



Chitosan (CS) / Polyvinyl alcohol (PVA) Films loaded with Novel Synthesized 1,2,3-Triazole Derivative for Cancer Treatment

Shrouk A. Osman ¹, Magda A. Abdallah ¹, Heba Salah ², Abdel Mohsen M. Soliman ³, Sobhi M. Gomha ¹, Heba Bassiony ⁴ and Huda R. M. Rashdan ^{5*}



CrossMark

¹ Department of Chemistry, Faculty of Science, University of Cairo, Giza, 12613, Egypt

² Polymers & Pigments Department, Industrial Chemical Institute, National Research Centre, 33 El-Behouth St. Dokki, Cairo, Egypt

³ Therapeutic Chemistry Department, Pharmaceutical and Drug Industries Institute, National Research Centre, 33 El-Behouth St. Dokki, Cairo, Egypt

⁴ Zoology Department, Faculty of Science, University of Cairo, Giza, 12613, Egypt

⁵ Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, 33 El Buhouth St, Dokki, Giza 12622, Egypt

In Loving Memory of Late Professor Doctor "Mohamed Refaat Hussein Mahran"

Abstract

4-Acetyl-1,2,3-triazole derivative 1 reacts with cyanoaceto-hydrazide in 2 ethanol to give 2-cyano-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acetohydrazide (3). The latter was submitted to react by condensation with benzaldehyde and its derivatives 5a-k in ethanol in the presence of piperidine to give the corresponding arylidene derivatives. Compound 5a reacts with a variety of heteroamines to give the corresponding annulated azolopyrimidine compounds. 9 and 13 and Additionally, 2-cyano-N-(substituted-1,2,3-triazol-4-yl)ethylidene)acetohydrazide reacts with 2-bis(methylthio)methylene malononitrile in ethanol and in the presence of potassium hydroxide to give triazolylhydrazonopyridine derivative. Treatment of the latter compound with hydrazine hydrate in ethanol under reflux led to formation of pyrazolopyridine derivative. The structure of all the newly synthesized compounds were fully identified using spectroscopic and elemental analysis. Moreover, the newly synthesized compounds were screened for their antiproliferative potential in vitro towards different human cell lines, including: colon (LoVo), liver (HEPG2) and breast (MCF7) cancer cell lines. The results revealed that compounds 5d, 5b, 5c and 5f are the most potent against tested cancer cell lines. Moreover, compound 5d was chosen for a further drug delivery study through using chitosan and PVA polymer film as drug carrier. This drug delivery system was used for in vitro anticancer evaluation compared to Doxorubicin. Colon (LoVo) cells were treated with various concentrations from the selected compound 5d loaded in CS/PVA drug delivery in comparison to doxorubicin to evaluate its anticancer activity.

Keywords :1,2,3-Triazoles, pyrazolopyridine,1,2,4-triazolopyrimidine, tetrazolopyrimidine, antiproliferation, drug delivery.

1. Introduction

Cancer stands as a leading cause of mortality, responsible for approximately 9 million deaths annually [1][2][3][4][5]. In the realm of cancer treatment, anticancer agents prove indispensable. As of the present, over 100 drugs have gained approval for this purpose [6]. Nevertheless, the swift emergence of drug resistance[7][8][9], coupled with the severe side effects associated with clinically employed anticancer drugs, remain a significant impediment to effective chemotherapy.

Consequently, there is an urgent imperative to investigate novel drugs that offer both reduced side effects and heightened efficacy. Heterocycles have emerged as pivotal components in the quest for

discovering anticancer medications. 1,2,3-Triazoles are heterocyclic compounds characterized by the presence of three nitrogen atoms within the ring structure[10][11][12][13][14]. These molecules exhibit stability and engage with biological targets through the formation of hydrogen bonds. Consequently, they serve as significant structural frameworks in the realm of drug discovery. Here is the rephrased and non-plagiarized version **Figure 1** displays drugs that incorporate the 1,2,3-triazole scaffold into their molecular structure [15][16][17][18].

1,2,3-Triazole derivatives have been documented to possess diverse pharmacological properties, including anticancer, antimicrobial[19], anti-

*Corresponding author e-mail: Huda R. M. Rashdan, hudadawoud20@yahoo.com

Receive Date: 29 January 2024, Revise Date: 09 March 2024, Accept Date: 10 March 2024

DOI: 10.21608/ejchem.2024.266304.9262

©2024 National Information and Documentation Center (NIDOC)

inflammatory, antitubercular, anti-HIV, and antiviral effects. In terms of their anticancer activity[7][20][21], 1,2,3-Triazoles have been observed to exert their effects through various mechanisms[22], as illustrated in **Figure 2**. These mechanisms involve the inhibition of crucial enzymes such as carbonic anhydrases (CAs), thymidylate synthase (TS), aromatase, tryptophan 2,3-dioxygenase (TDO), vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR)[18][23], all of which contribute to the progression of this life-threatening disease[12][12][13].

