Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Indole itself and its novel derivative affect PML cells proliferation via controlling the expression of cell cycle genes

Mojgan Noroozi Karimabad[1], Mehdi Mahmoodi[1,2], Abdollah Jafarzadeh[1,3], Ali Darehkordi[4], Mohammad Reza Hajizadeh[1,2], Hossein Khorramdelazad[1], Ahmad Reza Sayadi[5], Soudeh Khanamani Falahati-pour[6], Gholamhossein Hassanshahi[1,3*]

[1] Molecular Medicine Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[2] Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[3] Department of Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[4] Department of Chemistry, Faculty of Science, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran
[5] Social Determinants of Health Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[6] Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Correspondence to: ghassanshahi@gmail.com

Received February 14, 2018; **Accepted** March 19, 2019; **Published** March 31, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.3.6

Abstract: Recently the role of indole and pyran rings in carcinogenesis has been well studied. Here we studied the effects and the possible mechanisms of the action of basal indole (I3A) and its novel indole derivative (C19H15F3N2O) on inhibition of proliferation cells in acute promyelocytic leukemia NB4 cell line by examining the expression of cell cycle genes. We treated NB4 cells with concentration of C19H15F3N2O for 24-72 h. The MTT and PI/Annexin V examinations were employed for assessment of the proliferation and apoptosis of NB4 cells. Both of Cyclin D and P21 were detected by the Real-time PCR. The western blotting analysis was also performed to show the protein levels for P21. A difference was regarded significant if p-value was less than 0.05. MTT assay showed that 15.12-1000 µg/mL C19H15F3N2O caused a time and concentration-dependent inhibition of NB4 cell proliferation. Exposure to higher concentrations of C19H15F3N2O resulted in significantly increased apoptosis rate in NB4 cells. RT PCR showed that C19H15F3N2O has up-regulated the expression of P21 and down-regulated the expression of Cyclin D. Western blotting experiments also demonstrated that the P21 expression in C19H15F3N2O treated cells has significantly increased, where compared with either untreated control cells or I3A treated cells. This newly (C19H15F3N2O) was able to inhibit NB4 cells proliferation and causes apoptosis of these cells more than I3A, and these effects are probably facilitated via cell cycle arrest. C19H15F3N2O might probably be introduced as a promising organic therapeutic reagent against APL.

Key words: C19H15F3N2O; Gene; Cancer; Flow cytometry; Real-time PCR; Western blot.

Introduction

Around, 10% of acute myeloid leukemia (AML) sufferers are diagnosed as APL which was defined by having the t (15; 17), chromosomal translocation as a result of merging of PML gene into the RARα gene. The expression of resulted PML-RARα chimeric protein is a fundamental leading cause for leukemia-genesis. These include differentiation and cell cycle arrest and further apoptosis (1-3). This highly encouraged medical scientists, including our team to investigate and approach potential molecular target-based compositions, especially novel synthetic reagents, against APL and further developing novel synthetic products to target PML-RARα oncoprotein in a straightforward manner and remarkably increase the survival of patients suffering from APL (4, 5). The Indole-3-carbaldehyde (I3A) represents various functions against cellular proliferation and therefore serves as anti-cancer. The event of cellular proliferation is an important phenomenon that is associated with cancer development and continued progression. The G1 phase of cell cycle arrest was shown to be modulated by I3A in cancer cells. P21 and Cyclin D are key regulators of the cell cycle in tumor cells (6-8). The cyclin dependent kinases-1 (CDKs) has a pivotal role during the initial phases of the cell cycle. The cyclin D1- CDK4, in particular mediates a prevention point of a threshold which is deserved for all silent cells that might pass, in order to divide (9, 10). During this checkpoint, cyclin D1- CDK4 phosphorylates the RB protein, as the suppressor of cellular mitosis. Following phosphorylation the transcription factors (E2F) pro-devotion is taking place, which in turn stimulates the transition through the prevention point where the cells are committed to start a division program. Limiting of CDK4 with natural inhibitory proteins, such as CKIs which serves as a restriction factor against cellular mitosis and inhibits unrestricted cell growth in normal cells is achieved. The down expression or inactivation of CKIs is a frequent behavior of tumor cells. Subsequently, small size molecules are functioning as inhibitors of CDKs and initiate unlimited cell proliferation in a tumor (10). The tumor suppressor protein, p21 trans-activates multiple genes (11, 12), that are involved both in the events

