



Synthesis and characterization of new 1,2,4-triazole anticancer scaffold derivatives: *In Vitro* study



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Abstract

Four Schiff bases were obtained via one direct click reaction between 3-amino-1H-1,2,4-triazole and different substituted benzaldehyde. The synthesized compounds were characterized on the basis of their spectral data including IR spectra, mass spectroscopy and ¹HNMR. The synthesized compounds were tested against three human cancer cell lines to evaluate their *in vitro* anticancer activity, and also tested on Vero cells extracted from African green monkey kidney to investigate their side effect. The screening showed that the Schiff bases TB-NO₂ and TB-OCH₃ produced an effective anticancer activity against HEPG2, HCT-116, and MCF-7 cell lines. Further investigations had been conducted to determine the apoptotic and antifibrotic ability of TB-NO₂ and TB-OCH₃ on MCF-7 cells by determining the expression of Bax, Bcl-2, CTGF, and PDGF. Results of Bax/Bcl-2 ratio for TB-NO₂ and TB-OCH₃ confirmed the apoptotic effect of these compounds on MCF-7 cells. The reduction in CTGF and PDGF genes expression confirmed the regulatory effect of these compounds on MCF-7 cells growth.

Keywords: Anticancer activity; 1,2,4-triazole; Schiff base; cytotoxicity; In-vitro.

1. Introduction

Cancer, which defined as uncontrolled proliferation, invasion, and metastasis of abnormal cells, is considered to be one of the most challenges in the medical world [1, 2]. It represents the second lethal disease after cardiovascular diseases [3, 4]. The development and discovering of new efficient anticancer drugs with highly selectivity considered a large challenge face the modern community. Among the various ways of cancer treatment, the chemotherapy consider one of the most important methods in its management [2]. The restriction in the use of chemotherapy is related to the cytotoxicity of the used drugs, and their side effects on healthy cells. Thus, the discovery of new, efficient and safe therapeutic drugs is highly demanded [2]. The N-heterocyclic compounds like imidazole, triazole, pyrimidine, benzimidazole and indole showed significant biological activities [5-8]. A great attention is focused on 1,2,4 triazole derivatives as one of the most relevant discovered heterocyclic moiety in the

drug discovery that showed a wide and potential biological activity as anticancer [9-15], antimicrobial [9, 11], anti-inflammatory [11, 13], anticonvulsant [9, 11, 15], antiviral [10, 11], anti-tumor [12, 16], antifungal [9, 11]. Because of their ability to serve as building block for synthesizing many bioorganic-conjugates with different moieties as well as their higher tendency to form hydrogen bond, consequently improving toxicological, pharmacokinetic pharmacological as well as their physicochemical properties [17-19]. Subsequently 1,2,4- triazole derivatives were involved in many drugs like voriconazole, intracranazole and fluconazole that used in treatment of many diseases [20, 21]. Now a day, many researches focused on hybridization between two or more active moieties for enhancing the biological performance and overcome on the drug resistance, reduce toxicity with an enhancement of pharmacokinetic profiles for treatment of various diseases [22-25]. The Schiff bases moieties considered an important ligands and have the ability to coordinate with various metals ions as well

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as their easy preparation under simple one pot click chemistry [26-32]. The azomethine group is similar to the structures that present in the natural biological system, and it reported to have some biological activities like anti-bacterial, anti-viral, anti-inflammatory, anticancer, anti-HIV, anti-fungal, anti-fertility, and anti-tumor activities [33-40]. Nitro compounds showed recently antitumor activity as radiosensitizers in addition to their bio-reduction ability that release intermediate during the redox processes [41, 42] which enhance the antitumor activity [43]. Such findings oriented us to synthesize four novel 1,2,4 triazole derivatives based on Schiff base with variable functional groups. Such hybridization between 1,2,4-triazole moiety, Schiff base groups and some benzaldehyde derivative, we could enhance the anticancer efficiency on three different cancer cell lines which are HEPG2, HCT-116, and MCF-7 as well as avoiding the Multidrug resistance in cancer chemotherapy.

