



Original Research Article

Synthesis of New 1,2,4-Triazole Derivatives with Expected Biological Activities

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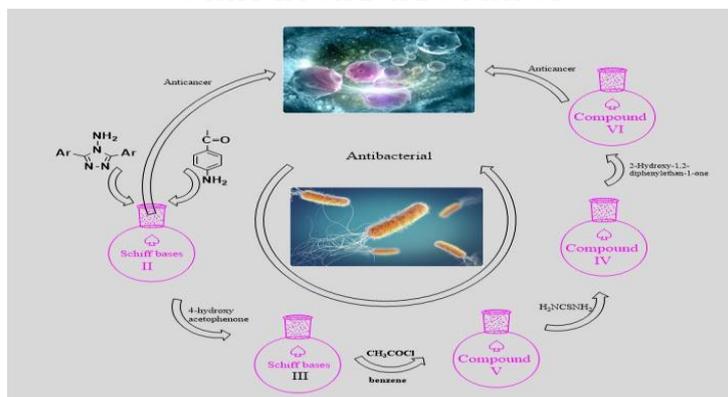
WRL-68

Cancer cell lines

ABSTRACT

To further study novel N-acyl, N-thiourea and imidazole derivatives were synthesized. We used a multi-step reaction protocol that began with symmetrical 4-amino-1,2,4-Triazole [I]. The new Schiff base [II] was derived by condensation of compound [I] with 4-aminoacetophenone in Ethanol and some drops from glacial acetic acid as a catalyst, whereas the Schiff base [III] was synthesized by reacting compound [II] with 4-hydroxyacetophenone in ethanol. N-acyl derivative [IV] was prepared by addition reaction of acetyl chloride to azomethine group of Schiff base [III] in dry benzene. Then, N-thiourea derivative [V] was prepared from reaction of thiourea with N-acyl derivative [IV] in alkaline solution. The third step involved cyclization reaction of derivative [V] with 2-hydroxy-1,2-diphenylethan-1-one in dimethyl formamide (DMF) to obtain new imidazole derivative [VI]. The synthesized compounds were characterized on the spectroscopic data and their physical properties. Also, we studied the anti-bacterial activity of the prepared compounds against three types of bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, some of which showed best antibacterial activity comparable with Ampicillin, as standard antibiotic. Furthermore, the cytotoxic effect of various concentrations of the prepared compounds [II] and [VI] was investigated against MCF-7 (human breast carcinoma cells), Hep G2 (human liver cancer cell line) and WRL-68 (human hepatic cell line), revealing a moderate activity at 400 µl/ml, which had no effect on the growth of normal WRL-68 cells, and confirmed the safety of using this type of molecules in medications.

GRAPHICAL ABSTRACT



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Introduction

Triazoles are five membered heterocyclic compounds with the chemical formula of $C_2H_3N_3$. Triazole has two isomers: 1,2,3-triazole and 1,2,4-triazole. The tautomeric forms of 1,2,4-triazoles are 1*H* and 4*H*-1,2,4-triazole is a pharmacologically significant nucleus [1,2]. Antibacterial, antifungal, anticancer, anti-inflammatory, antioxidant, antiviral, analgesic, anticonvulsant, anti-HIV, and biological properties were all found in 1,2,4-triazole derivatives [3-5]. Imidazole is a five-member heterocyclic ring with three carbon atoms and two nitrogen atoms in the positions number one and three. The behavior of one nitrogen is similar to that of a pyrrole-type nitrogen, while the other is similar to that of a pyridine-type nitrogen [6-7]. In drug development, the imidazole nucleus is an essential synthesis approach. The strong therapeutic effects of imidazole-related drugs have encouraged scientists to develop plenty of new chemotherapeutic medications [8,9]. According to the literature review, there is no evidence on the synthesis and investigation of the antibacterial and anticancer activities of these newly synthesized compounds [I-VI]. We synthesized and characterized novel compounds containing *N*-acyl, *N*-thiourea and imidazole units in the same symmetrical 1,2,4-Triazole molecule and also studied their antibacterial and anticancer activities in the hopes of developing new therapeutics.

