Antimicrobial and antiproliferative activities of novel synthesized 6-(quinolin-2-ylthio) pyridine derivatives with molecular docking study as multi-targeted JAK2/STAT3 inhibitors

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Abstract: Quinoline derivatives are attracting considerable interest due to their biological importance. In this paper, several 2-amino-4-aryl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile derivatives are synthesized by adopting a one-pot reaction of quinoline-2-thione, aromatic aldehydes, and malononitrile in the presence of sodium hydroxide in absolute ethanol. The structures of these newly synthesized compounds were determined using different spectroscopic techniques, including elemental analyses, IR, 1H NMR, and MS. The synthesized derivatives were screened for their antimicrobial and cytotoxic activities. Compounds **4a**, **4b**, **4d,** and **4e** exhibited promising antimicrobial activity compared to antibacterial and antifungal standard drugs. Additionally, **4f**, **4d,** and **4g** showed potent cytotoxic activity against both MCF-7 and A549 cells with IC_{50} values (6.39- 9.3 µM). Our molecular docking results of compound **4f** proves good binding affinity towards the three tested proteins as Jak2/STATA3 inhibition; are in accordance with the RT-PCR mRNA expressions of the compound against MCF-7 cells which downregulated the Jak2 and STAT3 genes, and this may be the proposed mode of action for anti-breast cancer activity.

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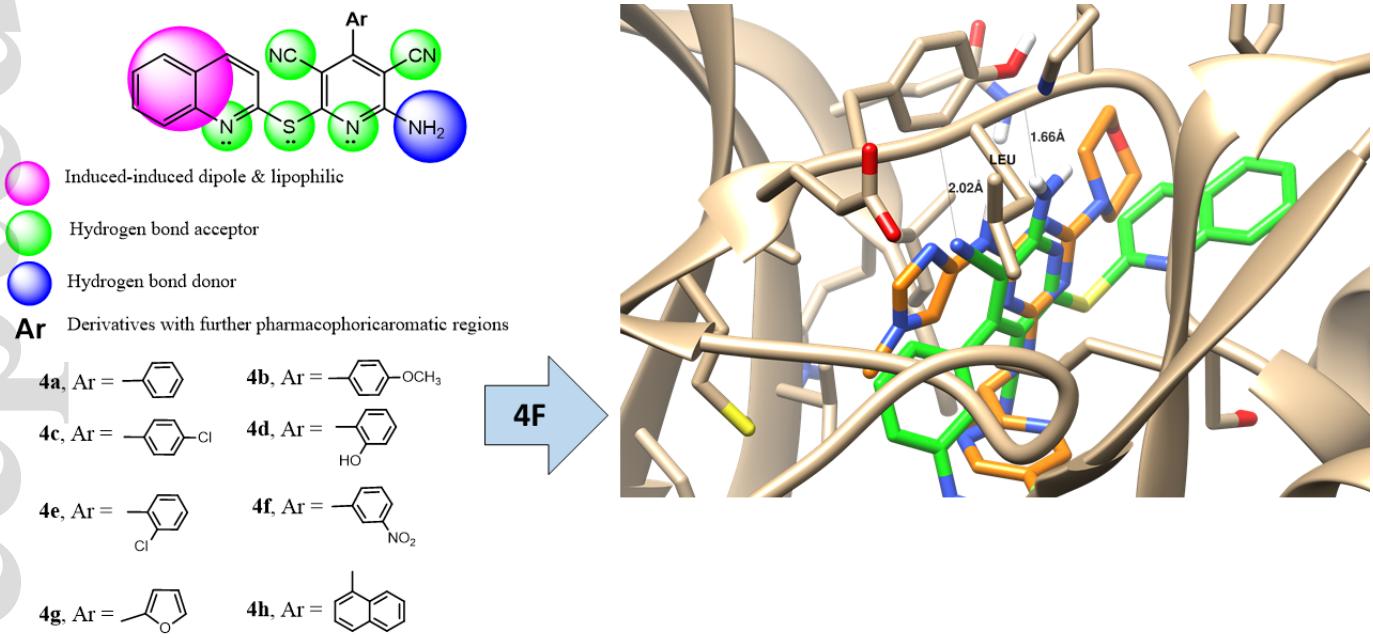
Highlights

- Novel 6-(quinolin-2-ylthio) pyridine derivatives were synthesized and spectroscopic characterized
- Some derivatives exhibited promising antibacterial and antifungal activities
- One derivative **4f** showed potent cytotoxic activity against MCF-7 and A549 with IC_{50} = **6.39** and **6.9** µM, respectively.
- Lead compound **4f** exhibited Jak2/STAT3 inhibition in both RT-PCR and *in silico* approaches

Graphical abstract



Compound **4f** exhibited potent cytotoxic activity against MCF-7 and A549 cell lines with IC_{50} = **6.39** and **6.9** µM, respectively with elucidated JAK2/STAT3 inhibition using gene expression and *in silico* approaches

1. Introduction

Over the last decades, quinolines have attracted much attention regarding their considerable biological and chemical activity [1–4]. Therapeutic potential of quinoline based heterocycles was recognized as a core structure in various pharmaceuticals such as; anti-inflammatory [5,6] antimicrobial [7,8], antimalarial [9,10] and antioxidant agents [11,12]. Recently, several quinoline derivatives are associated with antitumor [13,14], antiprotozoal [15], Antituberculosis [16,17] and antiulcer [18] activity. One of the most significant quinoline derivatives is quinoline-2-thiones, which is synthesized through thiation of corresponding quinoline-2-ones [19], 2-haloquinolines [20–22] or by using indium (III) reagent-mediated tandem Friedel Crafts alkenylation cyclization of 2-alkynylphenyl isothiocyanates [23]. Previous literature reported that quinoline-2-thiones have a variety of applications such as synthetic intermediates [24,25], bioactive molecules [26], sulfur-nitrogen mixed donor ligands [27–29], protective groups of thiols [30,31] and corrosion inhibitors [32] as well as fluorescent sensors for metals, pH and HNO [33]. Since pyridinedicarbonitrile nucleus is among the most widely used biologically and therapeutically important agents, this encourages us to attach it to quinoline ring system aiming to obtain novel compounds with anticipated biological activity [34,35].

