp. 957–964
Printed in the USA.

0965-0407/19 $90.00 + .00
DOI: https://doi.org/10.3727/096504019X15555428221646
E-ISSN 1555-3906
www.cognizantcommunication.com

FCY-302, a Novel Small Molecule, Induces Apoptosis in Leukemia and Myeloma Cells by Attenuating Key Antioxidant and Mitochondrial Enzymes

Prasanna Rajagopalan,*† Abdulrahim Hakami,*† Mohammed Ragab,* and Ashraf Elbessoumy*‡

*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha, Kingdom of Saudi Arabia
†Central Research Laboratory, College of Applied Medical Sciences, King Khalid University, Abha, Kingdom of Saudi Arabia
‡Department of Biochemistry, Faculty of Science, Alexandria University, Alexandria, Egypt

Arylidene analogs are well proven for biological activities. FCY-302, a novel small molecule belonging to this class, was screened for its biological efficacy in leukemia and myeloma cells. FCY-302 selectively inhibited proliferation of cancer cells with GI_{50} values of 395.2 nM, 514.6 Nm, and 642.4 nM in HL-60, Jurkat, and RPMI-8226 cells, respectively. The compound also increased sub-G_0 peak in the cancer cell cycle and favored apoptosis determined by annexin V assay. The compound decreased the antiapoptotic Bcl-2 levels and increased proapoptotic Bax proteins in leukemia and myeloma cell lines. FCY-302 attenuated the mitochondrial membrane-bound Na^+/K^+ ATPase, Ca^{2+} ATPase, and Mg^{2+} ATPase enzyme activities and significantly decreased activities of antioxidant enzymes like SOD, CAT, G_R, and GST in all the three cancer cells tested. Our findings suggest that FCY-302 inhibits the proliferation of leukemia and myeloma cancer cells by altering key mitochondrial and antioxidant enzymes, eventually driving them to apoptosis. These results drive focus on FCY-302 and its analogs to be developed as potential small molecules with bioactivities against cancer.

Key words: Antiproliferative; Antioxidant enzyme; Apoptosis; Arylidene; Leukemia; Mitochondrial enzyme; Myeloma

INTRODUCTION

Leukemia and myeloma are the two forms of cancers that remain as most dreadful conditions in their class. Both the forms of malignancies, once initiated, are characterized by uncontrolled proliferations and are unmet with significant medical need[1,2]. Current treatment regimens of acute leukemia and myeloma are very much limited to individual or combination chemotherapies with dose-limiting toxicities and drug resistance[3]. These limitations therefore open a scope for development of safer and efficacious drugs to treat leukemia and myeloma.

Small molecules are focused in the recent past as effective chemotherapeutics to tackle various diseases. Understanding of medicinal chemistry at the molecular level and development of high-throughput screening have resulted in many synthetic chemical compounds to be tested in vitro for bioactivity. These chemical compounds add value to current drug research with emphasis on effective, economical, and less toxic drugs. Chemotherapy considerably improves survivals rates and stops further malignancy but is mostly restricted by its toxic side effects[4]. To overcome resistance that malignant cells build against anticancer drugs, there is great interest in the search for new small molecule therapeutic agents[5].

Arylidenes are such small molecules, and their derivatives are widely proven effective for regulation of cancer cell cycle, antimolluscicidal, antidegenerative, hypoglycemic, and hypolipidemic activities[6–9]. Few derivatives of arylidene have also exhibited excellent cytotoxic potencies[10,11]. However, there is no literature evidence for arylidene derivatives possessing antiproliferative activity for leukemia and myeloma cells. As it is well proven that relationships exist among antioxidant status and antiproliferative properties in certain cancers[4], search of novel compounds targeting oxidant status and therapeutic efficacy against cancer will be largely rewarding. The current investigation was therefore undertaken to screen our lead molecule (2E)-7-methyl-2-(phenylmethylidene)-4-(propan-2-yl)-2,3-dihydro-1H-inden-1-one (internal product code

Address correspondence to Dr. Prasanna Rajagopalan, Ph.D., FICS, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Room number 3/151, Building C, King Khalid University, P.O. Box: 960, Guraiger, Abha 61421, Saudi Arabia. Tel: +966(0)17-241-9285; +966540924232; E-mail: rajagopalan@kku.edu.sa

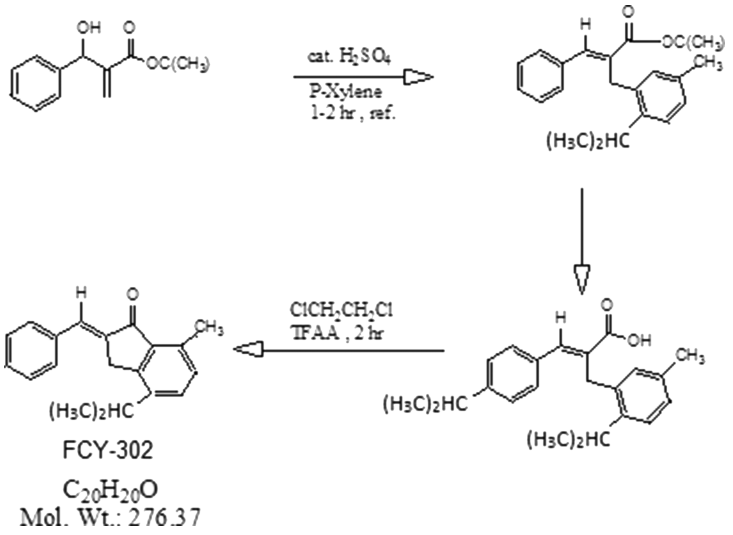

Figure 1. Structure and synthesis of FCY-302.