of apoptosis and cell division. The CDK inhibitor, p21, attaches to G1- CDK4/cyclin D complex and in turn facilitates the G1 arrest (13). Additionally, p21 restricts the G2/M phase via negatively affecting the Cdc2 activities as well (14, 15). Overexpression of P21 has been reported in several cancers (16-21) (26,–34). Mutations in p21 are not well identified (22, 23), however, exceptions were reported (24-26), that raising questions concerning the issue of enhanced p21 in some tumors. Although, knocking down of P21 was demonstrated to the empire the formation of in fibroblasts CDK4/cyclin D1 complex (27), studies have predicted that p21 is highly deserved for the formation of this complex. The P21 has promoted the complex formation. Its activity at the lower concentration required for inhibition of the complex (28). The restrictory effects of p21on normal cells is a frequent event, due to the fact that measures of CDK are constantly sustained (29) and cyclin level is closely modulated (30, 31). In tumors, the expression of CDK4/ cyclin D1 are increased (32-35) which allowing raised in complex and in turn retaining stoichiometry, in addition to elevating P21 level. P21 and cyclin D1 have been reported in various types of tumor (18), and their blocking inhibits tumor growth (36). Activated CDK4/ cyclin D1 phosphorylates retinoblastoma protein at Ser780 residue which is followed by releasing of E2F1 transcription of RB/E2F1 as an inhibitory complex (37-39). Consequently, E2F1 activates its own promoter (40) and in combination with several other targets such as cyclin A2, cyclin E1, and Myc, is involved in cell proliferation pathway(1, 41, 42). The I3A impacts on cancer cells, p27 and p21 have been well documented, however, cdk6-dependent activity was decreased (6, 7) and such an activation was required for I3A- dependent cell cycle arrest. In the current investigation, we have evaluated the effects of C19H15F3N2O and I3A as the basal indole on NB4 cells. To approach these are comparatively tested for their ability to observe weather if they are able to induce growth and cell cycle arrest of NB4 cells. Evaluation of both anti-leukemic potency and safety of the novel compound of C19H15F3N2O and basal indole might probably offer an interesting and promising clinical opportunity for the treatment of AML as well as other types of malignancy. The gene expression of cyclin D and P21 and their related gene products were also in this study investigated to explore some expected background molecular mechanistic events.

Materials and Methods

Preparation of C19H15F3N2O

As described in our previous works the method was applied for the preparation of C19H15F3N2O (44, 45) (Fig 1).

Cell culture protocol and method

A sample of the human NB4 cell line was prepared from pasture institute, Tehran-Iran. The prepared NB4 cells have initially been cultured in RPMI-1640 medium , inoculated with 10% fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), in humidified atmosphere containing 95% O2-5% CO2, at 37°C.

Methods for assessment of cell viability

Methyl-thiazolyl tetrazolium (MTT) assay

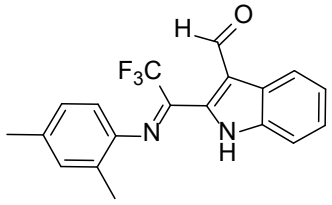
The viability of cells was determined using MTT-based techniques. To approach this, the NB4 cells were seeded onto the 96- well plates within a density of 1×10^4 cells/ well. The I3A and C19H15F3N2O were then added to the cultured cells at various concentrations of 15.12, 31.25, 62.5, 125, 250, 500 and 1000µg/mL alongside with vehicle control, the dimethyl sulfoxide (DMSO). Following indicated time point's 24-72h MTT assays were conducted. Briefly, 20 µl of 5 mg/mL of the MTT solution (Sigma, USA) was added to each separate well. Subsequently, the incubation of plates was further continued at 37°C for an additional period of 4 h. After 4h the supernatant medium was gently discarded and replaced by DMSO to dissolve the purple crystal. Finally relative absorbance for each well was detected at 570 nm by an ELISA reader system (ELISA reader, Bio Tek EIK 808, USA). Each sample was analyzed in triplicate (45).

Flow cytometric based cell analysis for apoptosis

The Annexin V-FITC / PI method was performed for the detection of cell death via apoptosis, as was recommended by manufacturers. To achieve that, cells were plated out in culture flasks (25 cm2) and were then incubated at 37 °C in humidified atmosphere containing, 95% O2-5% CO2for 24h. After 24h of culture, the culture supernatant was replaced by freshing prepared medium containing either C19H15F3N2O or I3A at IC50 concentration for 24, 48 and 72 h. Following an appropriate period of incubation duration cell lines have been harvested with centrifugation (1000 rpm for 5 min) and were further rinsed twice in fresh and cold PBS. The culture supernatant has gently been discarded and the resultant cell pellet was again re-suspended in the binding buffer. A volume of 5 µL annexin V-FITC along with 10 µL of PI (BD FACS Calibur- USA) was then added to each suspension for staining of NB4 cells. Suspensions were vortexed gently and incubation was continued in dark environment at RT for 10 min (eBioscience, USA) and cells were counted by a flow cytometry machine. All of these experiments were also repeated for three times.

The mRNA extraction and synthesis cDNA

The cellular total RNA was isolated by employing of a RNA purification kit (QIAGEN, Germany). Further extraction, the accuracy of the extracted RNA was assessed by electrophoresis on 1% agarose alongside



2-(1-((2,4-dimethylphenyl)imino)-2,2,2-trifluoroethyl)-1*H*-indole-3-carbaldehyde

Chemical Formula: $C_{19}H_{15}F_3N_2O$

Molecular Weight: 344.33

Figure 1. Shows the chemical structure of C19H15F3N2O.

with ethidium bromide staining and visualization of the RNA bands. The absorbance of resultant RNA was also read at 260 and 280 nm and the 260nm/280nm ratio was calculated for expecting protein contamination.

The DNase was used to remove DNA contamination from RNA. Further, the MMLV cDNA enzyme was applied for synthesizing strands from all mRNA molecules present in the specimens. OligoT Primer and Revert AID First Strand cDNA Synthesis (Bioneer, Korea) kits have also been employed to build cDNAs. Then, a reverse primer and a forward primer, as well as the Taq DNA polymerase enzyme, ATCG nucleotides and appropriate buffering media, and the Siber-green fluorescence color, DNA was synthesized from the investigated genes and reproduced in Applied Biosystems thermocycler (US).