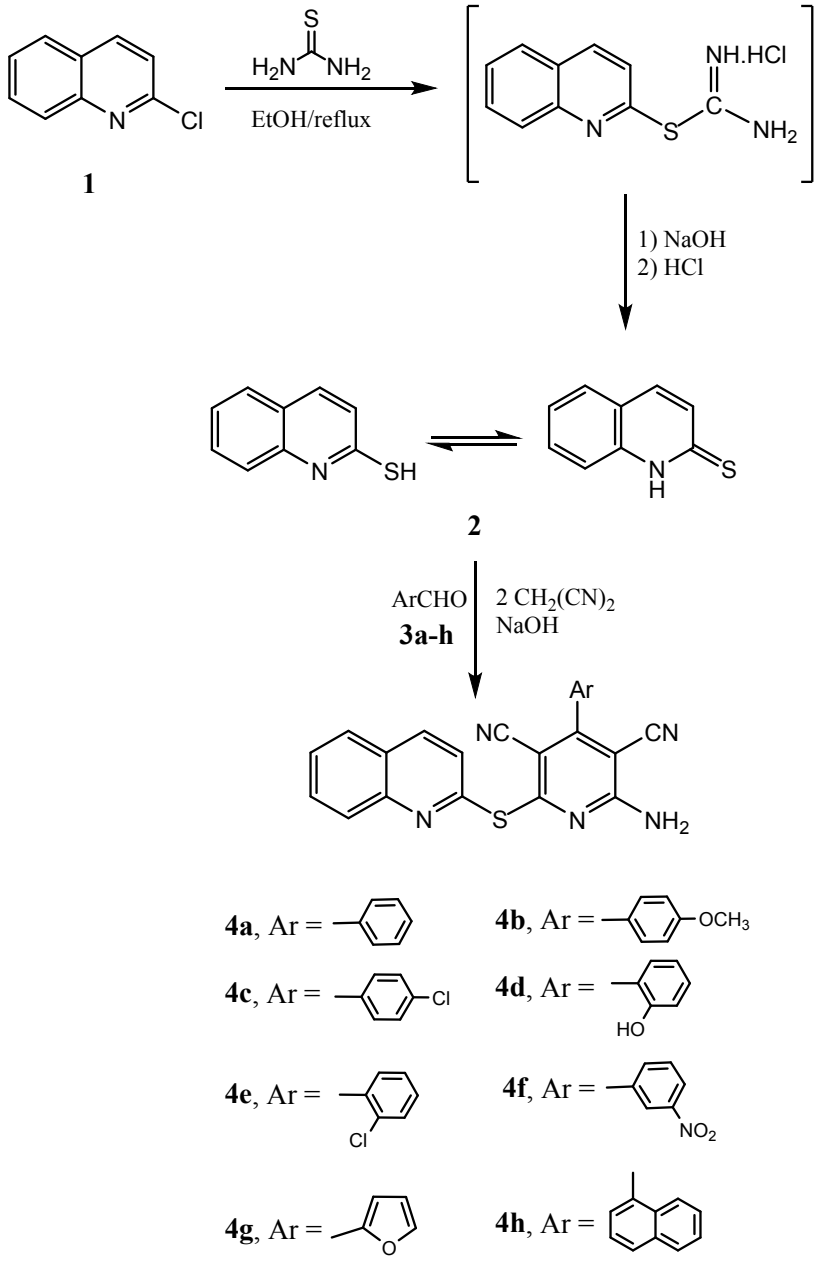
Kambe *et al*. [36] reported that the reaction of malononitrile with aromatic aldehyde and alkyl ketones in the presence of ammonium acetate and boiling benzene yields 2-Amino-4-aryl-3-cyanopyridines and the reaction was assumed to proceed by formation of arylidenemalononitrile firstly which in turn reacts with alkyl ketones. Similarly, Azizi and Haghayegh [37] found that benzylidenemlononitrile reacts with benzenethiol and malonoitrile in presence of the deep eutectic solvent composed of choline chloride: urea, 1:2 by ratio as a solvent and catalyst at 60 ºC for 80-240

minutes to give 2-amino-4-phenyl-3,5-dicyano-6-thoiphenylpyridine. In this context, we aimed to study the reaction of quinolinethione with aromatic aldehydes and malonoitrile to get 2-amino-4-(aryl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile derivatives, and to screen their antimicrobial and antiproliferative activities with elucidated mechanistic mode of action.

2. Results and discussion

2.1. Chemistry

This paper outlines synthesis of 2-amino-4-aryl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile derivatives **4a-h** as described in schemes 1, where 2-chloroquinoline **1** reacts with thiourea in refluxing ethanol for 3h to give quinolin-2-yl carbamimidothioate hydrochloride which is then added to solution of sodium hydroxide (5 M) followed by heating for 5 minutes. The previous solution is then acidified with hydrochloric acid to give quinoline-2-thione **2** in a good yield. Compound **2** reacts with two moles of malononitrile and one mole of aromatic aldehydes **3a-h** for 1 h in absolute ethanol catalyzed by sodium hydroxide to yield 2-amino-4-aryl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitriles **4a-h**. The reaction was firstly carried out using pyridine, piperidine, and triethylamine as a catalyst, and periodical reaction monitoring by TLC showed the formation of a new product, but the reaction did not proceed to completion even after 8 hours heating. However, using sodium hydroxide with heating for 1 hour resulted in the formation of desired derivatives this could be attributed to the increasing of nucleophilicity of sulfur atom through sodium thiolate formation.