FCY-302) for antioxidant and antiproliferative effects in cancer cells. A brief synthesis method of FCY-302 is indicated in Figure 1.

MATERIALS AND METHODS

HL-60, RPMI-8226, Vero, and Jurkat cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Iscove's modified Dulbecco's medium, Eagle's minimum essential medium, RPMI-1640 medium, and fetal calf serum (FCS) were purchased from Hi Media Corp (Mumbai, India). Annexin V and cell cycle analysis kits were from Millipore Corp. (Boston, MA, USA). Bcl-2, Bax, and β-actin antibodies were from Cell Signaling (Danvers, MA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and all other biochemicals were from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture

Vero cells were cultured in Eagle's minimum essential medium; RPMI-8226 cells were grown in Iscove's modified Dulbecco's medium. HL-60 cells and Jurkat cells were grown in RPMI-1640 medium. All complete growth medium was supplemented with 10% FBS, 100 U/ml of penicillin, and 100 U/ml streptomycin. Cells (passages 3–12) were maintained in a humidified atmosphere of 5% CO_2 incubator at 37°C until the confluence stage was attained. The medium was replaced every alternative day, and the maintenance was strictly in accordance with the standard methods.

Cell Proliferation Assay

Proliferation assay was performed as described by Mosmann[12] with modifications; 5,000 cells/well in 100 µl of RPMI media supplemented with 10% FBS and 1% penicillin–streptomycin were plated in triplicates in a 96-well plate. Cells were incubated overnight, and 50 µl of the compound containing the desired final concentration was added along with a DMSO blank. The cells were incubated at 37°C and 5% CO_2 for 72 h. MTT (15 µl of 5 mg/ml) was added and incubated for 3.5 h. Media was aspirated, and MTT was dissolved in 150 µl of DMSO, and absorbance was read at 560 nm with reference at 640 nm. Percent inhibition was calculated after subtracting day 0 MTT read. Results were analyzed using Graphpad Prism 6.0 software (La Jolla, CA, USA).

Cell Cycle Analysis

Cancer cells were seeded at a density of 0.5×10^6 cells per well in a six-well plate and incubated with FCY-302 at desired concentrations for 72 h at 37°C in a 5% CO_2 incubator. After incubation, cells were fixed in 70% ethanol and stored at −20°C until analysis. Cells were stained with Guava® Cell Cycle reagent according to the manufacturer's instructions, and 10,000 events were acquired on a Guava easyCyte™ flow cytometer. Data were analyzed using Express Pro software (Millipore), and percent cell population in different cell cycle stages with respect to control was calculated.

Annexin V Assay

The assay was performed using Annexin-V detection kit from e-Biosciences (Grand Island, NY, USA) as per the manufacturer's instructions as follows. Cells (0.5×10^6) were grown in six-well plates with complete growth media for 24 h until they are semiconfluent. Cells were treated with respective concentrations of FCY-302 and incubated in a CO_2 incubator for 48 h. Harvested

cells were washed twice with wash buffer and incubated with 0.25 µg/ml annexin V reagent in 1× binding buffer for 15 min. After a couple of washes with wash buffer, cells were resuspended back in binding buffer containing 0.5 µg/ml propidium iodide, and 10,000 events were acquired on a Guava easyCyte™ flow cytometer. Data were analyzed using InCyte software-Millipore. Early and late phase apoptotic cells were segregated with a quadriplot graph, and total percentage of apoptotic cells was represented using Graphpad Prism 6.0 software.

Western Immunoblotting

Western blotting procedure for determining the anti- and proapoptotic signaling proteins were carried out as described elsewhere[13] with minor modifications. Briefly, cancer cells were treated with desired concentrations of FCY-302 for 48 h and lysed in buffer containing 50 mM Tris, 150 mM NaCl, 0.5% deoxycholate, 0.1% SDS, 2 mM EDTA, 0.1% Triton X-100, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, and 5 µg/ml pepstatin A. Proteins were quantified using Coomassie Plus Protein Assay Reagent kit (Pierce, Rockford, IL, USA), and 20–40 µg of total cellular proteins from cell lysate was separated using 8%–15% SDS polyacrylamide gel electrophoresis. The proteins were further transferred to a nitrocellulose membrane, probed with the respective primary antibodies, and added with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies. Membrane stripping was done by stripping buffer at 50°C for 30 min and quantified. Protein bands were visualized using ECL reagents (Amersham Bioscience, Piscataway, NJ, USA) and exposed to Kodak X-Omat Blue XB-1 films (Rochester, NY, USA). Bands were quantified using ImageJ (Ver: 1.46, NIH) and normalized to actin.