Performing of the Real-Time PCR

By the using of specific primers Cyclin D and P21 (Table 1) , the expression of them was determined, employing RT PCR by a BIO-Rad Real-Time system (Bio-Rad Company, USA) . The RT-PCR temperature conditions were initial denaturation of templates at 95°C for 15 min, which was followed by 40 cycles of denaturation at 95° C for 15 s and annealing/extension at 58°C for 30 s. In this method, two integration and extension processes were coupled together and the PCR was performed in two steps. It is also important to be stated that ß-actin as a housekeeping gene was used as control, and the expression level of each gene was calculated according to the amount of ct and control ct in $2^{-\Delta\Delta Ct}$. The difference the number of target genes copies to the control gene; i.e. actin-β, was also calculated.

Western blotting

Whole cellular protein content was isolated from cells by (RIPA) buffer contained protease inhibitors. To measure protein level, the Bradford's protein measurement method was performed by a Bio-Rad protein assay reagent (Bio-Rad, San Diego, CA, USA), as manufacturer's recommended. Equal quantities of protein (20 µg in each well) were loaded and further isolated on12% SDS-PAG. Further isolation the gel, proteins were transferred onto the PVDF membranes in a semi-dry transfer system. Non-specific binding of proteins was blocked by a buffer containing 5% of skimmed milk which was dissolved in Tris-buffered saline and Tween-20 (TBST) at room temperature for an hour. Membranes containing proteins were then probed using primary human anti p21 antibody (cat. no. 687402; 1:500, Monoclonal Mouse IgG1 anti-human) (BioLegend; San Diego, USA), human anti β-actin antibody (cat. no. sc-47778; 1:100, Monoclonal Mouse IgG1 anti-human), (Santa Cruz Biotechnology Inc., USA) for an, overnight at a 4°C environment. At the next step the antibody probed membranes were washed up for three times in TBST.

Table 1. Contains the relative information of primers, including sequences for primers of studied genes.

Primer	Forward (5'→3')	Reverse (5'→3')
P21	GTGGACCTGTCACTGTCTTG	GGCGTTTGGAGTGGTAGAAA
Cyclin D	CCTCGGTGTCCTACTTCAAA	CTCTTTTTCACGGGCTCCAG
β-actin	GGGCATGGGTCAGAAGGATT	CGCAGCTCATTGTAGAAGGT

Membranes were then incubated in the presence of the secondary antibody: [goat anti- Mouse immunoglobulin g (IgG)/horseradish peroxidase (HRP); cat. no 405306; by 1:1,000 (BioLegend; San Diego, USA)] and anti β-actin antibody [goat anti- Mouse immunoglobulin g (IgG)/horseradish peroxidase (HRP); cat. no sc-516102; 1:1,500 (Santa Cruz Biotechnology Inc., USA)] for an additional hour. The ECL Plus reagent (purchased from Bio-Rad, San Diego, CA, USA) was used for the development of protein bands. The β-actin (as a housekeeping protein) was used as the internal control. These assays have also been repeated for three times (46).

Statistical analysis

The student t-test along with repeated analysis of variance employing SPSS software version 18 was applied for statically analysis of the data. Data are expressed as the mean ± standard deviation. The ddifference was statistically regarded significant if p-value was lower than 0.05.

Results

The viability of NB4 cells is altered in response to I3A and C19H15F3N2O based on the MTT assay

Both I3A and C19H15F3N2O have effectively decreased the NB4 cells viability at the approximately equal extent, nonetheless, the lower concentrations of C19H15F3N2O have more remarkably reduced the viability than what was observed for I3A.Present data propose that C19H15F3N2O has exhibited more suppressory effects, compared to I3A. The decreased NB4 cell lines viability further 24-72h of treatment with C19H15F3N2O was more whereby compared to I3A (Fig 2).

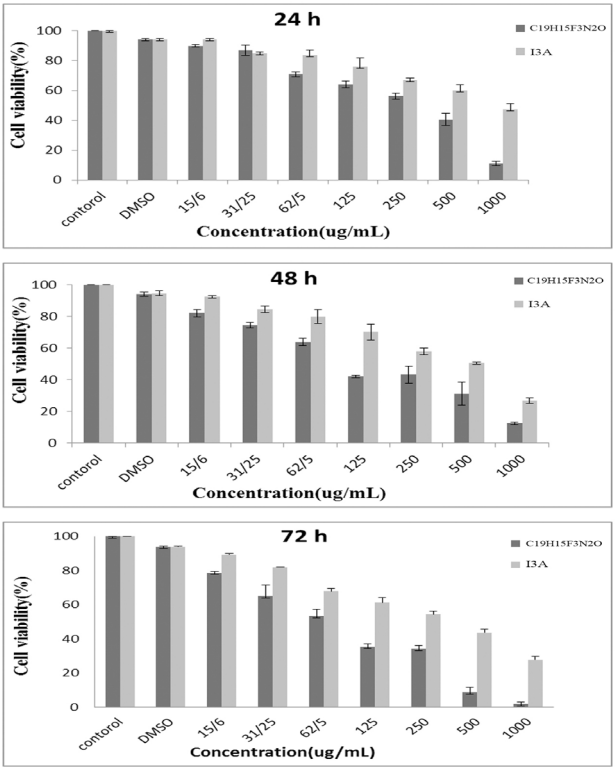


Figure 2. Demonstrates the Cell viability (%) of NB4 cells treated with C19H15F3N2O compared to I3A after 24 h 48 h and 72 h, using the MTT method ($P < 0.05$). All of the observed changes observed in the cell viability are significant ($P < 0.05$).