Material and Methods

General

The chemicals were provided from Aldrich, Merck and GCC Chemicals Co. On (Ir prestige-21) a Shimadzo, Fourier Transform Infrared Spectrometer (FT-IR) spectra were recorded using KBr discs; Bruker, Ultra Shield (500MHz), Switzerland, was used to record 1H NMR spectra.

General Synthetic Procedures

Synthesis of 3,5-bis (4-methoxyphenyl)-4H-1,2,4-triazole-4-amine [I]

4-methoxybenzohydrazide (6.64 g, 0.04 mol) was dissolved in dimethyl sulfoxide (10 mL), refluxed for 17 hrs., then distilled under reduced pressure, cooled, and put into ice water [10,11]. The

mixture was stirred for 12 hours at room temperature, filtered and washed with water, dried and recrystallized from aqueous ethanol (1:1).

Synthesis of (E)-4-(1-((3,5-bis(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl) imino)ethyl)aniline [II]

For eight hours, a mixture of the new compound [I] (2.69 g, 0.01 mol) and 4-aminoacetophenone (1.35 g, 0.01 mol) was refluxed in absolute ethanol (10 mL) with some drops of glacial acetic acid [12]. The reaction mixture was cooled to room temperature, then the solid was filtered and purified from ethanol.

Synthesis of 4-((E)-1-((4-((E)-1-((3,5-bis(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl)imino)ethyl)phenyl)imino)ethyl)phenol [III]

Equimolar amounts of 4-hydroxyacetophenone (1.36 g, 0.01 mol), compound [II] (4.13 g, 0.01 mol) were refluxed for 8 hrs. in 10 mL absolute ethanol with three drops of glacial acetic acid [13]. The resulting precipitate was filtered, dried, and the residue purified with ethanol.

Synthesis of N-(3,5-bis (4- methoxyphenyl)- 4H-1,2,4-triazol-4- yl)-N-(1-chloro -1-(4-(N-(1-chloro-1-(4-hydroxyphenyl) ethyl) acetamido) phenyl) ethyl) acetamide [IV]

To a stirred solution of compound [III] (5.31 g, 0.01 mol), 10 mL dry benzene was added drop by drop of acetyl chloride (0.025 mol) in an ice water bath with constant stirring for an hour [14]. After that, the reaction was refluxed for two hours. The solvent was evaporated, and the white precipitate was gently washed with water before being purified from diethyl ether.

Synthesis of (R)-1-(N-(4-((R)-1-(N-(3,5-bis(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl)acetamido)-1-(carbamimidoylthio)ethyl)phenyl)acetamido)-1-(4-hydroxyphenyl)ethyl carbamimidothioate [V]

To synthesize thiourea derivatives [V] [14], a mixture of compound [IV] (6.88 g, 0.01 mol), thiourea (1.52 g, 0.02 mol), anhydrous sodium carbonate (1.06g,0.01mol) and 20 mL acetone was heated for 6 hrs. with stirring. After cooling, the reaction solution was put into ice water, filtered and recrystallized from ethyl acetate.

Synthesis of *N*-(3,5-bis (4-methoxyphenyl) -4*H*-1,2,4-triazol-4-yl)-*N*-((*R*)-1-((4,5-diphenyl-1*H*-imidazol-2-yl)thio)-1-(4-(*N*-((*R*)-1-((4,5-diphenyl-1*H*-imidazol-2-yl)thio)-1-(4-hydroxyphenyl)ethyl) acetamido) phenyl) ethyl) acetamide [VI]

2-Hydroxy-1,2-diphenylethan-1-one (5.32 g, 0.02 mol) was added to a stirred solution of compound[V] (7.67 g, 0.01 mol) in dry DMF (10 mL). For 7 hrs., the reaction mixture was refluxed [15]. After cooling, a few drops of cold water were added and stirred until a precipitate. The crystals were filtered, dried and purified from ethyl acetate.