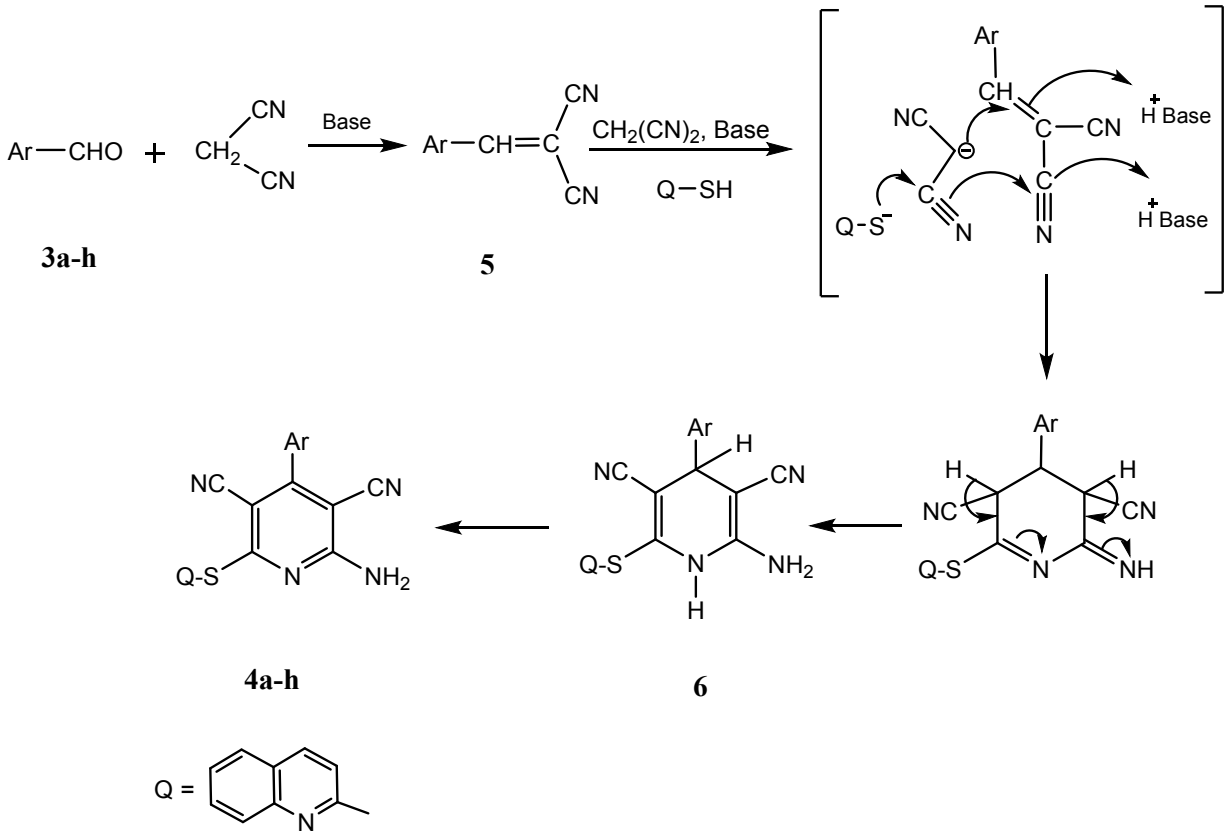
Scheme 1: Synthesis of derivatives **4a-h**

The reaction mechanism, scheme 2**,** of this pathway is discussed in accordance with previously suggested in the literature [38], the first step of this reaction involves the Knoevenagel condensation of aromatic aldehyde with malononitrile to give arylidenemlononitrile **5**. A molecule of malononitrile adds to compound **5** through Michael type addition, then the thiolate ion attacks -C≡N group of the

adduct giving dihydropyridine **6**. In situ oxidative aromatization of **6** affords 2-amino-4-aryl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile derivatives.

2.2. Spectral Elucidation

The structure of all the synthesized derivatives was confirmed by physical data, IR, 1H NMR, and Mass spectral data compared to previously reported in literature. Mass spectra of derivatives **4a-h** contain the molecular ion peaks matching their expected values. All compounds showed NH_2 peak in the range of 3471-3144 cm^{-1}, CH aromatic in the range of 3091-3054 cm^{-1}, cyano group in the range of 2216-2197 cm^{-1} and C=N in the range of 1635-1580 cm^{-1}. Compound **4d** shown OH peak at 3450 cm^{-1}. Also, derivative **4f** revealed C-NO_2 peak at 1533 and 1344 cm^{-1}. The ^{1}HNMR spectra of most compounds shown singlet signal at δ = 5.96 due to NH_2 group. Derivative **4d** has a singlet at δ = 8.6 ppm due to presence of NH_2 group; it also revealed a signal at 10.4 ppm assigned to OH group. H-4 of quinoline ring of most compounds give a doublet signal at δ = 8.3 ppm. Ar–H of most derivatives appeared as multiplets in the range of δ 6.82-7.56, 8.7-8.89, and 8.61 ppm. Finally, compound **4b** showed a signal at 3.82 due to OCH_3. All previous mentioned spectral data confirms without any doubt the structure of target compounds.



Scheme 2: Formation of derivatives **4a-h**

2.3. Antimicrobial Activity

Newly synthesized derivatives were screened *in vitro* for their antibacterial activities against two gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC6633), two gram-negative bacteria (*Salmonella typhimurium* ATCC14028 and *Escherichia coli* ATCC 8739). Moreover, antifungal activity was tested against *(Candida albicans* ATCC10231). Ciprofloxacin and Ketoconazole drugs were used as positive control for antibacterial and antifungal activity, respectively. Tested compounds and standard drugs were dissolved in DMSO, which was also used as negative control. Results of antimicrobial study are reported as zone of inhibition and are summarized in **Table (1)**, indicating that compounds **4a, 4b, 4d,** and **4e** possess promising activity against *Candida albicans*. Also, derivatives **4a**, **4d**, and **4e** gave a moderate activity against gram-negative bacteria. Furthermore, derivatives **4a**, **4b**, **4d,** and **4e** gave moderate activity against gram-positive bacteria. Whereas compounds **4c**, **4f** and **4h** are

inactive against all tested strains. The potency of the two derivatives **4d** and **4e** could be attributed to *o*-substitution of the phenyl ring, when compared to the *p*-, *m*- or unsubstituted phenyl ring.