2. Materials and methods

2.1. Chemicals and instrumentation

3-amino-1H-1,2,4-triazole, 3,4-dimethoxybenzaldehyde, p-chlorobenzaldehyde, p-nitrobenzaldehyde, and 4-(dimethylamino)benzaldehyde which used to synthesize four 3-amino-1H-1,2,4-triazole Schiff bases was purchased from Sigma-Aldrich chemical company. IR spectra of the synthesized compounds were obtained using BRUKER FTIR spectrophotometer (Germany Software: OPUS version 7.5). ¹HNMR spectra were measured with a BRUKER spectrometer Avance III 400 MHz (Swiss), and compounds were dissolved in dimethyl sulfoxide. The mass spectroscopy was conducted by ThermoScientific ISQ (Italy).

2.2. Materials of the Cell Lines Assay

Cell culture of MCF-7 (human breast adenocarcinoma), HCT-116 (human colorectal carcinoma), and HEPG2 (human hepatocellular carcinoma) cell lines obtained from the American Type Culture Collection (ATCC, Minnesota, USA) and were preserved at National Cancer Institute (NCI), Cairo, Egypt in RPMI1640 medium having 10% fetal bovine serum and 1% penicillin-streptomycin. The routine incubation of the cell lines is performed in 5% CO₂ in a humidified atmosphere at 37 °C.

2.3. General procedure for Synthesis of 3-(substituted phenylmethyleneamino)-1H-1,2,4-triazole derivatives.

10 mmol of each substituted benzaldehyde were reacted with 0.84 g (10 mmol) of 3-amino-1H-1,2,4-triazole in the presence of ethanol as solvent, and glacial acetic acid as catalyst. The mixture was allowed to reflux under stirring for 3 hrs. After cooling, the formed precipitate was separated out, followed by filtration, and finally recrystallization from anhydrous ethanol [44]. The scheme of synthesis is illustrated in Fig. 6.

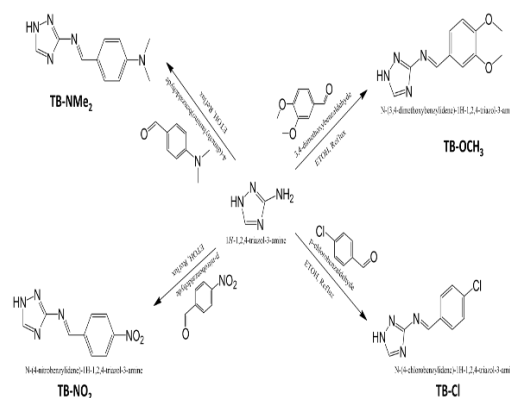


Figure 6. Synthesis of compounds TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃

(E)-N-(4-nitrobenzylidene)-1H-1,2,4-triazol-3-amine [TB-NO₂].

Color: Orange. FT-IR: 3149 (ν_{NH}), 1595 (ν_{C=N}), 1516 (ν_{C=C}); ¹HNMR (400 MHz, DMSO) δ: 14.13 (s, 1H, NH triazole proton), 9.37 (s, 1H, CH=N), 8.38 (d, J=8 Hz, 2H, 2,6-ArH), 8.28 (d, J=8 Hz, 2H, 3,5-ArH). ESI-MS (m/z, %): 217.14 (46.42 %) (Fig. S1, supporting information).

(E)-N-(4-(dimethylamino)benzylidene)-1H-1,2,4-triazol-3-amine [TB-NMe₂].

Color: Yellowish. FT-IR: 3306 (ν_{NH}), 1582 (ν_{C=N}), 1526 (ν_{C=C}); ¹HNMR (400 MHz, DMSO) δ: 13.8 (s, 1H, NH triazole proton), 9.67 (s, 1H, CH=N), 7.81 (d, J=8 Hz, 2H, 2,6-ArH), 6.81 (d, J=8 Hz, 2H, 3,5-ArH), 3.048 (s, 6H, N(CH₃)₂). ESI-MS (m/z, %): 215.19 (49.29 %) (Fig. S2, supporting information).

(E)-N-(4-chlorobenzylidene)-1H-1,2,4-triazol-3-amine [TB-Cl].

Color: Yellow. FT-IR: 3238 (ν_{NH}), 1589 (ν_{C=N}), 1491 (ν_{C=C}); ¹HNMR (400 MHz, DMSO) δ: 14.23 (s, 1H, NH triazole proton), 9.25 (s, 1H, CH=N), 8.05 (d, J=8 Hz, 2H, 3,5-ArH), 7.95 (d, J=8 Hz, 2H, 2,6-ArH). ESI-MS (m/z, %): 206.12 (19.85 %) (Fig. S3, supporting information).