Biological Evaluation

Antibacterial Activity:

The antibacterial activity of the synthesized compounds [I-VI] was tested by using the Well Diffusion Method [16], against three types of bacteria: *Staph. aureus* (G+), *Klebsiella pneumoniae* (G-) and *Pseudomonas aeruginosa* (G-

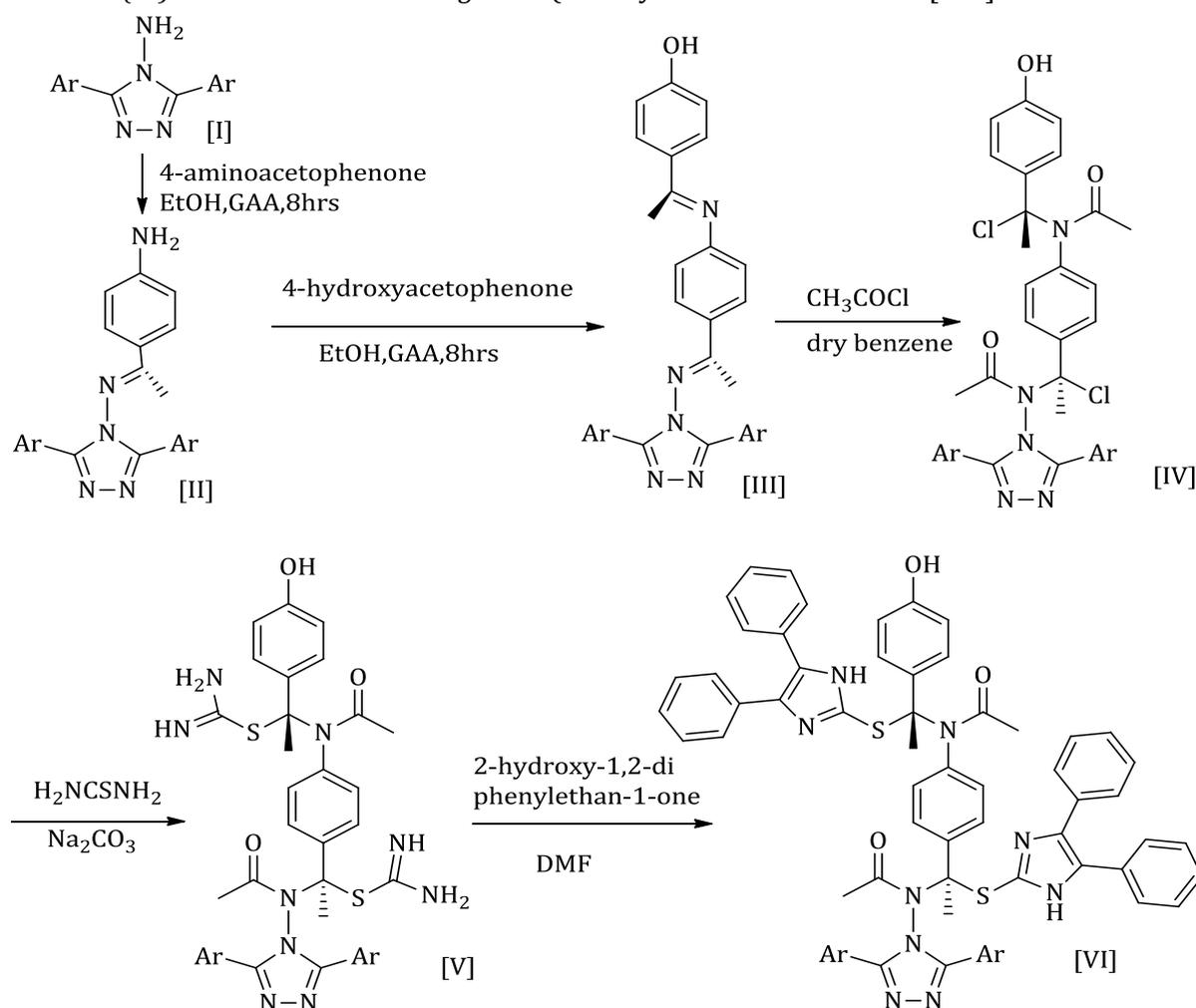
) and compared with Ampicillin, as antibiotic standard. All the tested compounds were dissolved in DMSO. The plates were then incubated at 37 °C and examined after 24 hrs.