Table (1): *In vitro* antimicrobial activity of newly synthesized compounds expressed as inhibition zones (I.Z) diameter*

Compound	Gram +ve		Gram -ve		Fungus
	S. aureus ATCC6538	*B. subtilis* ATCC6633	*E. coli* ATCC8739	*S. typhimurium* ATCC14028	*C. albicans* ATCC10231
	I.Z	I.Z	I.Z	I.Z	I.Z
4a	N/A**	10±0.9	8±0.47	10±0.72	9±0.79
4b	N/A	10±0.76	8±0.46	8±0.10	9±0.76
4c	N/A	N/A	N/A	N/A	N/A
4d	8±0.27	11±0.87	12±0.94	11±1.90	9±0.23
4e	8±0.47	10±0.84	11±1.01	9±0.76	10±0.29
4f	N/A	N/A	N/A	N/A	N/A
4g	5±0.32	6±0.12	5±0.97	4±0.32	5±0.85
4h	N/A	N/A	N/A	N/A	N/A
Ciprofloxacin	40±1.49	35±1.07	32±1.65	46±1.86	12±0.19
Ketoconazole	18±0.97	13±0.86	20±1.04	14±0.65	25±1.04

*Values are Mean±SD of measured inhibition zone diameter (mm) of three replicates.
**N/A no activity.

2.4. Cytotoxic activity against MCF-7 and A549 cell lines

Tested derivatives were screened for their cytotoxicity against two cancer cell lines; MCF-7 and A549 cell lines using their serial working concentrations (1, 10, 100, and 1000 μM). After 48 h of incubation, the percentage of cell viability was determined using MTT assay, and the IC_{50} values were calculated accordingly using GraphPad prism7. The results illustrated in **Table (2)** and **Fig. (1)** show potential cytotoxic effect for compounds **4f, 4d,** and **4g** IC_{50} values (6.39- 9.3 μM) against both MCF-7 and A549 cell lines. Our results are in accordance with Abbas et al [39] who screened a series of

synthesized quinoline derivatives as a potential anticancer activity with IC_{50} = 1.91–5.29 µM against A549 and K-562 cell lines.

Table (2): IC_{50} values of the tested derivatives against MCF-7 and A549 cell lines using MTT assay

Compounds	IC_{50} ± SD* (nM)	
	MCF-7	**A549**
4a	12.7±1.01	17.2±1.70
4b	11.6±0.87	9.7±0.65
4c	28.2±1.98	ND
4d	8.9±0.43	9.3±0.87
4e	30.1±1.79	>50
4f	6.39±0.23	6.9±0.23
4g	7.8±0.13	8.4±0.54
4h	13.41±1.10	12.4±0.99
5-FU	**5.8**±0.09	**4.5**±0.11

* Values are expressed as Mean±SD of 3 independent experimental runs, **ND**= Not Determined

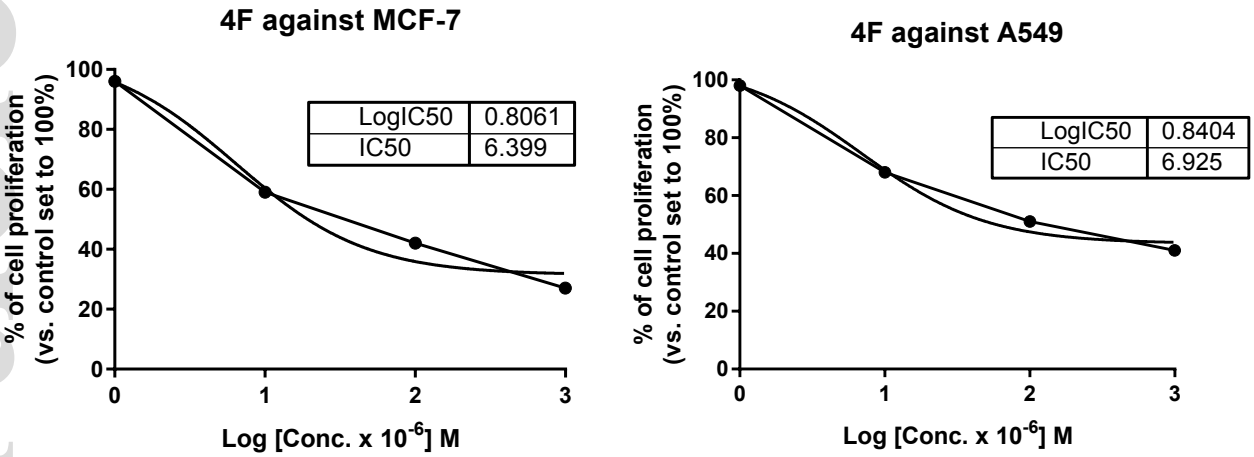


Fig. (1): MCF-7 cell line treated with a serial dilution of compound **4f** for 48 h. using MTT assay. Dose-response curve of the percentage of cell growth viability compared to control.

2.5. RT-PCR

The STAT3 signaling pathway was validated with apoptosis as a critical therapeutic target for cancer therapy. It can be activated by receptor-associated kinases (JAK) [40], so RT-PCR reaction was performed to monitor the apoptotic effect of compound **4f** on the mRNA expression of P53, MDM2, Bax as pro-apoptotic genes, Bcl-2 as anti-apoptotic gene, and Jak2/STAT3 genes as inhibition signaling pathway modulating cell death in MCF-7 cells. As shown in **Fig. (2)**, at the tested concentration, compound **4f** significantly elevated BAX (3.56-fold), P53 (5.91-fold), and MDM2 (4.42-fold) gene expressions. Also, it significantly inhibited anti-apoptotic gene BCl2 (0.79-fold), and dual inhibition Jak2/STAT3 with minimum fold of change 0.6. All mRNA expressions are related to the expression of β-actin as the housekeeping gene (fold of change=1). Our RT-PCR results for gene expression for pro-apoptotic, anti-apoptotic genes, and Jak2/STAT3 inhibition are in accordance with previous studies followed the same rational for elucidating the mechanistic mode of action [41–44]

