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The novel Indole-3-formaldehyde (2-AITFEI-3-F) is involved in processes of apoptosis induction?

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ABSTRACT

Aim and objectives: Balancing between Bax and Bcl-2 plays critical roles in both proliferation and self-renewal activation of cancer cells. Indole-3-formaldehyde derivatives limit the growth and facilitate cell death in different cell systems. In this study, we introduced a novel indole derivative (2-AITFEI-3-F) with tendency to facilitate apoptosis in NB4 line in comparison to basal Indole-3-formaldehyde (I3F).
Methods: The NB4 cells were cultured in RPMI1640 medium contained 2-AITFEI-3-F and I3F (15.12–1000 μg/mL) for 24, 48 and 72 h. Inhibition of cell proliferation was assessed by trypan blue staining technique and MTT assay. The fold changes of Bax/Bcl-2 expression against β-actin were determined by real-time-PCR technique. Western blotting analysis was also applied for evaluating the expression of Bax and Bcl2 at protein level. Data were analyzed by student t and repeated measure tests. Differences were considered significant if ($P < 0.01$).
Results: There was a significant difference in cell viability, when various concentrations of 2-AITFEI-3-F (but similar to I3F) were used for 24, 48 and 72 h in comparison to I3F regarding the cellular viability ($P < 0.05$). Real time PCR and Western blotting analysis indicated that the gene and protein expression level of Bcl-2 down-regulated while Bax was up-regulated in compare to untreated control cells and cells treated with I3F ($P < 0.01$).
Conclusion: According to these findings, the novel indole derivative 2-AITFEI-3-F probably triggered apoptosis of NB4 cells by modulating Bax/Bcl-2 ratio. Furthermore, the 2-AITFEI-3-F had markedly displayed anti-cancer activity than I3F.

1. Introduction

The acute promyelocytic leukemia (APL) was initially defined as a malignant hematopoietic disorder which is pathologically caused by both uncontrolled and unlimited proliferation in combination with lacked differentiation of leukemic stem cells [1,14]. In the past, APL was also described as the most malignant Acute myeloid (AML) subtype [1,14]. APL cells are responsive to chemotherapy (CT), however, evidence are in favor of the fact that CT is associated with bleeding disorders, leading to primitive early-mortality rates. Despite, APL is responsive to CT, the median duration of remission of APL is between 11 and 25 months, and only 35–45% of the patients are survived using CT, alone [2,6]. More than 90% of patients with APL patent enter complete clinically remission when being treated with all-trans-retinoic acid (ATRA) a strong differentiation [43]. Further of resistance to ATRA is a new problem against APL therapy. The mechanism(s) of relapse of APL remained as the most difficult clinical problem, which need to be solved [12,51]. Heterocyclic compounds attract significant attention in the chemical literature so far, due to both abundance in natural products as well as beneficial biological properties. A broad range of heterocycles are described to date, and, indole and pyran ring systems are of

Abbreviations: 2-AITFEI-3-F, novel synthesized Indole-3-formaldehyde; I3F, Indole-3-formaldehyde The indole-3-formaldehyde is the synonym of indole-3-carbaldehyde; NB4, Cell line name; APL, acute promyelocytic leukemia; AML, Acute myeloid; DMSO, Dimethyl sulfoxide; Bcl-2/Bax, A rheostat that regulates an anti-oxidant pathway and cell death; 2-AITFEI-3-F, 2-(1-((3,5-bis(trifluoromethyl)phenyl)imino)-2,2,2-trifluoroethyl)-1H-indole-3-formaldehyde; MTT, 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide; mRNA, Messenger RNA; PVDF, Polyvinylidene fluoride; RIPA, radio immune precipitation; IgG, immunoglobulin G; HRP, horseradish peroxidase; ECL, emitter-coupled logic; NF-Kb, nuclear factor kappa-light-chain-enhancer of activated B cells; CT, chemotherapy; UL, Up-left; UR, Up-right; LR, Low-right; LL, Low-left.

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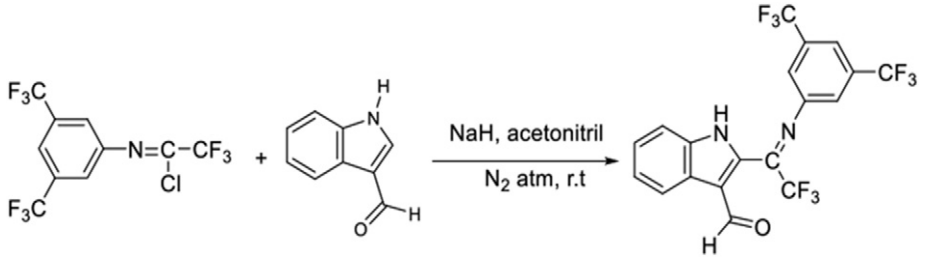

Fig. 1. Shows the structure of 2-AITFEI-3-F.