Cytotoxicity Activity

The compounds [II] and [VI] were screened for their cytotoxicity activity, using three types of cancer cell lines: MCF-7 (human breast carcinoma cells), Hep G2 (human liver cancer cell line) and WRL-68 (the human hepatic cell line). Freshney's protocol for cell culture media, reagents, and solutions were followed [17]. The viability of MCF-7, HepG-2 and normal cell line WRL-68 cells, after adding various concentrations of compounds [II] and [VI] was determined by using an ELISA reader at a wavelength of 575 nm, respectively.

Result and Dissection

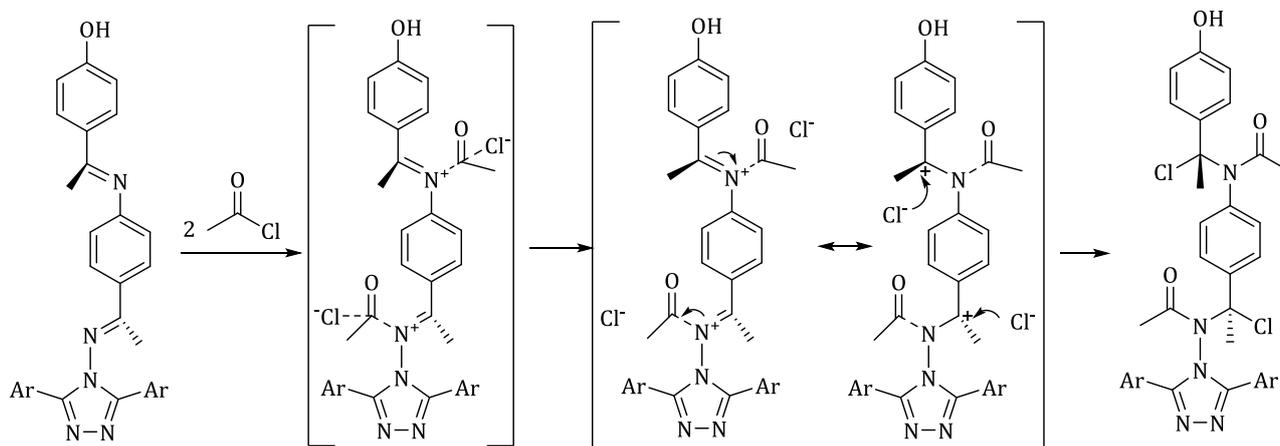
Scheme 1 shows the synthetic routes for the synthesis of derivatives [I-VI].



Scheme 1: Synthesis of compounds [I-VI]

The acid hydrazide (4-methoxybenzohydrazide) was condensed in the presence of dimethyl sulfoxide to produce the novel 3,5-bis (4-methoxyphenyl)-4*H*-1,2,4-triazole-4-amine [I]. Refluxing equimolar of 4-amino triazole [I] with 4-amino acetophenone in ethanol with some drops of glacial acetic acid yielded the new Schiff base [II], at the same procedure Schiff base [III]