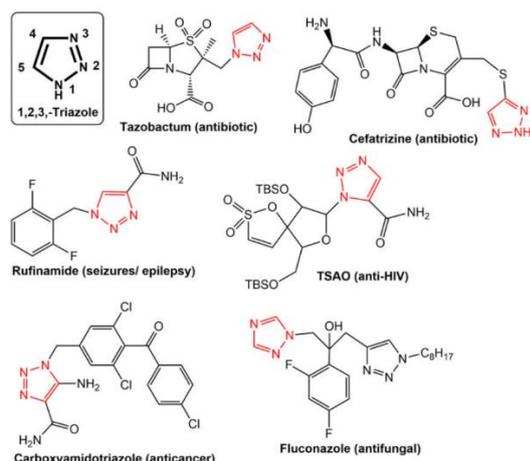


Figure 1. Biologically active 1,2,3-triazole drugs

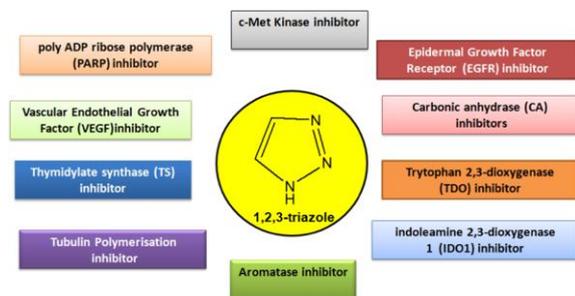


Figure 2. Mechanisms of action of 1,2,3-triazole hybrids

Considering the findings mentioned above, and as a part of our ongoing endeavor to discover novel compounds suitable for the development of effective, selective, and less toxic anticancer drugs, we present here the synthesis of a previously unreported compound: 2-cyano-N'-[1-(5-methyl-1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl) ethylidene] acetohydrazide. We also discuss their application in a range of heterocyclic transformations and assess their potential as anticancer agents.

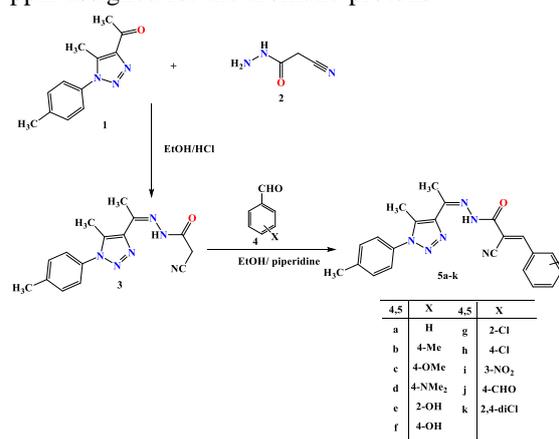
The present work investigated the cytotoxicity of the newly synthesized 1,2,3-triazole derivatives towards human liver (HEGP2), human colon

(LoVo) and breast (MCF7) cancer cell lines in comparison to Doxorubicin as traditional anticancer agent. The results indicated that the *in vitro* antiproliferative activity of the newly synthesized compounds was moderately significant and may be investigated for further *in vivo* and pharmacokinetic studies. Moreover, the most potent compound (**5d**) was chosen for a further drug delivery study through using chitosan and PVA polymer film as drug carrier. This drug delivery system was used for *in vitro* anticancer evaluation compared to Doxorubicin. Colon (LoVo) cells were treated with various concentrations from the selected compound (**5d**) loaded in CS/PVA drug delivery in comparison to doxorubicin to evaluate its anticancer activity.

2. Results and Discussion

2.1. Chemistry

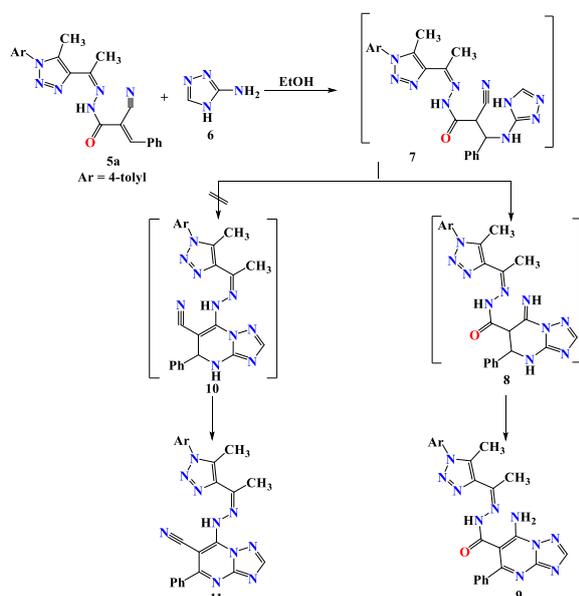
The required starting compound **3**, namely 2-cyano-N'-[1-(5-methyl-1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl) ethylidene] acetohydrazide was prepared by reaction of 1-[5-methyl-1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl] ethanone **1** with 2-cyanoacetohydrazide **2** in ethanol in the presence of few drops of HCl. Compound **3** reacts with benzaldehyde and its derivatives **4a-k** in ethanol in the presence of piperidine to give the respective arylidenes **5a-k** (**Scheme 1**). The structure of products **5a-k** was established by spectroscopic and elemental analysis data. For instance, **5a** ¹HNMR spectrum exhibits three singlet signals at δ 2.42; 2.48; 2.65 ppm for the three methyl groups, singlet signal at δ 7.50 ppm for the NH proton, in addition to signals at δ 7.42-7.48 ppm assigned for the aromatic protons.