(E)-N-(3, 4-dimethoxybenzylidene)-1H-1,2,4-triazol-3-amine[TB-OCH₃].

Color: Yellow. FT-IR: 3099 (ν NH), 1595 (ν C=N), 1516 (ν C=C); ¹HNMR (400 MHz, DMSO) δ : 14.04 (s, 1H, NH triazole proton), 9.14 (s, 1H, CH=N), 7.56 (d, J=8 Hz, H, 2-ArH), 7.13 (d, J=8 Hz, 1H, 3-ArH), 7.61 (s, 1H, 6-ArH), 3.8 (s, 6H, 2 OCH₃). ESI-MS (m/z, %): 231.13 (100 %) (Fig. S4, supporting information).

2.4. Sulforhodamine-B (SRB) Cytotoxic Assay

The evaluation of the anticancer activities of our triazole compounds is accomplished by sulforhodamine-B method.[45] It starts with seeding cells at a concentration of 3×10^3 cells/well in 96-well micro titer plates. Cells then left for 24 hours followed by incubation with our triazole compounds at concentrations of 12.5, 25, 50, and 100 μ g/ml for each cell line using DMSO as a control vehicle (1% v/v). These doses selection depended on single dose preliminary experiments by applying 100 μ g/ml of all synthesized triazole compounds on the three human cancer cell lines which are HEPG2, HCT-116, and MCF-7. Cells were incubated for 48 hours. Fixation of cells by 20% tri chloroacetic acid is then performed and followed by staining with 0.4% SRB dye. The optical density (O.D) then measured for each well spectrophotometrically at 570 nm by ELISA micro plate reader (TECAN sunriseTM, Germany). The determination of the mean survival fraction for each drug concentration was done as follows: O.D. of the treated cells/O.D. of the control cells. The calculation of IC₅₀ (concentration that provide 50% of cell growth inhibition) of each drug was performed using sigmoidal dose response curve-fitting models (Graph Pad Prizm software, version 5).

2.5. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Bax, Bcl-2, CTGF and PDGF mRNA expression in MCF-7 cells was determined quantitatively using quantitative real-time PCR. Extraction of total RNA was performed using triazole reagent (Invitrogen, Carlsbad, CA). The quality and the quantity of the RNA was determined using Nano drop (Thermo Fisher, UK). Single-stranded RNA was converted into complementary DNA using cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA). Thermal cycling was commenced using thermo cycler (Biometra, Germany) according to the following conditions: 25oC for 10 minutes, 37oC for

120 minutes, 85oC for 5 minutes, and 4oC for ∞ . Real-time PCR analysis was conducted using the thermo-cycler Step OneTM (Applied Biosystems). Each RT-reaction served as a template in a 20 μ L PCR reaction containing 0.2 μ mol/L of each primer and SYBR green master mix (Thermo Fisher Scientific, UK). Primer-set sequences are described in Table S1. Real-time PCR reactions were performed at 50oC for 2 minutes, 95oC for 10 minutes, followed by 45 cycles at 95oC for 15 minutes and 56oC for 1 minute. The mRNA levels of these genes were normalized to GAPDH (Δ CT). The Δ CT was calibrated against an average of the control sample.