Mitochondrial Membrane-Bound Enzyme Assays

HL-60, Jurkat, and RPMI-8226 cells were incubated with respective concentrations of FCY-302 for 48 h. The cells were then pelleted and washed once with phosphate buffer and resuspended in the same buffer and frozen. The suspension was thawed to break the cells and cooled in ice. The cell extract was made in 2.5 mM in $MgCl_2$ and centrifuged at 6,000×*g* for 10 min to remove the unbroken cells. The membrane fraction was collected by centrifugation at 50,000×*g* for 45 min. After washing twice and rehomogenization in the same buffer (ice cold), the membrane fraction was used for the estimation of Na^+/K^+ ATPase[14], Ca^{2+}, ATPase[15], and Mg^{2+} [16].

Statistical Analysis

Experiments were carried out at least in triplicate, and results are expressed as the mean±SD. Statistical analyses were performed using Graphpad Prism 6.0. GI_{50} and IC_{50} values were calculated using a nonlinear regression fit model with variable slope and plotted accordingly. Differences with a value of $p<0.05$ were considered statistically significant.

RESULTS

Antiproliferative Efficacy of FCY-302 in Cancer Cell Lines

We used acute promyelocytic leukemia cell line HL-60, myeloma cell line RPMI-8226, and acute T-cell leukemia cell line Jurkat to assess the antiproliferative/cytotoxic effect of the compound in cancer cells. To check out the nontoxic levels of the compound in normal cells and determine the selectivity toward cancer cells, nontumorigenic Vero cells were used. The compound inhibited the proliferation of HL-60, Jurkat, and RPMI-8226 cells with GI_{50} of 395.2 nM, 514.6 nM, and 642.4 nM, respectively (Fig. 2A). GI_{50} was 4,881 nM for Vero cells (Fig. 2A). FCY-302 was around 8-, 9-, and 13-fold more selective toward RPMI-8226, Jurkat, and HL-60 cells, respectively, in comparison with the normal Vero cells (Fig. 2B). We used the closest GI_{50} values; 400 nM FCY-302 in HL-60 cells, 520 nM in Jurkat cells, and 650 nM in RPMI-8226 cells for all the following assays.

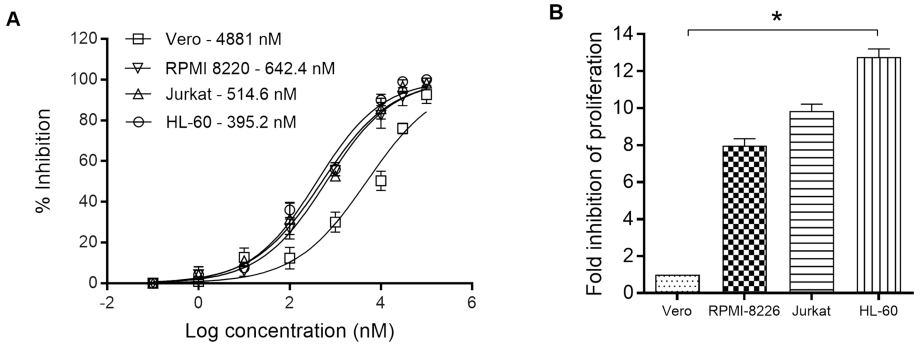

Figure 2. Efficacy of FCY-302 in cancer and normal cells. (A) GI_{50} values of FCY-302 in HL-60, Jurkat, RPMI-8226, and Vero cells showing antiproliferative effects by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. (B) Fold difference in antiproliferative efficacy by the compound between normal and cancerous cells. Results are average from three individual experiments performed in triplicates. *$p\leq0.05$.

Effects of FCY-302 on Cell Cycle Changes in Cancer Cells

To further elucidate the observed antiproliferative effects of the compound, we analyzed the effect of FCY-302 in the cell cycle of cancer cells. Appearance of a hypodiploid peak was observed in all the three types of cancer cells after FCY-302 treatment (Fig. 3). An increase in the percentage of sub-G_0 phase of cells with respective controls of 3%–15% to 14.23% in HL-60 cells (Fig. 3A), 3.46% to 11.93% in Jurkat cells (Fig. 3B), and 1.80% to 9.25% in RPMI-8226 cells (Fig. 3C) were observed.

FCY-302 Induced Apoptosis in Leukemia and Myeloma Cells

FCY-302 treatment resulted in the apoptotic indicator annexin V^+ cells of 32%, 28.87%, and 31.28% in HL-60, Jurkat, and RPMI-8226 cells, respectively (Fig. 4). FCY-302 treatment also decreased the antiapoptotic Bcl-2 levels and increased the proapoptotic Bax protein levels in HL-60, Jurkat, and RPMI-8226 cells (Fig. 5A), thereby significantly decreasing the Bcl-2/Bax ratio in all three cell lines (Fig. 5B).