Apoptosis of NB4 cells is facilitated by C19H15F3N2O and I3A

The NB4 cells apoptotic response to C19H15F3N2O and by flow cytometry method

For the detection of apoptosis, NB4 cells were treated by appropriate concentrations of I3A and C19H15F3N2O at the IC50 doses for 72 h and then were subjected to staining with Annexin V and PI to examine apoptotic effects of I3A and C19H15F3N2O. The flow cytometry results displayed that C19H15F3N2O has increased apoptosis of treated NB4 cells following 72 h in compare to I3A and control. As clearly is shown in Fig 3, the Annexin V related expression alongside with the apoptosis rate of NB4 cells following 72h treatment with I3A, C19H15F3N2O and untreated left control cells (Fig 3a and b).

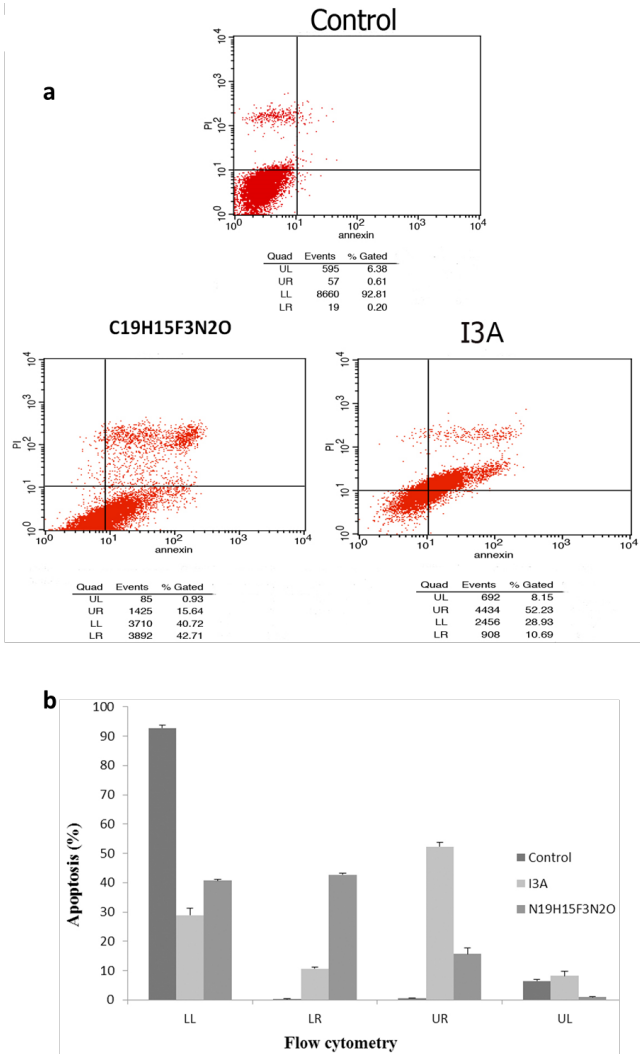


Figure 3. a. The Flow cytometry technique was applied for the quantification of determines apoptosis rates using the annexin V-FITC/PI staining method. The NB4 cells have received the relative IC50 concentration of C19H15F3N2O and I3A for 72 h. Stained cells are visible in four different groups of namely viable cells (LR), early apoptotic cells (LR), late apoptotic (LL) and finally necrotic cells (UL). The relative percentage of apoptotic (that were treated by C19H15F3N2O) was significantly increased whereby compared with both control and I3A treated population. **b.** Demonstrates the mean±SD between UL (necrotic cells), UR (late apoptotic cells), LL (normal live cells) and LR (early apoptotic cells) in untreated and treated cells with C19H15F3N2O in comparison with untreated cells after 24, 48 and 72 h.

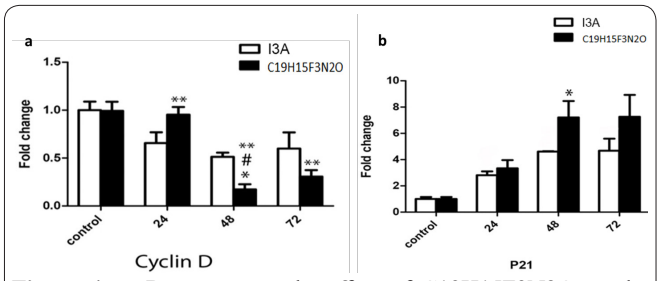


Figure 4. a. Demonstrates the effect of C19H15F3N2O on the mRNA expression of Cyclin D, in comparison to both untreated control cells and I3A treated cells.I3A. # = significant in compare to I3A ($P < 0.05$) * = significant in compare to control ($P < 0.05$). **= Significant in compare to different time points ($P < 0.05$). **b.** Demonstrates the effect of C19H15F3N2O on the mRNA expression of P21 in comparison to both untreated control cells. # = significant in compare to I3A ($P < 0.05$) * = significant in compare to control group ($P < 0.05$). **= Significant in compare to different time points ($P < 0.05$).