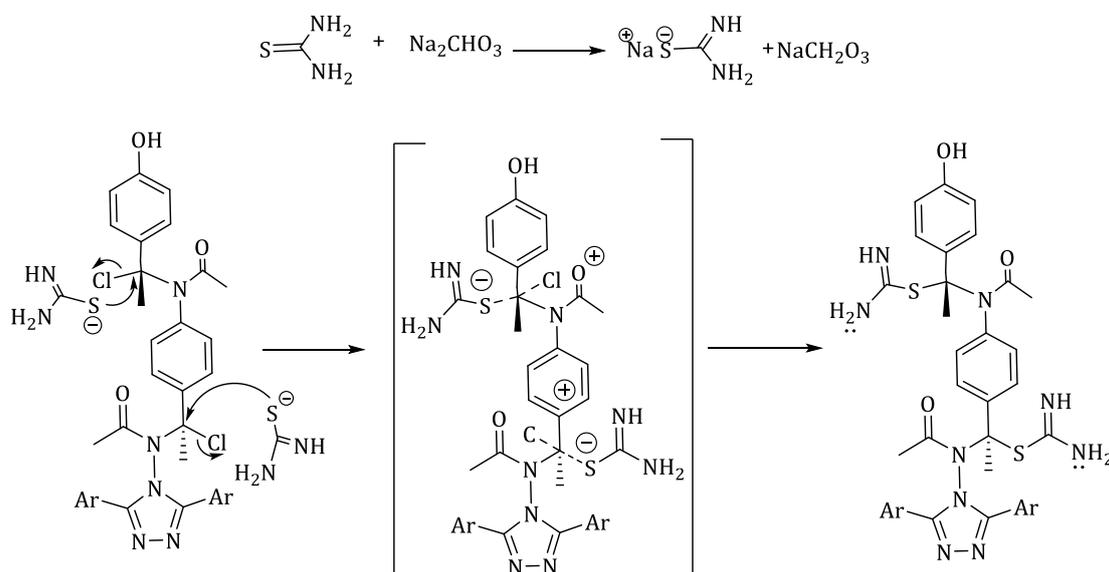
was synthesized. The new *N*-acetyl, *N*-thiourea and diphenyl imidazole derivatives [IV-IV] were synthesized by three-step reactions. *N*-acyl derivative [IV] was synthesized by addition reaction of acetyl chloride with Schiff base [III] in dry benzene. The following is an outline of the reaction mechanism suggested [14], as shown in Scheme 2:



Scheme 2: Proposed mechanism for the synthesis of *N*-acyl derivative [IV]

The second step involved the reaction of thiourea with *N*-acyl [IV] in anhydrous sodium carbonate in analar acetone to yield *N*-thiourea compound