FCY-302 Attenuated Mitochondrial and Antioxidant Enzyme Activities

In order to augment the observed antiproliferative and apoptosis-inducing effects of FCY-302 in cancer cell lines, we tested the effects of the compound on mitochondrial and antioxidant enzymes for all three cancer cell lines. Significant inhibition of Na^+/K^+ ATPase activity was observed in HL-60, Jurkat, and RPMI-8226 cells (Fig. 6A). There was a reduction in activities of Ca^{2+} ATPase post-FCY-302 treatment in these cancer cells (Fig. 6B). Mg^{2+} ATPase activities also remained inhibited, suggesting an alteration in the homeostasis of these ions (Fig. 6C). GSH was reduced in all three cancer cells after FCY-302 treatment (Fig. 7A). Activity levels of antioxidant scavenging enzymes like superoxide dismutase (SOD) catalase (CAT), glutathione reductase (G_R), and glutathione-S-transferase

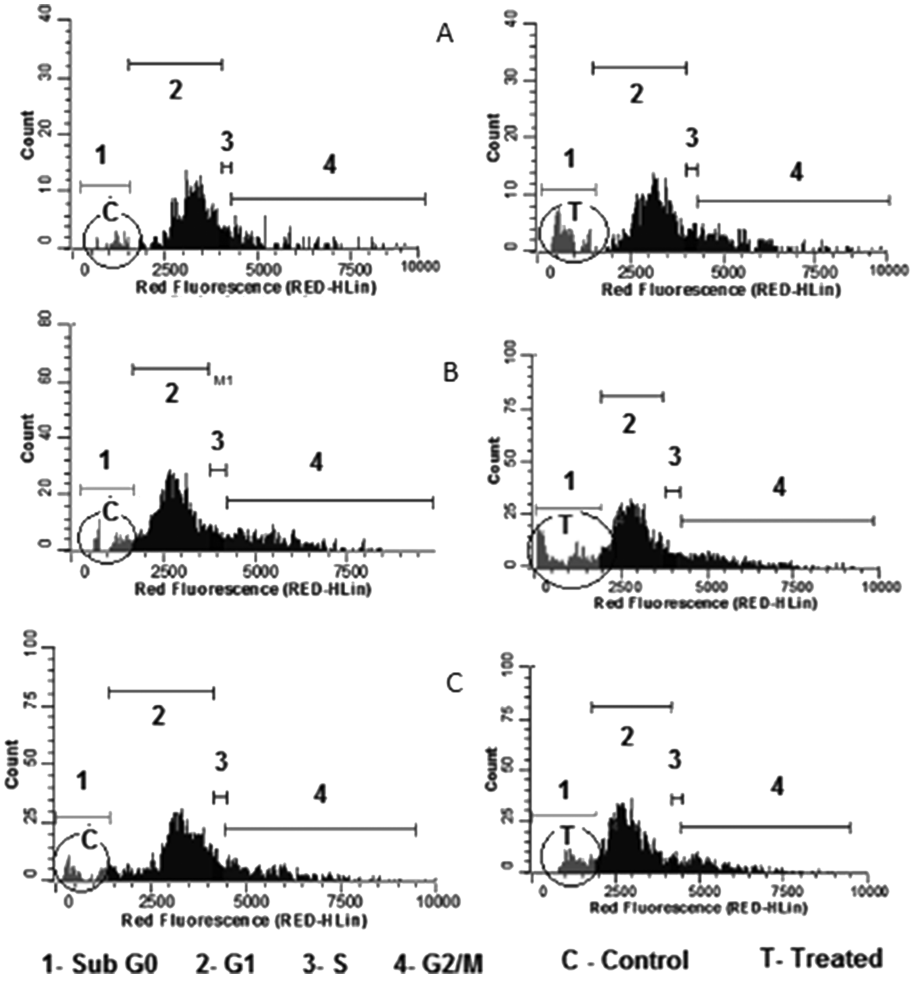

Figure 3. Cell cycle analysis of (A) HL-60, (B) Jurkat, and (C) RPMI-8226 cells after 72 h treatment of FCY-302. Representative cell cycle histograms from three individual experiments showing percentage sub-G_0 population in cancer cells after FCY-302 treatments.