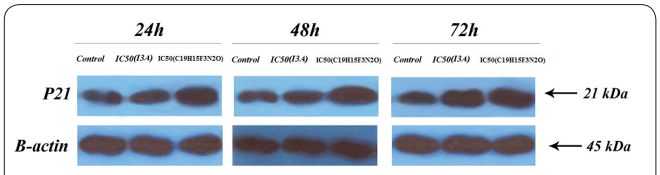


Figure 5. Effect of C19H15F3N2O on the protein expression of P21. The protein expression of P21 has markedly elevated comparison to control. Expression of β-actin was used as control and P21 is partial to β-actin.

Differential effects of I3A and C19H15F3N2O on the expression of Cyclin D and P21 genes

Our findings have demonstrated that the expression of cyclin D was considerably down-regulated following 24, 48 and 72h treatment by C19H15F3N2O ($P < 0.05$) in compare to I3A and untreated control cells (Fig 4a). The expression of P21 has followed an inverse fashion and has significantly up-regulated ($P < 0.05$) when cells were incubated for 24, 48 and 72h with C19H15F3N2O compared to both control and I3A (Fig 4b).

Regulatory effects of I3A and C19H15F3N2O on the protein expression of P21 by NB4 cells

Following 24, 48 and 72h of the culture of NB4 cells in presence of C19H15F3N2O the protein levels of P21 was considerably elevated, whereby compared with control (untreated) and I3A treated cells($P<0.01$). Thus, in a way it means that the increased protein level of P21 is in concert with its mRNA level, as confirmed by western blotting. As could be observed in fig-6 the protein band of P21 is localized in its predicted molecular size (20 kDa).The β-actin as a housekeeping gene was utilized as control and the expression of P21 is proportional to β-actin (Fig 5).

Discussion

Gene therapy for leukemia has emerged with ineffective results, so far. Therefore, research in the topic of key molecules involved in regulating of the leukemia cells growth has been considered as an important attraction for researchers and medical scientists to explore the underling mechanisms of leukemia and relatively

prospects of therapeutic gene therapy, these days. The current research was designed and aimed to investigate the role of P21 and Cyclin D in human leukemia cells. The P21 is considered as a, CKI that prevents the activities of CDK1, 2/cyclin-, and -CDK4/6 complexes, and thus serves as a checkpoint regulator for the development of G1 and S phases of cell cycle (47-49). Moreover, in G1 phase, the expression of P21 mediates cellular (50, 51). Cyclin D is expressed at G1/S transition phase where the diverse mitogenic signaling cascades are simultaneously activated to mediate engagement of the cell cycle machinery. Regardingly, the cyclin D1 down expression and its correlation with tumorigenesis in various cancers are well evidenced (52, 53). One fundamental property which was considered for the I3A is its ability to limit the growth of cancer cell lines (54-56). Synthesizing of novel anti-cancer combinations for anticancer purposes is a promising path by which the therapy of cancer might possibly be achieved. (51). To approach these synthesized novel derivatives of indole family, containing more fluorine methyl indole ring with attached phenyl ring is among properly suitable therapeutic compounds. Due to exhibiting lipophilic properties organo-fluorine compounds are highly electronegative fluorine which is an important factor for drug delivery, and this fluorine causing widespread application in the pharmaceutical industry, food and agriculture (57, 58). Attaching of the CF3 groups to these types of compounds elevates their antitumor activity, and relatively stabilizes these compounds and thus leading to important biological potency. Addition of phenylalanine amino acid also enhances its activity and as more as hydrophobic alkyl groups increase stopping cellular proliferation by several folds (59). Amines and their derivatives have indicated many specific biological features such as anti-bacterial, anti-fungal and anti-cancer. Addition of these substituents to nitrogen which presents in the indole ring of I3A molecule by the more hydrophobic alkyl groups showing maximum potency. According to these facts, C19H15F3N2O was designed so that exhibits a remarkably enhanced hydrophobic potency. Additionally, C19H15F3N2O was considerably more stable than I3A. This feature could possibly be gained as a result of the steric impact of the bulky CF3 group exchange, that present in its structure and