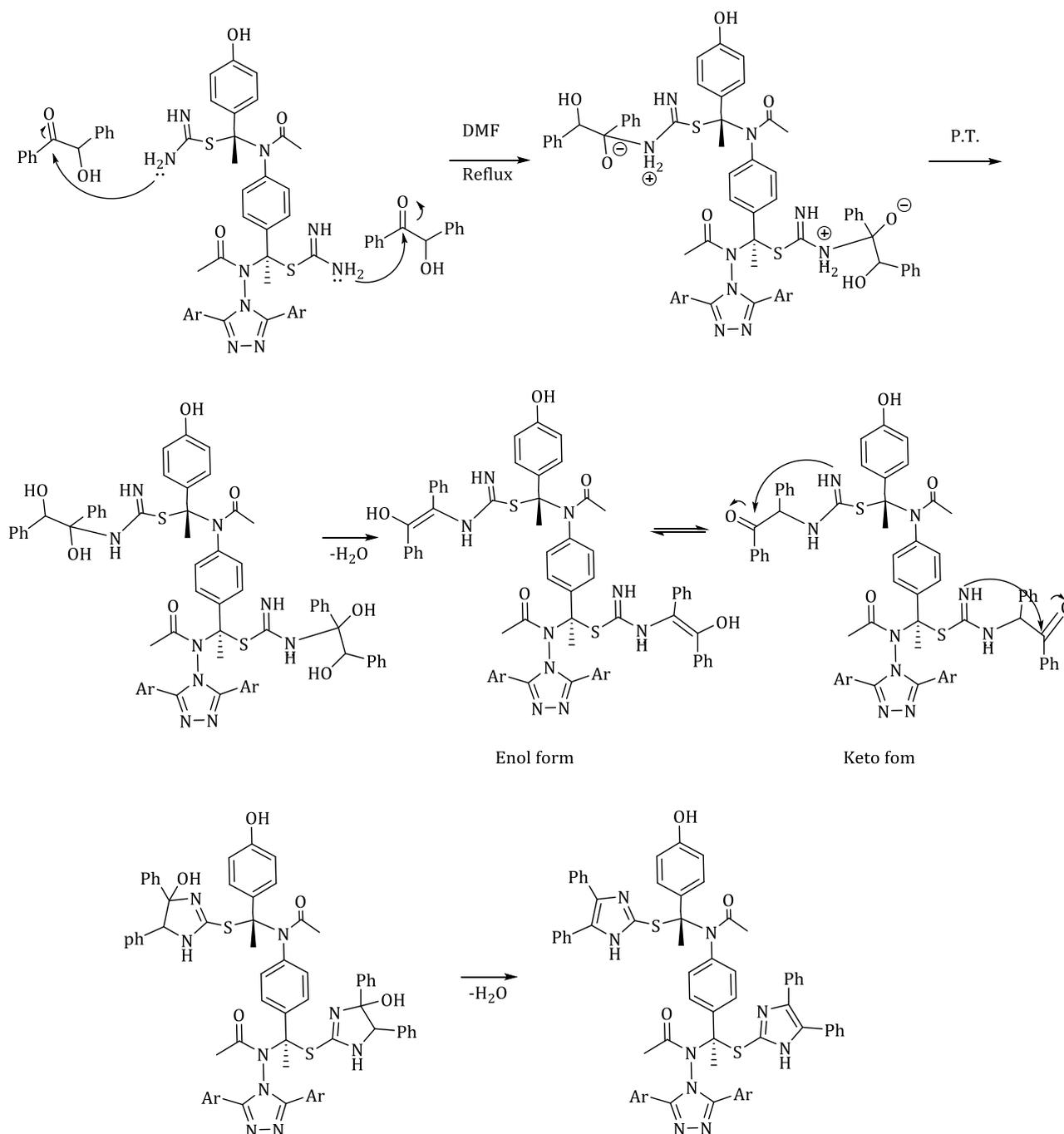
[V]. The next mechanism is proposed for this reaction [14], Scheme 3:



Scheme 3: Proposed mechanism for the synthesis of *N*-thiourea derivative [V]

The cyclization reaction of *N*-thiourea derivative with 2-hydroxy-1,2-diphenylethan-1-one in DMF caused the formation of a new imidazole derivative [VI] by intermolecular nucleophilic

substitution. Scheme 4 illustrated the suggested mechanism for obtaining the desired product [14].



Scheme 4: Proposed mechanism for the synthesis of imidazole derivative [VI]

Physical properties and spectral data were used to indicate the structures of all synthesized compounds, as shown in Table 1. These findings provide strong support for the formation of the structure for these molecules.

Biological Evaluation

The synthesized compounds [I-VI] showed good to moderate antibacterial activity against three types of bacteria. Compounds [IV], [V] and [VI] showed excellent inhibition of these bacteria, as shown in Table 2.

The cytotoxicity activity of compound [II] showed significant effects at concentration 400 $\mu\text{l/ml}$ against MCF-7 cell line Table 3, whereas the viability cells were (37.81%, 24.19%, 12.27, 5.71%, 4.05% and 5.13%) in concentration (400, 200, 100, 50, 25, 12.5 $\mu\text{g/ml}$), respectively, and IC₅₀=206.1, as shown in Figure 1.