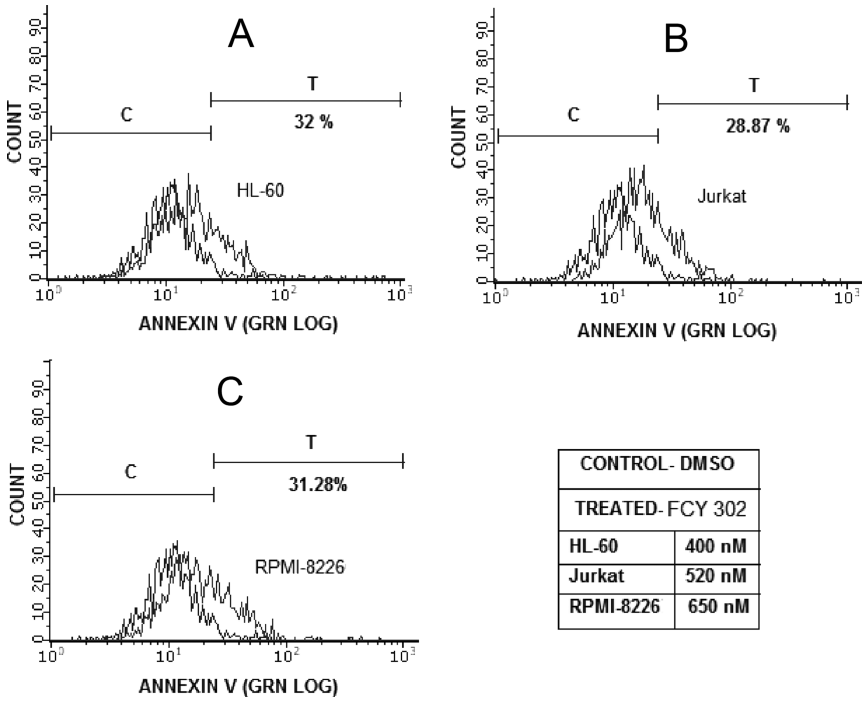

Figure 4. Representative histograms from annexin V assay post-FCY-302 treatment in (A) HL-60, (B) Jurkat, and (C) RPMI-8226 cells after 48 h. Results from three independent assays are shown.

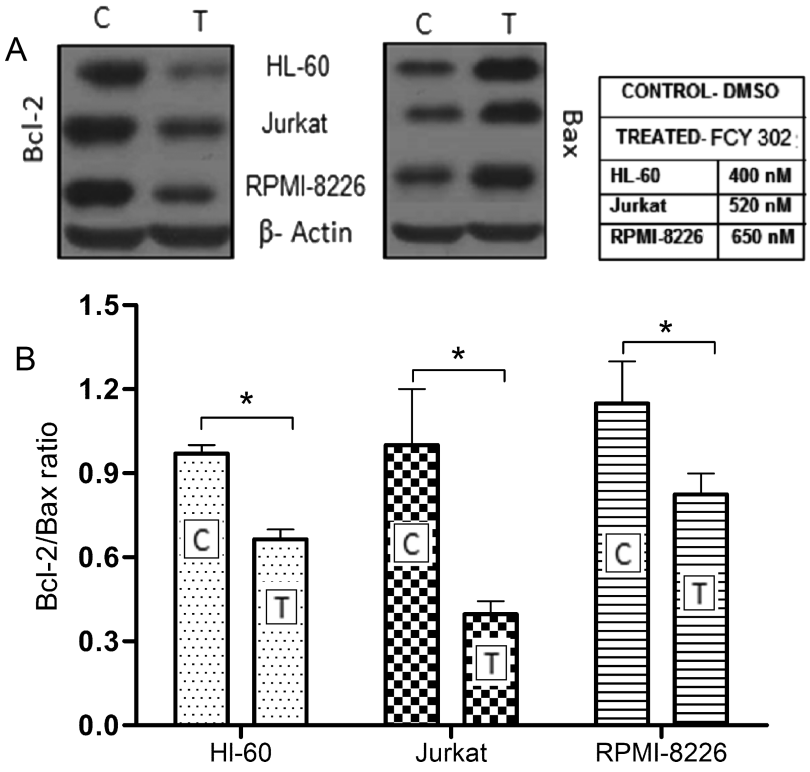

Figure 5. (A) Western blot of antiapoptotic and proapoptotic markers after various doses of FCY-302 treatment in HL-60, Jurkat, and RPMI-8226 cells for 48 h. (B) Bcl-2/Bax ratio of HL-60, Jurkat, and RPMI-8226 cells after FCY-302 treatment. Bands were quantified by densitometry using ImageJ (Ver: 1.46, NIH), normalized to actin, results were plotted with GraphPad Prism 6 software. Results are from three individual experiments in duplicates and were statistically significant at $*p < 0.5$.

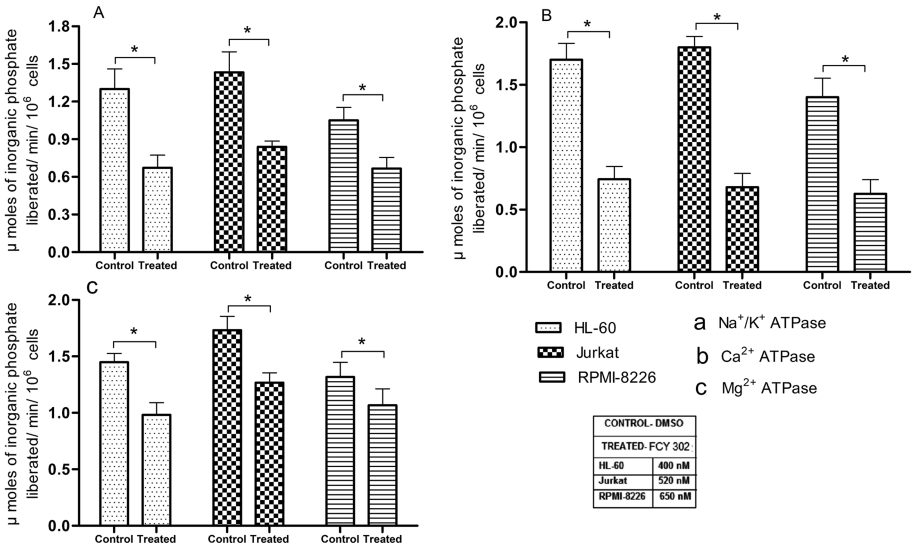

Figure 6. The levels of mitochondrial membrane-bound enzyme activities in FXY-302-treated HL-60, Jurkat, and RPMI-8226 cells at the end of 48-h treatment. (A) Na^+/K^+ ATPase activity, (B) Ca^{2+} ATPase activity, (C) Mg^{2+} ATPase activity. Data represent mean ± SD of three individual experiments performed in triplicate, and results were statistically significant at $*p<0.5$.