this may prevent oligomerization of the I3A derivative action. A key restriction for employing of I3A is the requirement of relatively elevated measures which relatively restricts cellular proliferation (51, 60-62). Comparatively, we observed that C19H15F3N2O is one of the strongest synthetic derivatives of I3A, to date, and has the ability to increase the cell cycle arrest by several folds in NB4 cells. C19H15F3N2O exhibits an IC50 value at nano-molar scale when is compared to the micro-molar scale concentrations of I3A, and this is able to overcome the problem of relatively high measures of I3A which is deserved for suppression of the NB4 cells proliferation. This property implicates the feasibility of C19H15F3N2O to be employed as a therapeutic compound against NB4 and possibly other tumor types in vitro and in vivo. Importantly, C19H15F3N2O maintained the hallmarks of anti-proliferative responses of I3A in NB4 cells. C19H15F3N2O is able to effectively inhibit the NB4 cell lines proliferation and this propose that this I3A derivative as a novel I3A derivative may potentially be used as a promising target for treating myeloid leukemia, especially the promyelocytic type. These observations implicate the use of C19H15F3N2O therapies and allowing lower doses, and hence, reduce the unwanted adverse effects of the previously available medicines. C19H15F3N2O elicits the potent anti-proliferative response of NB4 cells, indicating that this synthetic indolic compound is biologically active in the in vivo environment. The anti-proliferative activities of C19H15F3N2O at effective low concentrations in vitro may make it as a promising candidate to be developed for therapy of the other types of cancers in future as well. In current study, we have examined the role of P21 and Cyclin D in the arrest of the cell cycle pathway in presence of I3A and its novel derivative, the C19H15F3N2O. To our best knowledge, this study is the first investigation that has addressed a critical role for I3A and C19H15F3N2O in the mediation of the P21 and cyclin D expression, to date. We have observed that the level of P21, mRNA expression was increased while the cyclin D expression was inversely reduced in NB4 cells, whereby treated by C19H15F3N2O in compare to both control and I3A. The effects of C19H15F3N2O on the p P21 protein expression has also been examined in NB4 cells that received compound for various duration varying from 24 and 48 to 72 h. Western blotting analysis revealed that C19H15F3N2O has also strongly upregulated the P21 protein expression by NB4 cells, ,while, the C19H15F3N2O concentration has been increased. Although, there exists no report within the literature regarding the regulatory effects of C19H15F3N2 but Mauro De Santi and colleagues reported that both P21 and cyclin D are amongst genes responsible for restricting the processes of cellular proliferation. P21 is introduced as a general inhibitor for CDK family and is able to block development of cell cycle during either the G1/S or G2/M phase (43). Our results showed that there were approximately several folds of increased potency for C19H15F3N2O when compared to I3A. Regardingly the effects of I3A on NB4 cells has regulated the expression of plenty of genes mainly for the cell cycle, such as reducing cyclin D1 expression levels and increasing P21 (63). Overall, theanalysis of our data determined that both C19H15F3N2O and I3A could be considered as a promising therapy method for tumors. Collectively, findings of this investigation are indicative for the fact that C19H15F3N2O prevents cell cycle progression via P21-Cyclin D-dependent pathway by probably equilibrating of the stoichiometry between CDK4/cyclin D1 and P21 with decreasing cell cycle activation. The demonstrated elevated level of the expression P21 in primary cancers together with findings from this research on the functions of P21 propose the possibility that P21 can favor faster cell growth in tumor conditions with enhancing cell cycle. Since P21 is overexpressed in different tumors, utilization of cell cycle diagnostic and therapeutic perspective in tumors, this overexpression could be further explored.