Also, the cytotoxicity activity of compound [VI] showed significant effects at concentration 400 $\mu\text{l/ml}$ against Hep G2 cell line Table 4, whereas the viability cells were (61.23%, 54.47%, 38.39 % ,

20.87%, 4.28% and 5.98 %) in concentration and IC₅₀=82.60, as shown in Figure 2. (400, 200, 100, 50, 25, 12.5µg/ml), respectively,

Table1: The physical properties, FT-IR and ¹HNMR spectral data synthesis compounds [I-VI]

Comp. No.	m.p. °C	Color	Yield%	IR(v, cm ⁻¹) KBr	¹ HNMR(DMSO) (δ, ppm)
[I]	158-160	light beige	60	3263, 3170 (NH ₂ asy., sy.), 1685 (C=N triazol ring), 1604(C=C arom.)	3.82 (s, 6H, OCH ₃), 5.76 (s, 2H, NH ₂), 7.01-7.99(dd, 8H, Ar-H)
[II]	193-195	Gray-yellow	76	3394, 3224(NH ₂ asy., sy.), 1681 (C=N endo cyclic), 1654 (C=N exo cyclic), 1600, 1577(C=C arom.)	2.43 (s, 3H, CH ₃), 3.83 (s, 6H, OCH ₃), 6.03 (s, 2H, NH ₂), 6.65-7.91 (m, 12H, Ar-H)
[III]	128-130	orange	80	3350 (OH), 1678 (C=N endo cyclic), 1600 (C=N exo cyclic), 1577(C=C arom.)	2.38 (s, 6H, CH ₃), 3.83 (s, 6H, OCH ₃), 6.90-8.49 (m, 16H, Ar-H), 9.80 (s, 1H, OH)
[IV]	162-164	white	60	3380 (OH), 1782(N-C=O), 1689 (C=N endo cyclic), 1600(C=C arom.), 705(C-Cl)	1.62 (s, 6H, CH ₃), 2.55 (s, 3H, CH ₃ CO), 3.88 (s, 6H, OCH ₃), 7.01-8.17 (m, 16H, Ar-H), 10.03 (s, 1H, OH)
[V]	198-200	pale yellow	64	3385 (OH), 3375,3278,3170 (NH ₂ asy.,sy.& NH), 837(C-S)	1.65 (s, 6H, CH ₃), 2.87 (s, 3H, CH ₃ CO), 3.75 (s, 6H, OCH ₃), 6.00 (s, 2H, NH ₂), 7.86(s, 1H, NH), 6.80-7.99 (m, 16H, Ar-H), 10.62 (s, 1H, OH)
[VI]	130-132	golden yellow	68	3483 (OH), 3406 (NH), 1640 (C=N), 1620 (C=Cimidazol ring), 759(C-S)	2.73 (s, 6H, CH ₃), 2.90 (s, 3H, CH ₃ CO), 3.81(s, 6H, OCH ₃), 7.13 -7.51 (m, 10H, Ar -H) 7.52-8.11 (dd, 16H, Ar-H), 8.25 (s, 1H, NH), 10.60 (s, 1H, OH)

Table 2: Antibacterial screening data (zone of inhibition in mm) for compounds [I]-[VI]

Comp. No.	<i>Staph.aurus</i> (G+)	<i>Klebsiella Pneumoniae</i> (G-)	<i>Pseudomonas Aeruginosa</i> (G-)
[I]	25	13	20
[II]	25	20	19
[III]	27	24	18
[IV]	27	29	19
[V]	22	25	24
[VI]	22	27	19
Ampicillin	22	22	23

The results of this study showed the compounds [II] and [VI] affecting against MCF-7 and Hep G2 cancer cells (in vitro), respectively, and at high concentration did not affect the growth of normal cells WRL-68, which confirms the safety of the

utilize of compounds in drugs used according to a dose-dependent treatment. This efficacy needs to be studied, detected and analyzed in future studies on factors that inhibit the growth of cancer cells [18,19].