(GST) were also found to be decreased with FCY-302 treatment in the three cell lines tested (Fig. 7B–E).

DISCUSSION

Novel chemotherapeutics for leukemia and myeloma are needs of the hour, and biologically active small molecule has been the key research focus in recent past[17]. FCY-302 is shown to inhibit the proliferation of three different cancer cells in the current investigation. Observation of 8- to 13-fold potency in the tested cancer cells over non-cancerous Vero cells clearly indicates the selectivity of FCY-302 to inhibit cancer cell proliferation with a reasonable therapeutic window in vitro. Appearance of the hypodiploid peak in cell cycle analysis post-FCY-302

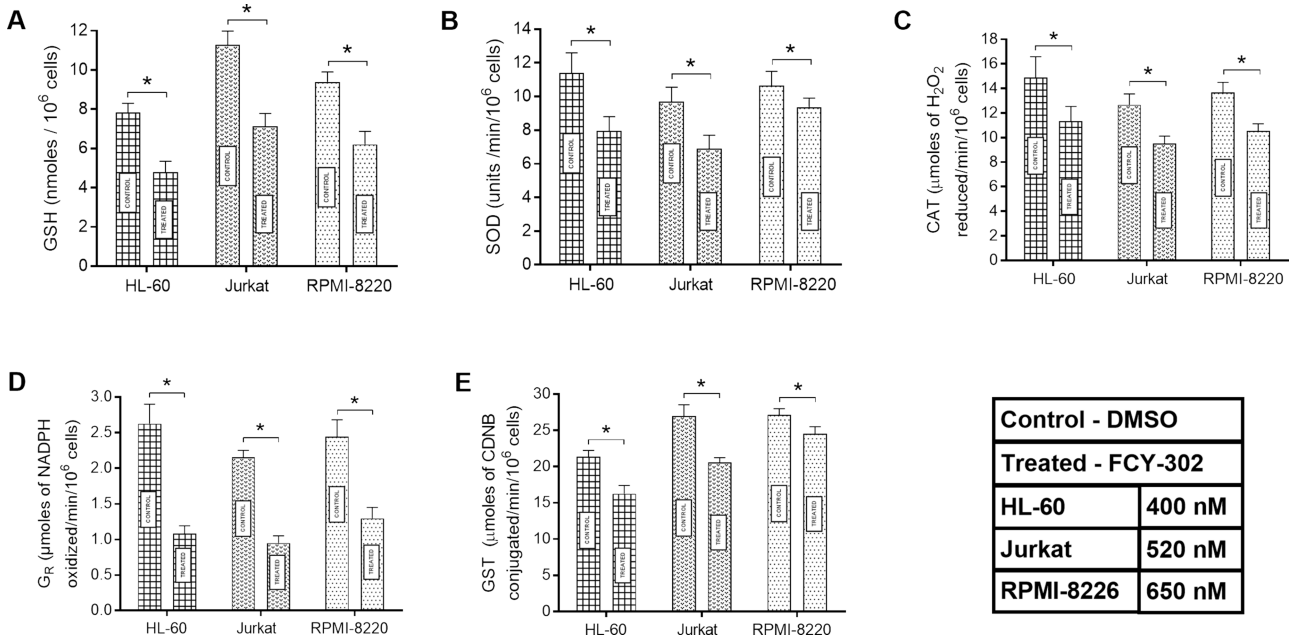

Figure 7. Antioxidant status of FCY-302-treated cancer cells. Levels of (A) GSH, activities of (B) superoxide dismutase (SOD), (C) catalase (CAT), (D) glutathione reductase (G_R), and (E) glutathione-S-transferase (GST) post-FCY-302 treatments. Results are expressed as mean value ± SD. $*p \leq 0.05$.

treatment indicates DNA damage induced by the compound in cancer cells[18].

Annexin V binds with high affinity to phospotidyl serine of the mitochondrial membrane when exposed to apoptotic cell surface and differentiates healthy cells from apoptotic cells by considering the cellular membrane integrity[19]. The occurrence of annexin V^+ cells after FCY-302 treatment indicated apoptotic induction by the compound in leukemia and myeloma cells. It is reported that apoptotic events in cancer cells are closely regulated by the antiapoptotic Bcl-2 protein and the proapoptotic Bax protein[20]. Overexpression of the Bcl-2 proteins is believed to provoke proliferation and confer resistance in many cancer cell types[20]. On the other hand, studies also indicate that upregulation of the Bax protein is responsible for apoptotic induction[21]. The current investigation supports these claims with downregulation of the Bcl-2 and upregulation of Bax proteins, thereby favoring apoptosis in the cancer cells.