3. Results and Discussion**3.1. Spectral confidence of the synthesized triazole derivatives**

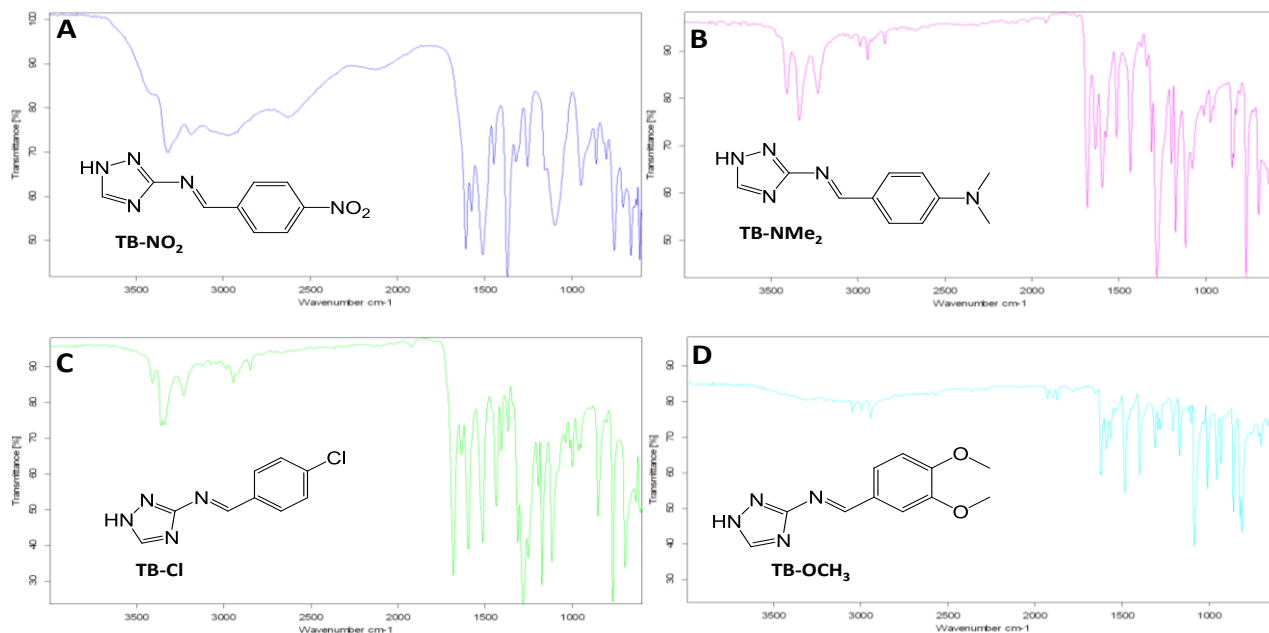
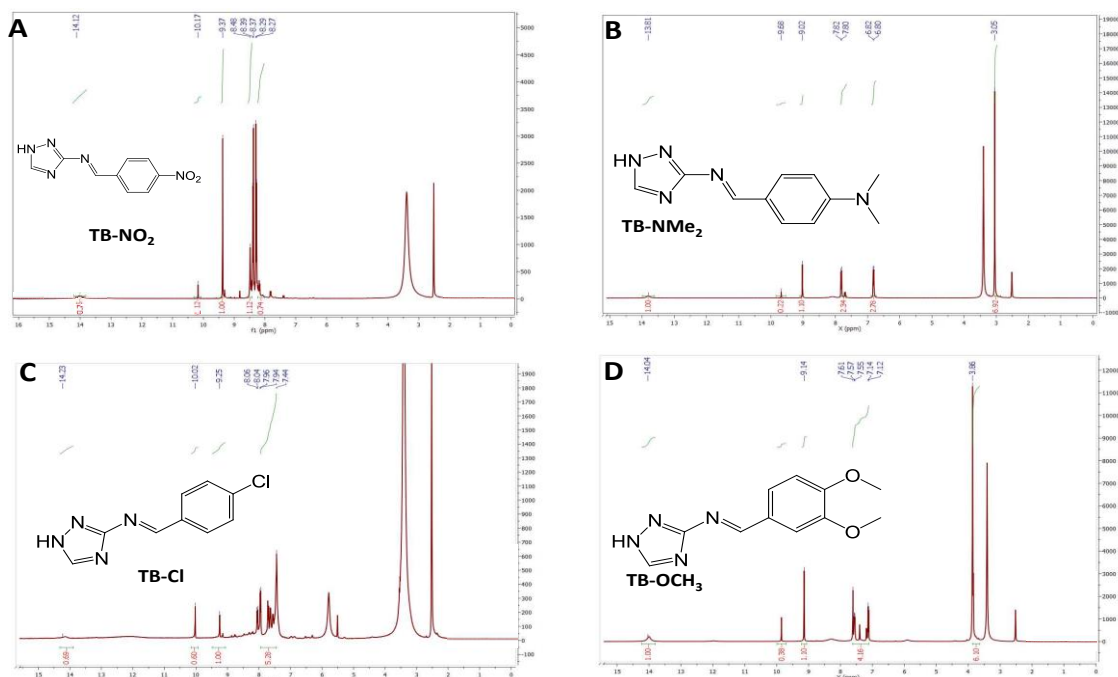
Four 1,2,4-triazole derivatives based on Schiff bases were synthesized by reacting 3-amino-1H-1,2,4-triazole with four different substituted aromatic aldehydes. The reactions were accomplished in the presence of ethanol as solvent. The physical and analytical data of the synthesized four Schiff bases TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃ were depicted in **Table 1**.