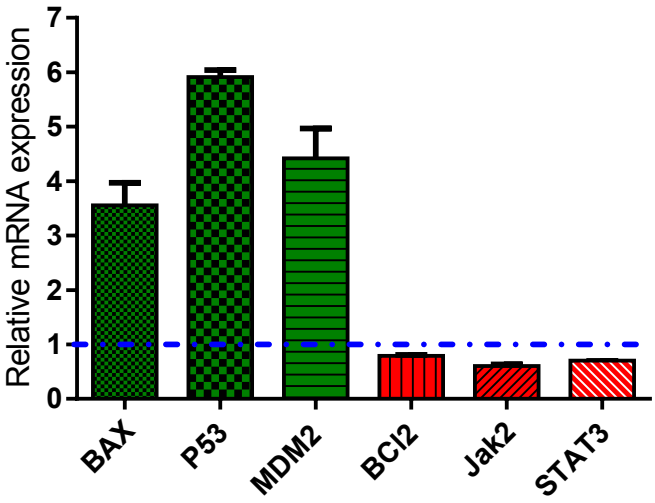


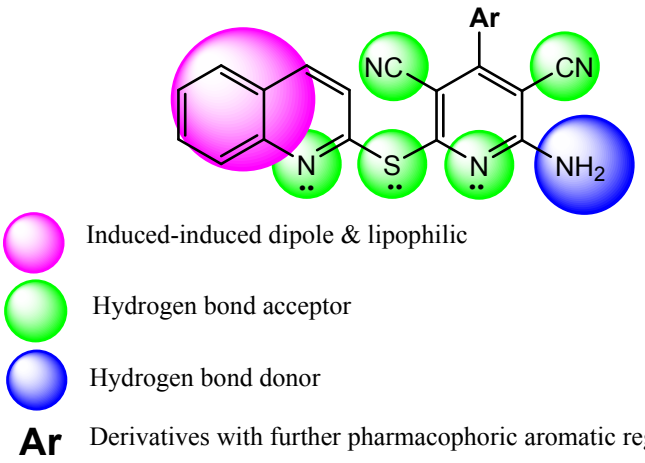
Fig. (2): RT-PCR gene expression analysis of upregulated genes; "BAX, P53, MDM2" (Green bars), and downregulated genes "BCL2, Jak2, and STAT3" (Red bars) for MCF-7 cell line treated with compound **4f** (IC_{50}=6.39 µM, 48h) versus control. β-actin as housekeeping gene (Fold of change=1) as the dashed line

2.6. Molecular docking study

The molecular docking study was performed to investigate the binding interactions towards three proteins as Jak2/STAT3 inhibition to elucidate their mechanism of action inhibitors. Proteins 3ZMM, 4C62, and 5AEP, whose crystal structures complexed with their co-crystallized ligands, were easily accessible from the Protein Data bank [45–47]. The chosen PDB structures have proper resolution ≤ 2.5 and with acceptable R-value. The co-crystallized ligands of the studied proteins form (1-2) hydrogen bonds with Leu 932 as the key amino acid for interaction. **Table (3)**, summarize the overall ligand-receptor interactions with binding energies of the tested derivatives insides the three protein binding sites, while other derivatives couldn't bind. As seen in **Table (4)** with 3D images, compound **4f** was docked inside the protein active site of the studied proteins. It formed one to two hydrogen bonds with bond length ≤ 2 ºA through the functional groups either as hydrogen bond acceptor/donor with the key amino acid Leu 932 like their co-crystallized ligand with binding energy -13.42 to -17.19 Kcal/mol. Additionally, compound **4f** it formed lipophilic interactions with the nonpolar amino acids (Leu 983, Leu 855, Val 863, Pro 933, Met 929, Ala 880, and Leu 932) inside the receptor pocket. Our molecular

docking results of compound **4f** proving good binding affinity towards the three tested proteins as Jak2/STATA3 inhibition.; are in accordance with the RT-PCR mRNA expressions of the compound against MCF-7 cells which downregulated the Jak2 and STAT3 genes, and this may be the proposed mode of action for anti-breast cancer activity.

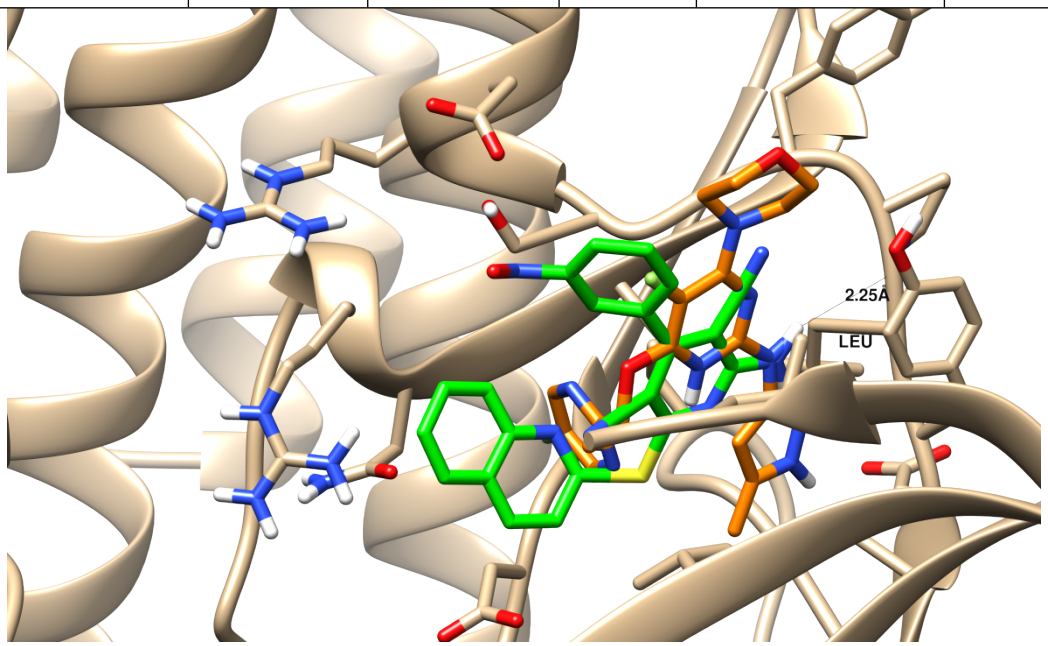
Table (3): Summarized ligand-receptor interaction of the docked compounds inside the receptor binding sites 3ZMM, 4C62, and 5AEP