Studies indicate a tight correlation between the Bcl-2, Bax proteins, and mitochondrial membrane integrity[22]. The susceptibility of the cells to undergo apoptosis is controlled by Bcl-2 protein by regulating the membrane integrity of the mitochondria, while Bax helps in permeabilization of the mitochondrial membrane favoring apoptosis[22]. Bcl-2/Bax ratio is therefore a key event for apoptotic induction mediated by mitochondria. Our observations of decrease in the Bcl-2/Bax ratio by FCY-302 treatment can therefore be attributed to mitochondria-mediated apoptosis in leukemia and myeloma cells. Therefore, it could be inferred that the increased levels of Bax and decreased levels of Bcl-2 would have led to loss of mitochondrial potential leading to cell suicide and making the cells bioenergetically deficient[23]. These claims were further confirmed by the decrease in the mitochondrial membrane-bound enzyme activities upon FXY-302 treatment, which was in line with previously published research[24,25].

To further augment our observations, we checked the status of key antioxidant enzymes after FCY-302 treatment in cancer cells. In aerobic organisms, reactive oxygen species (ROS) including O_2^-, OH^-, and H_2O_2 are continuously generated by mitochondrial action, which remains a potent threat to cell survival by inducing oxidative stress. To detoxify ROS, the mitochondria have developed/adapted several defense mechanisms by combined action of antioxidant enzymes[26] like SOD, CAT, GST, and G_R. Out of these enzymes, SOD catalyzes the dismutation of the superoxide anion to H_2O_2, while catalase favors the conversion of H_2O_2 to water and oxygen[27]. Though appearing hostile, oxidative stress could be beneficial by inducing apoptosis in cancer cells[28,29]. Studies indicate oxidative stress and free radicals that are created by chemotherapeutic agents and could be helpful in treatments of leukemia and myeloma[30,31]. The activity levels of SOD, CAT, GST, and G_R were markedly reduced in all the tested cells following FCY-302 treatment. These overall observations are in par with the report of Chang et al., who demonstrated the anticancer effect of a traditional medicine by induction of oxidative stress leading to the loss of mitochondrial membrane potential, downregulation of Bcl-2, and upregulation of Bax protein[32].

CONCLUSION

Outcomes of this study indicate excellent antiproliferative activity of FCY-302 in models of leukemia and myeloma cells. The activity was supported by attenuation of key mitochondrial and antioxidant enzymes to favor apoptosis. These findings drive more attention toward this compound for further investigation and development of analogs to test biological efficacy against cancer types.

ACKNOWLEDGMENTS: This work was funded by The Deanship of Scientific Research, King Khalid University, Abha, Saudi Arabia (Approval Number G.R.P-88-1439). The authors are duly thankful to Mr. Mohammad Ussain, Department of Chemistry, College of Science, King Khalid University, for his help rendered during this study. The authors declare no conflicts of interest.

REFERENCES

1. Modak H, Kulkarni SS, Kadakol GS, Hiremath SV, Patil BR, Hallikeri U, Gai PB. Prevalence and risk of leukemia in the multi-ethnic population of North Karnataka. Asian Pac J Cancer Prev. 2011;12:671–5.
2. Herrmann H, Blatt K, Shi J, Gleixner KV, Cerny-Reiterer S, Müllauer L, Vakoc CR, Sperr WR, Horny HP, Bradner JE, Zuber J, Valent P. Small-molecule inhibition of BRD4 as a new potent approach to eliminate leukemic stem-and progenitor cells in acute myeloid leukemia (AML). Oncotarget 2012;3:1588.
3. Mateen S, Tyagi A, Agarwal C, Singh RP, Agarwal R. Silibinin inhibits human nonsmall cell lung cancer cell growth through cell-cycle arrest by modulating expression and function of key cell-cycle regulators. Mol Carcinog. 2010;49(3):247–58.
4. Schmitz KJ, Otterbach F, Callies R, Levkau B, Hölscher M, Hoffmann O, Grabellus F, Kimmig R, Schmid KW, Baba HA. Prognostic relevance of activated Akt kinase in node-negative breast cancer: A clinicopathological study of 99 cases. Mod Pathol. 2004;17(1):15–21.
5. Ozdemir A, Turan-Zitouni G, Kaplancikli ZA, Tunali Y. Synthesis and biological activities of new hydrazide derivatives. J Enzyme Inhib Med Chem. 2009;24(3):825–31.
6. Geronikaki A, Eleftheriou P, Vicini P, Alam I, Dixit A, Saxena AK. 2-Thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinones as new agents with SHP-2 inhibitory action. J Med Chem. 2008;51(17):5221–8.
7. Abdelrazek FM, Metz P, Kataeva O, Jäger A, El-Mahrouky SF. Synthesis and molluscicidal activity of new chromene and pyrano[2,3-c]pyrazole derivatives. Arch Pharm. (Weinheim) 2007;340(10):543–8.
8. Ottanà R, Maccari R, Ciurleo R, Vigorita MG, Panico AM, Cardile V, Garufi F, Ronsisvalle S. Synthesis and in vitro evaluation of 5-arylidene-3-hydroxyalkyl-2-phenylimino-