Spectral characterization of the prepared triazole derivatives were established by IR, ¹HNMR and mass spectra as outlined in the **Fig. 1**, **Fig. 2** and Figs. S1-S4 (supporting information) respectively. The, confirm the successful preparation of the four triazole derivatives; TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃. The **Figs. 1A-1D** show formation of a band in the range 1582-1595 cm^{-1} referring to formation the Schiff base C=N, as well as the disappearance of the corresponding aldehydes carbonyl C=O group around 1710 cm^{-1} indicating the complete conversion of the aldehyde into corresponding Schiff base triazole derivatives. The band around 3300 cm^{-1} corresponds to the secondary NH confined in the triazole ring.

The ¹HNMR spectra (**Figs.2A-2D**), confirm the chemical structure of the synthesized four Schiff bases. The singlet band at range δ = 9.1-9.6 refer to the new formed Schiff base proton in all synthesized four Schiff base compounds, while the Schiff base proton confined in the triazole ring showed a singlet band at range δ = 9.7-10.2. Some other present bands identify the chemical structure such as the aromatic protons in the δ = 6.80-8.38 and triazole proton (-NH) with a singlet band at δ = 13.8-14.2.

Table 1: the physical properties of the synthesized four Schiff base compounds.

Schiff Base	Empirical formula	Melting point, °C	Color	Shape	Molecular weight	Solubility
TB-NO ₂	C ₉ H ₇ N ₅ O ₂	243	Orange	Powder	217.18	Ethanol, DMSO
TB-NMe ₂	C ₁₁ H ₁₃ N ₅	240	Yellowish	Powder	215.25	Ethanol, DMSO
TB-Cl	C ₉ H ₇ ClN ₄	215	white	Powder	206.63	Ethanol, DMSO
TB-OCH ₃	C ₁₁ H ₁₂ N ₄ O ₂	165	white	Powder	232.23	Ethanol, DMSO

**Figure 1:** FTIR spectra of the synthesized 1,2,4-triazole Schiff base derivatives; (A) is TB-NO₂, (B) is TB-NMe₂, (C) is TB-Cl and (D) is TB-OCH₃.**Figure 2:** ¹H NMR spectra of the synthesized 1,2,4-triazole Schiff base derivatives; (A) is TB-NO₂, (B) is TB-NMe₂, (C) is TB-Cl and (D) is TB-OCH₃.

3.2. Anticancer activity

The cytotoxicity of the synthesized four 1,2,4-triazole Schiff base derivatives TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃ were tested against three cancer cell lines which are human liver cancer cell (HEPG2), human colorectal cancer (HCT-116) and human breast cancer (MCF-7) also they were examined against Vero cells extracted from the kidney of African green monkey to insure their safety on normal cell line. The percentage of inhibition growth of from 100 µg/mL triazole derivatives TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃ using SRB assay are outlined in the **Table 2** and configured in the **Fig. S5 (supporting information)**. The *IC*₅₀ that correspond to the concentration which can induce inhibition of the cancer cells growth by 50% had also determined for TB-NO₂ and TB-OCH₃, as they considered most potent against cancer cells than TB-NMe₂ and TB-Cl as provided from **Table 2**. The *IC*₅₀ results of TB-NO₂, TB-OCH₃ and Doxorubicin are given as mean ± SD (**Table 3**) and exploited in a graph (**Fig. S6**, Supporting information) on HEPG2, HCT-116, MCF-7, and Vero cells. The results clarified no significant toxicity for the tested compounds is observed on normal cell line. In future, further *in vivo* investigation is required to insure the safety of the synthesized compounds.

Among the wide range of 1,2,4-triazole derivatives that previously synthesized and evaluated as anticancer drugs by researchers, our synthesized TB-NO₂ and TB-OCH₃ anticancer could be considered of a great attention when compared with those studies (For clarification, the chemical structure of 1,2,4-triazole derivatives that mentioned in Table 3, have been outlined in the Fig. S7, supporting information). Chowrasia *et al.* [46] synthesized a series synthesized as series of fluorinated 3,6-diaryl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (2a–2i) and studied their anticancer activity against MCF-7. The tested compounds 2a–2i showed *IC*₅₀ of 30.2, 22.1, 23, 25.2, 29.1, 28.8, 27.5, 29.4, and 26.5 µM respectively. All compounds showed *IC*₅₀ higher than our synthesized compounds TB-NO₂ and TB-OCH₃.