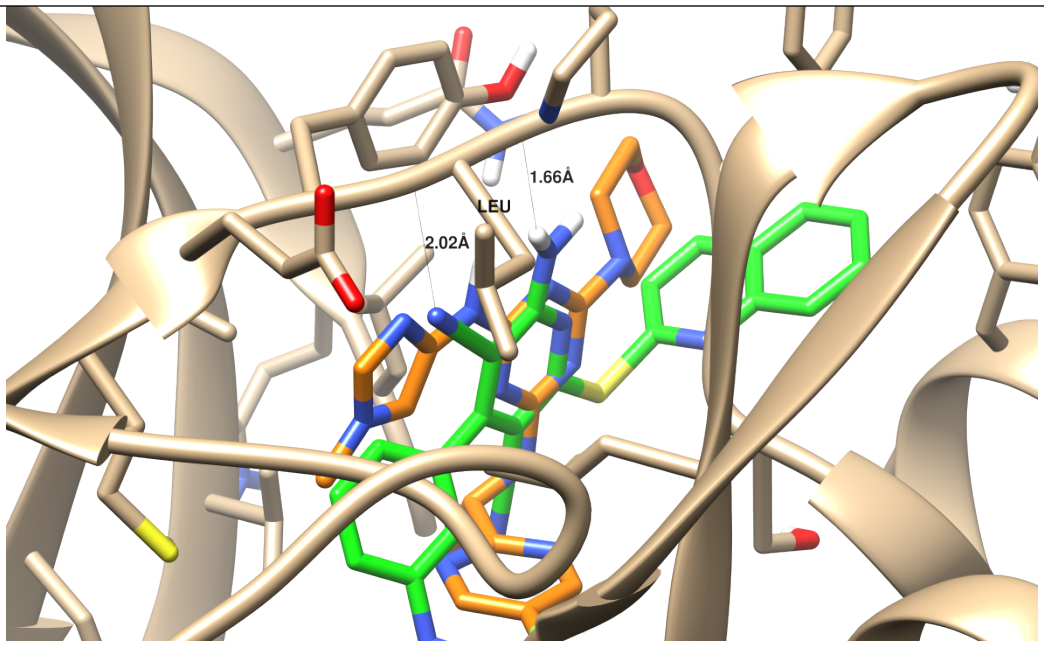


Protein (PDB code)	Tested compounds	Binding energy (Kcal/mol)	Interactive amino acids
3ZMM	**4b**	-10.90	**1** HB with Leu 932
	4d	-14.77	**2** HB with Leu 932
	4f	-13.60	**1** HB with Leu 932
	4g	-20.03	**2** HB with Leu 932
	4h	-13.77	**2** HB with Leu 932
4C62	**4a**	-15.32	**2** HB with Leu 932
	4d	-15.02	
	4f	-17.19	
	4g	-12.92	
5AEP	**4f**	-13.42	**2** HB with Leu 932

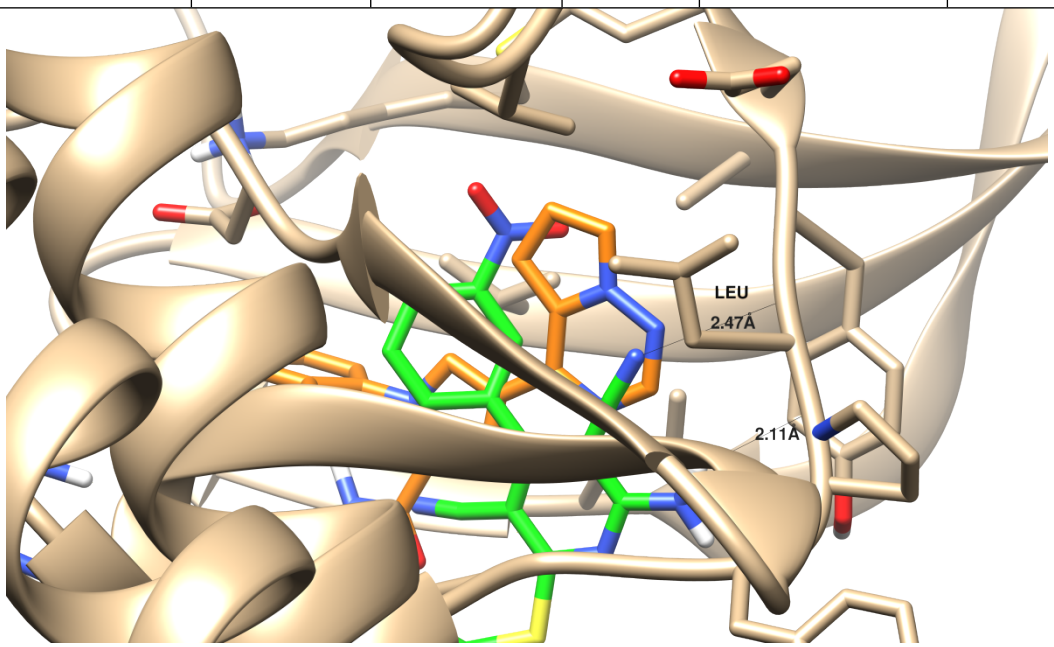
*Other derivatives couldn't bind inside the receptors binding sites.

Table (4): Analysis of ligand-receptor interactions of the **4f**-docked compound inside 3ZMM, 4C62, and 5AEP proteins as Jak2 inhibitor

Protein (PDB code)	Binding affinity (Kcal/mol)	Type on interaction	Bond length (°A)	Interaction moiety involved	Amino acid
3ZMM	**-15.32**	**H-donor**	**2.25**	**N-H**	**C=O Leu 932**
					
4C62	**-17.19**	H-acceptor H-donor	2.02 1.66	**C≡N** **N-H**	**N-H Leu 932** **C=O Leu 932**



5AEP	**-13.42**	H-acceptor H-donor	2.47 2.11	**C≡N** **N-H**	**N-H Leu 932** **C=O Leu 932**



Superimposed **4f**-docked compound (Green), and the co-crystalized ligand (Orange) of the three studied 3ZMM, 4C62, and 5AEP proteins

3. Experimental

3.1. Instruments

Melting points were determined in open-glass capillaries and are uncorrected. IR spectra were recorded on a Bruker Vector 22 Germany spectrometer (KBr). 1H NMR spectra were recorded on (Bruker) 400 MHz spectrometer using TMS as an internal reference. Mass spectra were obtained at 70 eV using Shimadzu QP-2010 Plus mass spectrometer. Completeness of reactions was checked by thin-layer chromatography (TLC) on silica gel F254 aluminum sheets (Merck), and visualized by UV lamp at 254–365 nm.