paramountcy important. A broad range several biological activities are linked to natural and synthetic compounds including a substituted indole nucleus, making it a suitable building block for many therapeutic reagents [39,46]. It is well established that indole structural nucleus exists in natural products, pharmaceuticals, functional materials, and agrochemicals [39]. Analogues of indole represent dramatic therapeutic effects in medicinal chemistry, vary from cancer therapy [28], antioxidant [41], to anti-rheumatoid, and anti-HIV [3,40]. Progression of bladder cancer, lung and colon cancer, mammary tumors, prostate cancer, and breast tumors are reported to be in habited indole derivative as well [35]. Multiple indole derivatives serve as potent scavengers of free radicals [5]. A particularly useful field of medicinal research is synthesizing and application of organofluorine substances [24]. This field of study is now being as an interesting expanding and productive area of research, with increasing number of novel publications, reviews topics and monographs [13]. Additionally, synthetic, organofluorine substances are widely utilized in the pharmaceutical industry, materials science, and agriculture due to the unique biological functions that are devoted by the fluorine atom [24,30]. The Indole-3-formaldehyde is introduced as a promising anti-cancer [19]. The I3F has displayed cancer suppressing, such as cell cycle inhibition, apoptosis, as well as decreasing tumor invasion through modulation of cellular signals such as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), Bcl-2, Bax pathways several cancerous cell systems [11,22,23,33]. These days induction of apoptosis is considered as potentially promising method for either cancer prevention or treatment furring various steps. Thus, induction of apoptosis by such chemical substances can follow an excellent approach for termination development and progression of carcinogenesis, in addition to removing genetically damaged, reinitiated, or neoplastic cells. Bax and Bcl-2 are Longley described as key regulators of apoptosis in cancer cells [21,26]. Bcl-2 and Bax are regulated by NF-κB, underlying its importance on cell death in cancer cells [25,44].Prolonged activation of Bcl-2 and Bax is well evidenced in several cancer types and is believed that it induces gene products and allowing these cells to escape apoptosis [9,16]. Continuous and constitutive Bcl-2 and Bax activation is occurred in the most of (90%) childhood APL tumors which strongly proposes fundamental part for these factors in leukemia. Cell survival either by apoptosis inhibition or by stimulating proliferation [18]. Compelling evidences are supportive to believe that subverting Bcl-2 and Bax activation would be a beneficial strategy to restore or enhance apoptosis in APL cells. Several other confirmatory evidences revealed that various natural compounds have represented anti-cancer properties against APL through perturbing multiple cellular signaling pathways [8]. Laxmi and colleagues focused on the anticancer activity of Indole-3-formaldehyde derivatives [19] and displayed that Indole-3-formaldehyde an important role in antitumor activities [17,36,42]. The 2-Phenyl-1H-indole-3-carbaldehyde derivatives inhibited the growth of MDA-MB-231 and MCF7 breast cancer cells and evaluated for anticancer activity [10,17,42]. In a systematic structure–activity analysis, investigators demonstrated that the addition of substituents to the nitrogen at position 1 in the indole ring due to the more hydrophobic alkyl groups in its structure, I3F exhibited the highest tendency in combination with increased potency of the growth arrest response up to several folds [7,17,49] This is suggesting that substituents that elevate lipophilicity and modify the indole ring nitrogen may improve the activity of I3F. In the present experimental study, we have developed and assessed the effect of 2-AITFEI-3-F (Fig. 1) on NB4 cell by examining the expression and exploring of the balancing Bcl-2 and Bax genes in mRNA and protein levels to unmark the possible molecular mechanism which might be involved.

2. Material and methods

2.1. Preparation of the 2-AITFEI-3-F

The chemical compound was synthesized according to the following procedure [7]: Briefly, in a typical experimental procedure, a 50 mL dry round-bottomed flask equipped with a nitrogen inlet was charged with 5 mL of dry acetonitrile, 0.145 g (1.0 mmol) of Indole-3-formaldehyde and 0.24 g (1.0 mmol) of NaH. The resultant solution was left on shaker under nitrogen atmosphere and at room temperature for 30 min, then a solution of N-(3,5-bis(trifluoromethyl)phenyl)-2,2,2-trifluoroacetimidoyl chloride (1.0 mmol) was gently and dropwise added by a syringe. The mixture was stirred at room temperature for 20 h under N_2 atmosphere and was then filtered in a new container. Further to discarding the solvent the crude product was purified by recrystallizing from ethanol (twice) to gain the product (2-(1-((3,5-bis(trifluoromethyl) phenyl)imino)-2,2,2-trifluoroethyl)-1H-indole-3-formaldehyde.

This compound was a white solid yield with the following characterizations, M.P = 78–80 °C, Yield = 89%, FT-IR (KBr) υ_{max} = 1698, 1673, 1557 cm^{-1}. ^1H NMR (DMSO-d_6 500 MHz) δ = 10.11 (s, 1H), 9.94 (s, 1H), 8.21 (s, 2H), 8.05 (d, 1H), 7.92 (d, 1H), 7.51 (d, 1H), 7.26 (m, 2H) ppm. Anal. Calcd for $C_{19}H_9F_9N_2O$ (452.27): C, 50.46; H, 2.01; N, 6.19%. Found: C, 50.17; H, 1.94; N, 6.24%.

2.2. Cell culture

The NB4 cells were purchased from the National Cell Bank of Iran (NCBI, Tehran, Iran) and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Bio-Rad, San Diego, CA, USA), and 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C

Table 1
Indicates the sequences of the employed primers in this study.

Primer	Forward (5′ → 3′)	Reverse (5′ → 3′)
Bcl-2	GGTGAACTGGGGGAGGATG	CGTACAGTTCCACAAAGGCATC
Bax	AGGATGCGTCCACCAAGAA	CGGCCCCAGTTGAAGTTGC
β-Actin	GGGCATGGGTCAGAAGGATT	CGCAGCTCATTGTAGAAGGT