4-thiazolidinones with antidegenerative activity on human chondrocyte cultures. Bioorg Med Chem. 2007;15(24): 7618–25.
9. da Costa Leite LFC, Veras Mourão RH, de Lima M do CA, Galdino SL, Hernandes MZ, de Assis Rocha Neves F, Vidal S, Barbe J, da Rocha Pitta I. Synthesis, biological evaluation and molecular modeling studies of arylidene-thiazolidinediones with potential hypoglycemic and hypolipidemic activities. Eur J Med Chem. 2007; 42(10):1263–71.
10. Bansal R, Guleria S. Synthesis of 16E-[3-methoxy-4-(2-aminoethoxy) benzylidene] androstene derivatives as potent cytotoxic agents. Steroids 2008;73(14):1391–9.
11. Pati HN, Das U, De Clercq E, Balzarini J, Dimmock JR. Molecular modifications of 2-arylidene-1-indanones leading to increased cytotoxic potencies. J Enzyme Inhib Med Chem. 2007;22(1):37–42.
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65(1–2):55–63.
13. Rajagopalan P, Alahmari KA, Elbessoumy AA, Balasubramaniam M, Suresh R, Shariff ME, Chandramoorthy HC. Biological evaluation of 2-arylidene-4, 7-dimethylindan-1-one (FXY-1): A novel Akt inhibitor with potent activity in lung cancer. Cancer Chemother Pharmacol. 2016; 77(2):393–404.
14. Solomson LP, Liepkalns VA, Spector A. Changes on Na+ K+ ATPase activity of Ehrlich ascites tumor cells produced by alteration of fatty acid composition. Biochemistry 1976;15:892–7.
15. Hjerten S, Pan H. Purification and characterization of two forms of a low affinity Ca 2+ ATPase from erythrocyte membrane. Biochem Biophys Acta 1983;728:218–88.
16. White MD, Ralston GB. Purification of water soluble Mg2+ ATPase from human erythrocyte membranes. Biochem Biophys Acta 1980;599:569–79.
17. Mateen S, Tyagi A, Agarwal C, Singh RP, Agarwal R. Silibinin inhibits human nonsmall cell lung cancer cell growth through cell-cycle arrest by modulating expression and function of key cell-cycle regulators. Mol Carcinog. 2010;49(3):247–58.
18. Nouri K, Yazdanparast R. Proliferation inhibition, cell cycle arrest and apoptosis induced in HL-60 cells by a natural diterpene ester from Daphne mucronata. DARU J Fac Pharm. 2011;19(2):145–53.
19. van Engeland M, Ramaekers FC, Schutte B, Reutelingsperger CP. A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells in culture. Cytometry 1996;24(2):131–9.
20. Moretti L, Li B, Kim KW, Chen H, Lu B. AT-101, a pan-Bcl-2 inhibitor, leads to radiosensitization of non-small cell lung cancer. J Thorac Oncol. 2010;5(5):680–7.
21. Agarwal C, Tyagi A, Kaur M, Agarwal R. Silibinin inhibits constitutive activation of Stat3, and causes caspase activation and apoptotic death of human prostate carcinoma DU145 cells. Carcinogenesis 2007;28(7):1463–70.
22. Park SY, Shin JN, Woo HN, Piya S, Moon AR, Seo YW, Seol DW, Kim TH. DOBI is cleaved by caspases during TRAIL-induced apoptotic cell death. BMB Rep. 2009;42(8):511–5.
23. Wong BS, Hsiao YC, Lin TW, Chen KS, Chen PN, Kuo WH, Chu SC, Hsieh YS. The in vitro in vivo apoptotic effects of Mahonia oiwakensis on human lung cancer cells. Chem Biol Interact. 2009;180(2):165–74.
24. Chunxin W, Youle RJ. The role of mitochondria in apoptosis. Annu Rev Genet. 2009;43:95–118.
25. Gassbarini A, Borie AB, Faghali H, Francavilla A, Van Thiel D. Effect of anoxia on intracellular ATP, Na+, Ca2+, Mg2+ and cytotoxicity in rat hepatocytes. J Biol Chem. 1994;267:6654–63.
26. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med. 2000;29:222–30.
27. Oberley LW, Buettner GR. Role of superoxide dismutase in cancer: A review. Cancer Res. 1979;39:1141–9.
28. Kumar S, Yedjou CG, Tchounwou PB. Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL-60) cells. J Exp Clin Cancer Res. 2014;33:42.
29. Udensi UK, Tchounwou PB. Dual effect of oxidative stress on leukemia cancer induction and treatment. J Exp Clin Cancer Res. 2014;33:106.
30. Battisti V, Maders LD, Bagatini MD, Santos KF, Spanevello RM, Maldonado PA, Brulé AO, Araújo Mdo C, Schetinger MR, Morsch VM. Measurement of oxidative stress and antioxidant status in acute lymphoblastic leukemia patients. Clin Biochem. 2008;41:511–8.
31. Sallmyr A, Fan J, Rassool FV. Genomic instability in myeloid malignancies: Increased reactive oxygen species (ROS), DNA double strand breaks (DSBs) and error-prone repair. Cancer Lett. 2008;270:1–9.
32. Chang C, Zhu YQ, Mei JJ, Liu SQ, Luo J. Involvement of mitochondrial pathway in NCTD-induced cytotoxicity in human hepG2 cells. J Exp Clin Cancer Res. 2010;29:145.