3.2. Synthesis

3.2.1. Synthesis of 2-amino-4-aryl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4a-h):

Quinoline-2-thione (1 mmol), aldehyde derivatives **3a-h** (1 mmol), malononitrile (2 mmol) and sodium hydroxide (1 mmol) reacted in ethanol at refluxing temperature for 1 h. Then reaction mixture was cooled and the formed precipitate was filtered and recrystallized from ethanol giving pure products.

3.2.2. 2-Amino-4-phenyl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4a) Yellow solid; yield 79 %; mp 222-224 °C; IR (KBr, cm^{-1}): 3427, 3210 (NH_2), 3052 (CH_{arom}), 2206 (CN), 1635 (C=N), 1454 ($C=C_{arom}$); MS (m/z): 379 [M^+, 0.16 %]; ^1H NMR (DMSO-d6): δ (ppm) = 5.96 (s, 2H, NH_2), 6.83–7.53 (m, 10H, $H_{aromatic}$), 8.3 (d, 1H, H-4 of quinoline). Anal. Calcd for $C_{22}H_{13}N_5S$ (379.44): C, 69.64; H, 3.45; N, 18.46. Found: C, 69.54; H, 3.36; N, 18.35.

3.2.3. 2-Amino-4-(4-methoxyphenyl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4b) Yellow solid; yield 81 %; mp >300 °C; IR (KBr, cm^{-1}): 3471, 3193 (NH_2), 3059 (CH_{arom}), 2923 (CH_{aliph}), 2202 (CN), 1635 (C=N), 1467 ($C=C_{arom}$); MS (m/z): 409 [M^+, 0.31 %]; ^1H NMR (DMSO-d6): δ (ppm) = 3.82 (s, 3H, OCH_3), 5.96 (s, 2H, NH_2), 6.83 –7.59 (m, 9H, $H_{aromatic}$), 8.3 (d, 1H, H-4 of quinoline). Anal. Calcd for $C_{23}H_{15}N_5OS$ (409.47): C, 67.47; H, 3.69; N, 17.10. Found: C, 67.32; H, 3.54; N, 16.95.

3.2.4. 2-Amino-4-(4-chlorophenyl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4c) Yellow solid; yield 76 %; mp >300 °C; IR (KBr, cm^{-1}): 3372, 3216 (NH_2), 3091 (CH_{arom}), 2216(CN), 1580

(C=N), 1467 (C=C_{arom}); MS (m/z): 413 [M^+, 0.21 %], 411[M^+-2, 0.26%], 270[Base Peak, 100%]; ^{1}H NMR (DMSO-d6): δ (ppm) = 5.96 (s, 2H, NH_2), 6.82 –7.56 (m, 9H, $H_{aromatic}$), 8.3 (d, 1H, H-4 of quinoline). Anal. Calcd for $C_{22}H_{12}ClN_5S$ (413.88): C, 63.84; H, 2.92; N, 16.92 Found: C, 63.70; H, 2.78; N, 16.78.

3.2.5. 2-Amino-4-(2-hydroxyphenyl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4d) Yellow solid; yield 81 %; mp 190-192 °C; IR (KBr, cm^{-1}): 3450 (OH), 3323, 3144 (NH_2), 3059 (CH_{arom}), 2213(CN), 1606 (C=N), 1538, 1470 (C=C_{arom}); MS (m/z): 395 [M^+, 0.56 %]; ^{1}H NMR (DMSO-d6): δ (ppm) = 10.4(s,1H, OH), 8.9 (d, 1H, H-4 of quinoline), 8.6 (s, 2H, NH_2), 7.2 –7.8, 8.4 and 8.7–8.89 (m, 9H, $H_{aromatic}$). Anal. Calcd for $C_{22}H_{13}N_5OS$ (395.44): C, 66.82; H, 3.31; N, 17.71. Found: C, 66.70; H, 3.19; N, 17.59.

3.2.6. 2-Amino-4-(2-chlorophenyl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4e) Yellow solid; yield 87 %; mp 118-120 °C; IR (KBr, cm^{-1}): 3324, 3217 (NH_2), 3054 (CH_{arom}), 2216 (CN), 1623 (C=N), 1570, 1475 (C=C_{arom}); MS (m/z): 413 [M^+, 0.23 %], 411[M^+-2, 0.28%], 270[Base Peak, 100%]; ^{1}H NMR (DMSO-d6): δ (ppm) 5.96 (s, 2H, NH_2), 6.82 –7.56 (m, 9H, $H_{aromatic}$), 8.3 (d, 1H, H-4 of quinoline). Anal. Calcd for $C_{22}H_{12}ClN_5S$ (413.88): C, 63.84; H, 2.92; N, 16.92. Found: C, 63.70; H, 2.78; N, 16.78.

3.2.7. 2-Amino-4-(3-nitrophenyl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4f) Yellow solid; yield 77 %; mp 180-182 °C; IR (KBr, cm^{-1}): 3329, 3193 (NH_2), 3056 (CH_{arom}), 2208(CN), 1629 (C=N), 1533, 1344 (NO_2), 1482 (C=C_{arom}); MS (m/z): 424 [M^+, 0.26 %]; ^{1}H NMR (DMSO-d6): δ (ppm) 5.96 (s, 2H, NH_2), 6.82 –7.81 and 8.61(m, 9H, $H_{aromatic}$), 8.32 (d, 1H, H-4 of quinoline). Anal. Calcd for $C_{22}H_{12}N_6O_2S$ (424.44): C, 62.26; H, 2.85; N, 19.80. Found: C, 62.11; H, 2.70; N, 19.65.

3.2.8. 2-Amino-4-(furan-2-yl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4g) dark brown solid; yield 92 %; mp 195-197 °C; IR (KBr, cm^{-1}): 3442, 3190 (NH_2), 3059 (CH_{arom}), 2926, ($CH_{stretch}$), 2197(CN), 1624 (C=N), 1588, 1495 (C=C_{arom}) ; MS (m/z): 369 [M^+, 0.77 %]. Anal. Calcd for $C_{20}H_{11}N_5OS$ (369.40): C, 65.03; H, 3.00; N, 18.96 Found: C, 64.89; H, 2.86; N, 18.82.