A series of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazoles and some novel 5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles bearing 3,4,5-trimethoxyphenyl moiety were synthesized and screened against different cancer cell lines including HEPG2 and MCF-7 by Zhao *et al.*, [47] with *IC*₅₀ for most compounds is >200 µM regarding

HEPG2 and MCF-7. For MCF-7, only one compound showed *IC*₅₀ less than our synthesized TB-NO₂ and TB-OCH₃ with *IC*₅₀= 10.92 µM, and for HEPG2, three compounds were more potent than TB-NO₂ and TB-OCH₃. While Li *et al.* [48], prepared substituted-phenyl-1,2,4-triazol-3-thione analogues with modified D-glucopyranosyl residues and examined their cytotoxicity on MCF-7 and Bel-7402. The *IC*₅₀ for compounds 5a–5d are higher than TB-NO₂ and TB-OCH₃, while compounds 1a–1d showed lower *IC*₅₀. Kamel *et al.*, [49] synthesized a series of new N-substituted-3-mercapto-1,2,4-triazoles (3a,b and 7a–d), triazolo[1,3,4]thiadiazines (5a,b) and triazolo[1,3,4]thiadiazoles (4a–d, 6 and 8a–d) and screened for their cytotoxicity against a variety of cancer cell lines. For N-substituted-3-mercapto-1,2,4-triazoles 3a,b and 7a–d, the *IC*₅₀ values are 0.053, 0.089, 2.022, 1.299, 1.230, and 2.782 µM for HEPG2 respectively, while the *IC*₅₀ values of MCF for the same compounds are 2.102, 1.092, 3.231, 1.672, 2.170, 1.592 µM respectively. Pei-LiangZhao *et al.*, [50], synthesized as series of 3-alkylsulfanyl-4-amino-1,2,4-triazole (8p–2x) and showed *IC*₅₀ over 100 on HEPG-2 & HCT-116 µM..

3.3. Assessment of Apoptosis in MCF-7 cells

In our study, we determined the expression of mRNA of Bax and Bcl-2 relative to GADPH to investigate the apoptotic effect of TB-NO₂ and TB-OCH₃ on MCF-7 cells in the dose of *IC*₅₀. Bax and Bcl-2 are members of Bcl-2 family that have a pivotal role in tumor progression or inhibition of apoptotic pathway. The **Fig. 3**, shows the expression of Bax and Bcl-2 proteins for control and treated MCF-7 cells. In case of treatment with TB-NO₂ triazole schiff base, an insignificant increase in the expression of Bax was observed, which is an apoptotic promoter and insignificant reduction in the expression of Bcl-2 and is apoptosis inhibitor. TB-OCH₃ caused a significant expression of Bax and also significant increase in Bcl-2 expression.

The Bax/Bcl-2 ratios for both TB-NO₂ and TB-OCH₃ determine the apoptotic ability of these compounds. The Bax/Bcl-2 ratios of the newly synthesized compounds as shown in the **Fig. 4**, are significantly higher than Bax/Bcl-2 ratio of the control which confirms the apoptotic effect of our synthesized triazole derivatives.

Table 2: The inhibition % for compounds TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃ against human cancer cell lines.

Concentration, 100µg/mL	Inhibition (%) (Mean ± SD)		
	HEPG2	HCT-116	MCF-7
Doxorubicin	83 ± 4.3	85 ± 3.5	78.6 ± 2.5
TB-NO ₂	67.76 ± 0.75	70.6 ± 0.56	73.52 ± 0.8
TB-NMe ₂	51.2 ± 0.81	68.37 ± 0.21	68.77 ± 1.29
TB-Cl	59.40 ± 0.82	66.19 ± 0.45	65.61 ± 0.6
TB-OCH ₃	71.33 ± 0.96	73.25 ± 0.44	74.91 ± 0.78

Table 3: The IC₅₀ for compounds TB-NO₂, TB-OCH₃ and Doxorubicin against human cancer cell lines with comparison against some relevant studies.