Table 3: The viability of MCF-7 cells, and WRL-68 cells after adding various concentrations of compound [II]

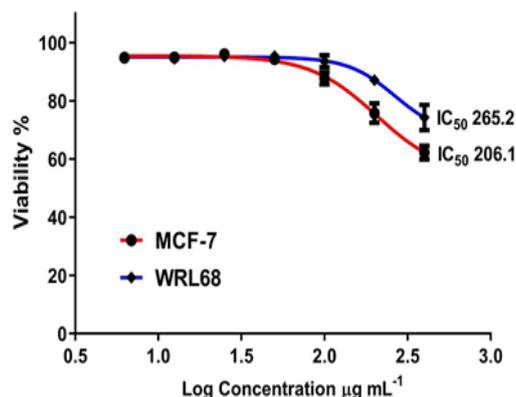
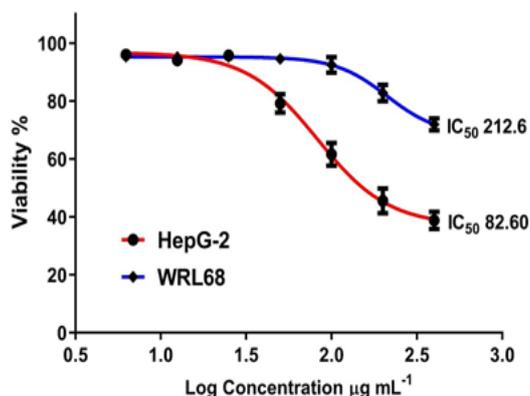
Concentration $\mu\text{g mL}^{-1}$	Mean Inhibition (%) \pm SD		Sig.	pValue
	MCF-7	WRL68		
400	37.81 \pm 2.38	25.73 \pm 4.34	**	\leq 0.0001
200	24.19 \pm 3.33	12.89 \pm 1.46	**	\leq 0.0001
100	12.27 \pm 2.09	6.4 \pm 2.10	**	\leq 0.0001
50	5.71 \pm 0.82	4.67 \pm 1.18	NS	\leq 0.0001
25	4.05 \pm 0.90	4.78 \pm 0.82	NS	\leq 0.0001
12.5	5.13 \pm 0.29	5.52 \pm 1.55	NS	\leq 0.0001
6.25	5.17 \pm 0.71	4.98 \pm 0.83	NS	\leq 0.0001

** : $p \leq 0.0001$, NS: Non-Significant, SD: Standard Deviation

Table 4: The viability of Hep G2 cells, and WRL-68 cells after adding various concentrations of compound [VI]

Concentration $\mu\text{g mL}^{-1}$	Mean Inhibition (%) \pm SD		Sig.	p Value
	Hep G2	WRL68		
400	61.23 \pm 2.99	28.05 \pm 2.10	**	\leq 0.0001
200	54.47 \pm 4.25	17.25 \pm 2.84	**	\leq 0.0001
100	38.39 \pm 3.97	7.56 \pm 2.71	**	\leq 0.0001
50	20.87 \pm 3.18	5.44 \pm 1.10	**	\leq 0.0001
25	4.28 \pm 0.81	4.78 \pm 0.82	NS	\leq 0.0001
12.5	5.98 \pm 0.98	4.82 \pm 0.41	NS	\leq 0.0001
6.25	4.05 \pm 0.53	4.71 \pm 1.05	NS	\leq 0.0001

** : $p \leq 0.0001$, NS: Non-Significant, SD: Standard Deviation

**Figure 1:** Cytotoxic activity of compound [II] on MCF-7 cell line**Figure 2:** Cytotoxic activity of compound [VI] on Hep G2cell line

Conclusion

The aim of the research was to create *N*-acyl, *N*-thiourea, and imidazole derivatives by a series of

reactions that began with symmetrical 4-amino-1,2,4-Triazole. The antibacterial activity of all of the synthesized compounds [I-VI] was tested in

vitro. MCF-7, Hep G2 and WRL-68; cancer cell lines were used to estimate the cytotoxic effect of different concentrations of the created compounds [II] and [VI].

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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