3.2.9. 2-Amino-4-(naphthalen-1-yl)-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile (4h) yellow solid; yield 74 %; mp >300 °C; IR (KBr, cm^{-1}): 3407, 3205 (NH_2), 3052 (CH_{arom}), 2204 (CN), 1597

(C=N), 1449 (C=C_{arom}); MS (m/z): 429 [M^+, 1.20 %]. Anal. Calcd for $C_{26}H_{15}N_5S$ (429.50): C, 72.71; H, 3.52; N, 16.31. Found: C, 72.58; H, 3.39; N, 16.19.

3.3. Antimicrobial Activity

New compounds were evaluated for their antibacterial activities by agar diffusion technique [48] against two gram positive bacteria (*Staphelococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC6633), two gram negative bacteria (*Salmonella typhimurium* ATCC14028 and *Escherichia coli* ATCC 8739) at a concentration 100 µg/ml. Also, the antifungal activity was tested against (*Candida albicans* ATCC10231). DMSO was used as a solvent and negative control. Ciprofloxacin and Ketoconazole at concentration of 100 µg/mL in DMSO were used as positive control. After incubation period, the growth inhibition zones diameters were carefully measured in millimeter.

3.4. Cytotoxic assay

Cytotoxic activity of the tested derivatives was made against two cancer cell lines, lung (A549) and breast (MCF-7) using MTT assay. Each cell line was cultured in a proper complete medium composed of DMEM, supplemented with 10% FBS, and 1% Antibiotic (Penicillin/Streptomycin (1:1)) according to the standard cell culture work. Cells were treated with four working concentrations of each compound and 5-FU as standard (1, 10, 100, and 1000 µM) [49–51]. Data were calculated as percent of cell viability relative to control, then IC_{50} was calculated using GraphPad prism 7.

3.5. RT-PCR

MCF-7 cells were treated with compound **4f** (6.39 µM, 48 h), then, total RNA was extracted from both treated and non-treated cells using Qiagen RNA extraction. Nanodrop spectrophotometer was used to measure the purity of the RNA. cDNA synthesis was performed, followed by the qPCR test in a single tube [52]. The sequence of primer pairs is displayed in **Table (5).** Genes BAX, P53, MDM2, BCL2, Jak2, STAT3, and β-actin as a housekeeping gene are selected to be tested [50,52,53]. The obtained results were cycle threshold (Ct), and relative quantitation of each tested gene, and hence calculation of the (**ΔΔ**Ct).

Table (5): Forward and reverse primers used in RT-PCR assay.

	Gene	Forward	Reverse
	BAX	5'- GTTTCATCCAGGATCGAGCAG -3'	5'- CATCTTCTTCCAGATGGTGA -3'
Upregulated	**P53**	5`-GTGGTTTCTTCTTTGGCTGG-3`	5`-CTTTGAGGTGCGTGTTTGTG-3`
	MDM2	5`-TCTAGGAGATTTGTTTGGCGT-3`	5`-TCACAGATGTACCTGAGTCC-3`
	BCL2	5`-GAGGATTGTGGCCTTCTTTG-3`	5`-ACAGTTCCACAAAGGCATCC-3`
Downregulated	**Jak2**	5′- GTGTGGAGATGTGCCGCTAT-3′	5′- GCACTGTAGCACACTCCCTT-3′
	STAT3	5′- GCAGTTTAGACAGGGAGGGG-3′	5′- CACTGTCTCTGGGGCTGAAG-3′
Housekeeping	**β-actin**	5`-GAGCCGCCGATCCACACG-3`	5`-GCACTCTTCCAGCCTTCCTTCC-3`

3.6. Molecular docking

All molecular modeling studies were performed on a computational software basis using the (MOE 2008-10 Chemical Computing Group, Canada) towards three proteins as Jak2 inhibitors; 3ZMM, 4C62, and 5AEP whose crystal structures complexed with their co-crystallized ligands were easily accessible from the Protein Data bank. Principles of modeling regarding receptor and ligand preparation, and molecular docking were carried out according to Nafie et al. [54] Each ligand-receptor complex was tested for binding interaction analysis, 3D images were taken by Chimera as a visualizing software.

4. Conclusion

In conclusion, we have described an efficient and straightforward protocol for synthesis of 2-amino-4-aryl-6-(quinolin-2-ylthio)pyridine-3,5-dicarbonitrile derivatives **4a-h** with good yields. All synthesized compounds **4a-h** have been investigated for their *in vitro* antibacterial and antifungal activities. Among the synthesized compounds, **4a, 4b, 4d,** and **4e** possess promising activity against *Candida albicans*. Also, derivatives **4a**, **4d**, and **4e** gave a moderate activity against gram-negative bacteria. Furthermore, derivatives **4a**, **4b**, **4d,** and **4e** gave moderate activity against gram-positive bacteria. Whereas compounds **4c**, **4f,** and **4h** are inactive against all tested strains. The potency of the two

derivatives **4d** and **4e** could be attributed to *o*-substitution of the phenyl ring, when compared to the *p*-, *m*- or unsubstituted phenyl ring. Additionally, **4f**, **4d,** and **4g** exhibited potent cytotoxic activity against both MCF-7 and A549 cell with IC_{50} values (6.39- 9.3 μM), and the investigated Jak2/STAT3 inhibition through the gene expression analysis was also proved through the molecular docking studies by having good binding affinities towards the three tested proteins of Jak2/STAT3. Some of the synthesized derivatives might be good candidates for the investigated biological activities

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Conflict of interest

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Electronic supporting information

The spectroscopic characterization charts of the investigated compounds together with some docking results are provided as electronic supporting information at

Author's contribution

Sebaey Mahgoub, and **Atef M. Amer** synthesized the entire series of derivatives with characterization of structure elucidation. **Mohamed S. Nafie** initiated the idea and design of the biology part by carrying out *in vitro* cytotoxic screening, RT-PCR analysis, and *in silico* molecular docking. All authors contributed to data analysis and manuscript writing in their corresponding parts. **Mohamed S. Nafie** carried out the linguistic revision for the whole manuscript and validated it in the final submitted form, additionally, he followed up the publication process from submission through the review to acceptance.

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