Compounds	Cytotoxicity (IC ₅₀ , µM)			Ref.
	HEPG-2	HCT-116	MCF-7	
Doxorubicin	4.97	6.8	6.3	
TB-NO ₂	46.7	83	18.49	Current work
TB-OCH ₃	43.9	90.5	20.4	Current Work
2a-2i	---	---	22-30	[46]
5-10	>200	---	>200	[47]
5A-5B, 6A-6B/7Ai-7Aviii	---	74.1-300/19.2-300	87-300/38-300	[51]
1, 2, 3 and 4	45.3, 36.4, 33.9 and 40	---	---	[16]
8p-8x	>100	>100	---	[50]

The chemical structure of compounds in Refs [39, 16, 40-42] was outlined in Fig. S7.

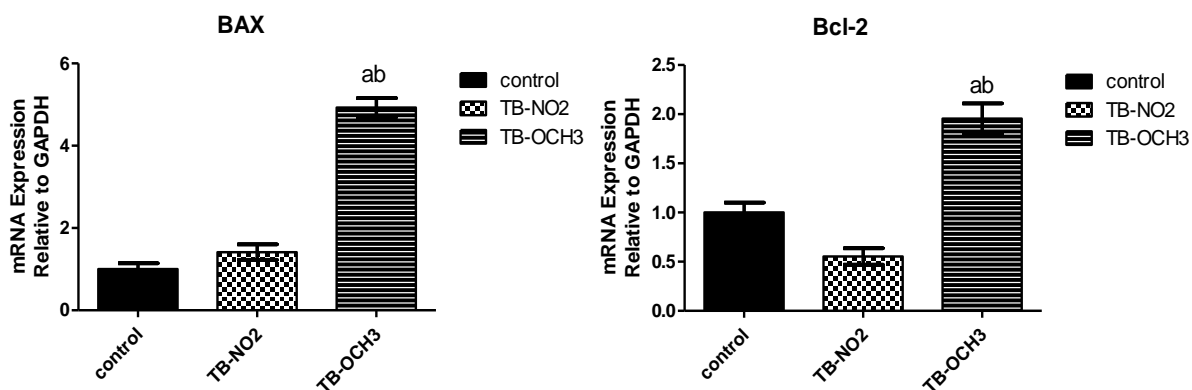


Figure 3: Effect of TB-NO₂ and TB-OCH₃ in Bax and Bcl-2 mRNA expression in MCF-7 cells. Results are expressed as means ± SD of two independent experiments performed in duplicate. Statistical significance of results was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. (a) Significantly different from the control group and (b) significantly different from TB-NO₂ at P<0.05.

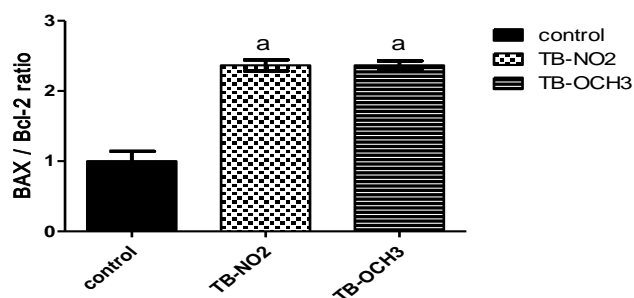


Figure 4: Bax / Bcl-2 ratio for TB-NO₂ and TB-OCH₃ in MCF-7 cells. Results are expressed as means ± SD of two independent experiments performed in duplicate. Statistical significance of results was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. (a) Significantly different from the control group at P<0.05.

3.4. Determination of mRNA expression of CTGF and PDGF in MCF-7 cells

As well known, the connective tissue growth factor CTGF and Platelet-derived Growth Factor PDGF play a potential role regarding the cell growth and survival. So the mRNA expression of these genes was determined to investigate the effect of TB-NO₂ and TB-OCH₃ on the MCF-7 cells growth regulation. For CTGF gene, the TB-NO₂ significantly promotes the down-regulates of the gene expression with an

insignificant down-regulation triggered by the TB-OCH₃ relative to control. That's trend suggests a protective and antifibrotic effect for the triazole TB-NO₂. The TB-NO₂ promotes a down-regulated insignificantly regarding to expression of PDGF gene, while TB-OCH₃ doesn't exert any change relative to the control. These results conclude that TB-NO₂ and TB-OCH₃ promote apoptosis in MCF-7 cells through the regulation of growth factors genes.