Conflict of Interest

None of the authors of present the study declared conflict of interest.

Acknowledgments
This project was financially supported by a grant from the Rafsanjan University of Medical Sciences.

Author Contribution
- Study Design: Gholamhossein Hassanshahi, Mojgan Noroozi Karimabad
- Data Collection: Mojgan Noroozi Karimabad, Hossein Khorramdelazad
- Statistical Analysis: Ali Darekordi, Ahmad Reza Sayadi, Hossein Khorramdelazad
- Data Interpretation: Mohammad Reza Hajizadeh, Mehdi Mahmoodi
- Manuscript Preparation: Abdollah Jafarzadeh, Gholamhossein Hassanshahi, Soudeh Khanamani Falahatipour
- Literature Search: Mojgan Noroozi Karimabad, Mehdi Mahmoodi
- Funds Collection: Gholamhossein Hassanshahi.

References

1. Hiebert SW, Lipp M, Nevins JR. E1A-dependent trans-activation of the human MYC promoter is mediated by the E2F factor. Proc Natl Acad Sci India Sect . 1989;86(10):3594-8.
2. Mistry AR, Pedersen EW, Solomon E, Grimwade D. The molecular pathogenesis of acute promyelocytic leukaemia: implications for the clinical management of the disease. Blood Rev. 2003;17(2):71-97.
3. Sheikhrezaei Z, Heydari P, Farsinezhad A, Fatemi A, Khanamani Falahati-Pour S, Darakhshan S, et al. A New Indole Derivative Decreased SALL4 Gene Expression in Acute Promyelocytic Leukemia Cell Line (NB4). Iran Biomed J. 2018;22(2):99-106.
4. Wang Z-Y, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood. 2008;111(5):2505-15.
5. Primo MN, Bak RO, Schibler B, Mikkelsen JG. Regulation of pro-inflammatory cytokines TNFα and IL24 by microRNA-203 in primary keratinocytes. Cytokine. 2012;60(3):741-8.
6. Nguyen HH, Aronchik I, Brar GA, Nguyen DH, Bjeldanes LF, Firestone GL. The dietary phytochemical indole-3-carbinol is a natural elastase enzymatic inhibitor that disrupts cyclin E protein processing. Proc Natl Acad Sci India Sect . 2008;105(50):19750-5.
7. Moiseeva EP, Heukers R. Indole-3-carbinol-induced modulation of NF-κB signalling is breast cancer cell-specific and does not correlate with cell death. Breast Cancer Res Treat. 2008;109(3):451-62.
8. Cram EJ, Liu BD, Bjeldanes LF, Firestone GL. Indole-3-carbinol inhibits CDK6 expression in human MCF-7 breast cancer cells by disrupting Sp1 transcription factor interactions with a composite element in the CDK6 gene promoter. J Biol Chem. 2001;276(25):22332-40.
9. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Fibroblasts and their transformations: Connect-tissue cell fam. 2002.
10. Aubry C, Patel A, Mahale S, Chaudhuri B, Maréchal J-D, Sutcliffe MJ, et al. The design and synthesis of novel 3-[2-indol-1-yl-ethyl]-1H-indole derivatives as selective inhibitors of CDK4. Tetrahedron Lett. 2005;46(9):1423-5.
11. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, et al. WAF1, a potential mediator of p53 tumor suppression. Cell. 1993;75(4):817-25.
12. Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. Genes Dev. 1993;7(7a):1126-32.
13. Harper JW, Elledge SJ, Keyomarsi K, Dynlacht B, Tsai LH, Zhang P, et al. Inhibition of cyclin-dependent kinases by p21. Mol Biol Cell. 1995;6(4):387-400.
14. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, et al. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science (New York, NY). 1998;282(5393):1497-501.
15. Taylor WR, Stark GR. Regulation of the G2/M transition by p53. Oncogene. 2001;20(15):1803-15.
16. Adrover E, Maestro ML, Sanz-Casla MT, del Barco V, Cerdan J, Fernandez C, et al. Expression of high p53 levels in colorectal cancer: a favourable prognostic factor. Br J Cancer. 1999;81(1):122-6.
17. Gasparini G. Prognostic variables in node-negative and node-positive breast cancer—editorial. Prognostic variables in node-negative and node-positive breast cancer: Springer; 1998. p. 411-21.
18. Rey MJ, Fernández PL, Jares P, Muñoz M, Nadal A, Peiró N, et al. p21WAF1/Cip1 is associated with cyclin D1CCND1 expression and tubular differentiation but is independent of p53 overexpression in human breast carcinoma. J Pathol. 1998;184(3):265-71.
19. Abbas T, Dutta A. p21 in cancer: intricate networks and multiple activities. Nature Reviews Cancer. 2009;9(6):400-14.
20. Aaltomaa S, Lipponen P, Eskelinen M, Ala-Opas M, Kosma V. Prognostic value and expression of p21 (waf1/cip1) protein in prostate cancer. The Prostate. 1999;39(1):8-15.
21. Biankin AV, Kench JG, Morey AL, Lee C-S, Biankin SA, Head DR, et al. Overexpression of p21WAF1/CIP1 is an early event in the development of pancreatic intraepithelial neoplasia. Cancer Res. 2001;61(24):8830-7.
22. Shiohara M, El-Deiry WS, Wada M, Nakamaki T, Takeuchi S, Yang R, et al. Absence of WAF1 mutations in a variety of human malignancies. Blood. 1994;84(11):3781-4.
23. Shiohara M, Koike K, Komiyama A, Koeffler HP. p21WAF1 mutations and human malignancies. Leuk Lymphoma. 1997;26(1-2):35-41.
24. Balbín M, Hannon GJ, Pendás AM, Ferrando AA, Vizoso F, Fueyo A, et al. Functional analysis of a p21WAF1, CIP1, SDI1 mutant (Arg94→ Trp) identified in a human breast carcinoma Evidence that the mutation impairs the ability of p21 to inhibit cyclin-dependent kinases. J Biol Chem. 1996;271(26):15782-6.
25. Bahl R, Arora S, Nath N, Mathur M, Shukla NK, Ralhan R. Novel polymorphism in p21waf1/cip1 cyclin dependent kinase inhibitor gene: association with human esophageal cancer. Oncogene. 2000;19(3):323.
26. Cazier J-B, Rao S, McLean C, Walker A, Wright B, Jaeger E, et al. Whole-genome sequencing of bladder cancers reveals somatic CDKN1A mutations and clinicopathological associations with mutation burden. Nat Commun. 2014;5.
27. Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, et al. The p21Cip1 and p27Kip1 CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J. 1999;18(6):1571-83.
28. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. Genes Dev. 1997;11(7):847-62.
29. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif. 2003;36(3):131-49.
30. Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell. 1983;33(2):389-96.
31. Noroozi MK, Mahmoodi M, Jafarzadeh A, Darekordi A, Hajizadeh MR, Hassanshahi G. Molecular targets, anti-cancer properties and potency of synthetic indole-3-carbinol derivatives. Mini Rev Med Chem. 2018.
32. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature. 1994;369(6482):669.
33. Buckley MF, Sweeney K, Hamilton J, Sini R, Manning D, Ni-

cholson R, et al. Expression and amplification of cyclin genes in human breast cancer. Oncogene. 1993;8(8):2127-33.
34. Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, et al. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res. 1994;54(7):1812-7.
35. An H-X, Beckmann MW, Reifenberger G, Bender HG, Niederacher D. Gene amplification and overexpression of CDK4 in sporadic breast carcinomas is associated with high tumor cell proliferation. Am J Pathol. 1999;154(1):113-8.
36. Dai M, Al-Odaini AA, Fils-Aimé N, Villatoro MA, Guo J, Arakelian A, et al. Cyclin D1 cooperates with p21 to regulate TGFβ-mediated breast cancer cell migration and tumor local invasion. Breast Cancer Res. 2013;15(3):R49.
37. Kitagawa M, Higashi H, Jung H-K, Suzuki-Takahashi I, Ikeda M, Tamai K, et al. The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2. EMBO J. 1996;15(24):7060.
38. Hanahan D, Weinberg RA. The hallmarks of cancer. cell. 2000;100(1):57-70.
39. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer cell. 2002;2(2):103-12.
40. Takahashi Y, Rayman JB, Dynlacht BD. Analysis of promoter binding by the E2F and pRB families in vivo: distinct E2F proteins mediate activation and repression. Genes Dev. 2000;14(7):804-16.
41. Yam C, Fung T, Poon R. Cyclin A in cell cycle control and cancer. Cell Mol Life Sci. 2002;59(8):1317-26.
42. Geng Y, Eaton EN, Picon M, Roberts JM, Lundberg AS, Gifford A, et al. Regulation of cyclin E transcription by E2Fs and retinoblastoma protein. Oncogene. 1996;12(6):1173-80.
43. De Santi M, Galluzzi L, Lucarini S, Paoletti MF, Fraternale A, Duranti A, et al. The indole-3-carbinol cyclic tetrameric derivative CTet inhibits cell proliferation via overexpression of p21/CDKN1A in both estrogen receptor-positive and triple-negative breast cancer cell lines. Breast Cancer Res. 2011;13(2):R33.
44. Darehkordi A, Rahmani F, Hashemi V. Synthesis of new trifluoromethylated indole derivatives. Tetrahedron Lett. 2013;54(35):4689-92.
45. Karimabad MN, Mahmoodi M, Jafarzadeh A, Darehkordi A, Hajizadeh MR, Khorramdelazad H, et al. The novel Indole-3-formaldehyde (2-AITFEI-3-F) is involved in processes of apoptosis induction? Life Sci. 2017;181:31-44.
46. Karimabad MN, Mahmoodi M, Jafarzadeh A, Darehkordi A, Hajizadeh MR, Khorramdelazad H, et al. Evaluating of OCT-4 and NANOG was differentially regulated by a new derivative indole in leukemia cell line. Immunol Lett. 2017;190:7-14.
47. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, et al. WAF1, a potential mediator of p53 tumor suppression. Cell. 1993;75(4):817-25.
48. Gartel AL, Radhakrishnan SK. Lost in transcription: p21 repression, mechanisms, and consequences. Cancer Res. 2005;65(10):3980-5.
49. Child ES, Mann DJ. The intricacies of p21 phosphorylation: protein/protein interactions, subcellular localization and stability. Cell cycle. 2006;5(12):1313-9.
50. Rössig L, Jadidi AS, Urbich C, Badorff C, Zeiher AM, Dimmeler S. Akt-dependent phosphorylation of p21Cip1 regulates PCNA binding and proliferation of endothelial cells. Mol Cell Biol. 2001;21(16):5644-57.
51. Aggarwal BB, Ichikawa H. Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. Cell cycle. 2005;4(9):1201-15.
52. Masamha CP, Benbrook DM. Cyclin D1 degradation is sufficient to induce G1 cell cycle arrest despite constitutive expression of cyclin E2 in ovarian cancer cells. Cancer res. 2009;69(16):6565-72.
53. Jung H, Shin J-H, Park Y-S, Chang M-S. Ankyrin repeat-rich membrane spanning (ARMS)/Kidins220 scaffold protein regulates neuroblastoma cell proliferation through p21. Mol Cells. 2014;37(12):881-7.
54. Nguyen HH, Lavrenov SN, Sundar SN, Nguyen DH, Tseng M, Marconett CN, et al. 1-Benzyl-indole-3-carbinol is a novel indole-3-carbinol derivative with significantly enhanced potency of antiproliferative and anti-estrogenic properties in human breast cancer cells. CHEM BIOL INTERACT. 2010;186(3):255-66.
55. Cover CM, Hsieh SJ, Tran SH, Hallden G, Kim GS, Bjeldanes LF, et al. Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling. J Biol Chem. 1998;273(7):3838-47.
56. Garcia HH, Brar GA, Nguyen DH, Bjeldanes LF, Firestone GL. Indole-3-carbinol (I3C) inhibits cyclin-dependent kinase-2 function in human breast cancer cells by regulating the size distribution, associated cyclin E forms, and subcellular localization of the CDK2 protein complex. J Biol Chem. 2005;280(10):8756-64.
57. Ryu C-K, Lee JY, Park R-E, Ma M-Y, Nho J-H. Synthesis and antifungal activity of 1H-indole-4, 7-diones. Bioorg Med Chem Lett. 2007;17(1):127-31.
58. Blum CA, Xu M, Orner GA, Fong AT, Bailey GS, Stoner GD, et al. β-Catenin mutation in rat colon tumors initiated by 1, 2-dimethylhydrazine and 2-amino-3-methylimidazo [4, 5-f] quinoline, and the effect of post-initiation treatment with chlorophyllin and indole-3-carbinol. Carcinogenesis. 2001;22(2):315-20.
59. Gul W, Hamann MT. Indole alkaloid marine natural products: An established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. Life Sci. 2005;78(5):442-53.
60. Safe S, Papineni S, Chintharlapalli S. Cancer chemotherapy with indole-3-carbinol, bis (3′-indolyl) methane and synthetic analogs. Cancer Lett. 2008;269(2):326-38.
61. Firestone GL, Sundar SN. Minireview: modulation of hormone receptor signaling by dietary anticancer indoles. Mol Endocrinol. 2009;23(12):1940-7.
62. Firestone GL, Bjeldanes LF. Indole-3-carbinol and 3-3'-diindolylmethane antiproliferative signaling pathways control cell-cycle gene transcription in human breast cancer cells by regulating promoter-Sp1 transcription factor interactions. J Nutr. 2003;133(7 Suppl):2448s-55s.
63. Zhou T, Li G, Cao B, Liu L, Cheng Q, Kong H, et al. Downregulation of Mcl-1 through inhibition of translation contributes to benzyl isothiocyanate-induced cell cycle arrest and apoptosis in human leukemia cells. Cell Death Dis. 2013;4(2):e515.