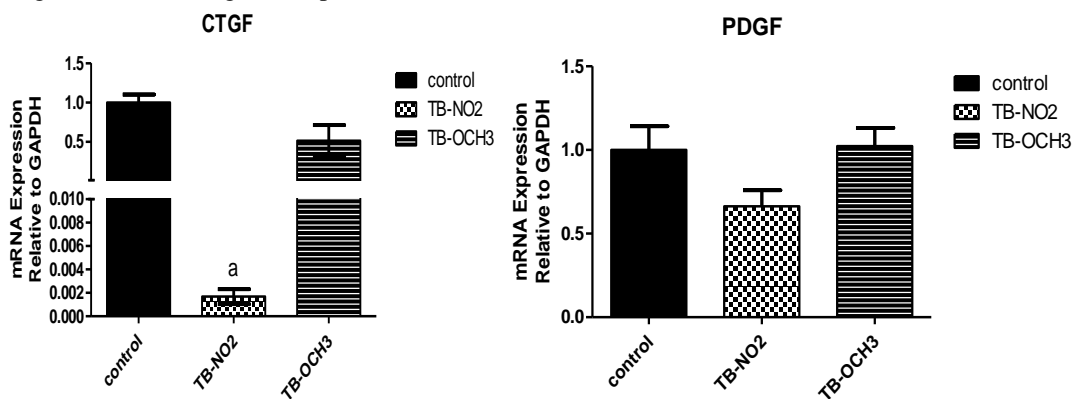


Figure 5: Effect of TB-NO₂ and TB-OCH₃ on CTGF and PDGF mRNA expression. Results are expressed as means \pm SD of two independent experiments performed in duplicate. Statistical significance of results was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. (a) Significantly different from the control group at $P < 0.05$.

3.5. Structure-anticancer activity relationship

By analyzing the results obtained on the HEPG2, HCT-116 and MCF-7 that depicted in both Tables 2 & 3, we can correlate the effect of a chemical structure of the synthesized TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃ on the tested cancer cells. As known, the molecular configuration and chemical structure of any drug candidate plays a significant role in the therapeutic efficacy, therefore a relatively minor modifications in the chemical structure promotes a major influence on the medicinal efficacy [51, 52]. Therefore regarding to this study we attempt to recognize the functional groups which are important for pronounce the anticancer activity toward the HEPG2, HCT-116 and MCF-7 cancer cells. The triazole Schiff base derivatives were obtained via condensation between the amino group at the position 3 of 1,2,4-triazole ring with the some aromatic aldehydes with different function groups at the para-position like nitro, dimethyl amino, chloro and methoxy groups leading to formation of four triazole Schiff base TB-NO₂, TB-NMe₂, TB-Cl and TB-OCH₃.

Among these compounds both TB-OCH₃ and TB-NO₂ showed highest inhibition against all the tested cancer cells, hence they were chosen for estimating their IC_{50} values as outlined in the **Table 3**. The presence the electron withdrawing groups in the para position of the phenyl ring can increase their anti-proliferation activity and so increasing their anticancer activity [53]. Based on these finding we expect that the activity of TB-NO₂ > TB-Cl > TB-OCH₃ > TB-NMe₂. But this not the only factor that could effect on the activity of the synthesized Schiff base 1,2,4-triazole derivatives, but the polarity of the substituted play an important role. As more the synthesized triazole derivatives possess both hydrophobic and hydrophilic nature (amphipathic property) could enhance their penetrability through the cell membranes and allowing effectively interaction with biomolecules such as RNA, proteins and DNA [51]. Based on that the synthesized TB-OCH₃ and TB-NMe₂ could have the highest anti-proliferation activity due to their higher polarity. Finally the net effect of the polarity and electron withdrawing effect predominate the activity as anti-proliferation. From the results included in the **Tables 2 & 3**, the drug candidate TB-OCH₃ shown a potent effect against the tested cancer cells HEPG2, HCT-116 and MCF-7.

4. Conclusion

In summary, we prepared four Schiff bases of 3-amino-1*H*-1,2,4-triazole and evaluated their anticancer activity against three human cancer cell lines which are HEPG2, HCT-116, and MCF-7. The in vitro screening proved that 1,2,4-triazole derivatives possess a great potency in cancer fight. Two derivatives of this study TB-NMe₂ and TB-Cl produced a moderate anticancer activity against cancer cell lines. The derivatives TB-NO₂ and TB-OCH₃

6. Conflicts of interest

The authors declare no conflict of interest.

7. Acknowledgements

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