Scheme 1. Synthesis of arylidene derivatives **5a-k**

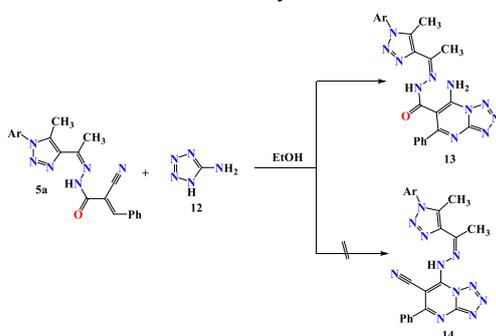
Since compound **5a** has an electrophilic site, it was used for synthesis of novel annulated azolopyrimidines *via* its reaction with nucleophilic reagents like heteroamines. Thus, reaction of arylidene derivative **5a** with 4*H*-1,2,4-triazol-3-amine **6** in ethanol afforded triazolopyrimidine derivative **9** (**Scheme 2**) and not product **11**. The

mechanism of formation of compound **9** was outlined in **Scheme 2**, where the reaction starts by Michael addition to give intermediate **7** which undergoes tandem intramolecular cyclization and tautomerization to give the final product **9**. The other pathway of the reaction which leads to formation of product **11** was discarded based on the spectral and elemental analyses data. The ^1H NMR spectrum of the product **9** displayed three singlet signals at δ 2.43; 2.62; 2.75 ppm for the three methyl groups, singlet signal at δ 7.44 for the NH_2 group, singlet signal at δ 7.87 ppm for the NH proton, in addition to the peaks assigned for the aromatic protons (see experimental). In addition, the mass spectrum of compound **9** showed a molecular ion peak at $m/z=466$ which is in accordance with its molecular formula $\text{C}_{24}\text{H}_{22}\text{H}_{10}\text{O}$



Scheme 2. Synthesis of triazolopyrimidine **9** derivative

Similarly, compound **5a** reacts with *1H*-tetrazol-5-amine **12** in ethanol to give tetrazolo [1,5-*a*] pyrimidine derivative **13** and not **14** (**scheme 3**). Compound **13** was also well confirmed by IR, ^1H NMR and mass spectral data (see experimental) together with elemental analysis.

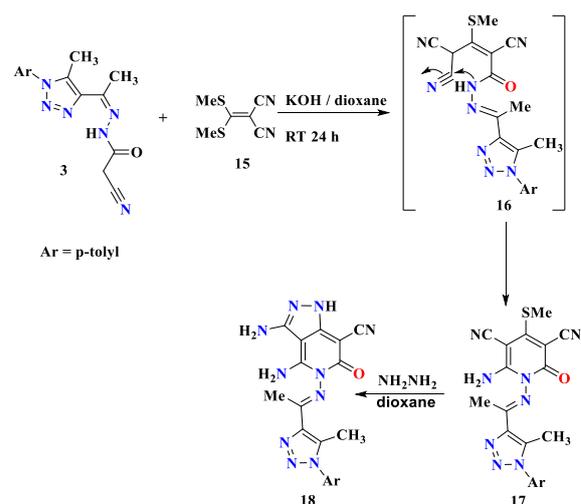


Scheme 3. Synthesis of tetrazolopyrimidine derivative **13**

Moreover, our study was extended to synthesize another novel 1,2,3-triazole based heterocycles, using compound **3** as building block for their formation. Thus, reaction of 2-cyano-*N*-[1-(5-methyl-1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)ethylidene]acetohydrazide **3** with 2-[bis(methylthio) methylene]malononitrile **15** in dioxane in the presence of potassium hydroxide at room temperature for 24 hrs. afforded the final product **17** via the non-isolable intermediate **16**. The mechanism of formation of **17** was outlined in **Scheme 4**. The structure designated for the product **17** was evidenced by spectral (IR, ^1H NMR, Mass) and elemental analysis data. The ^1H NMR spectrum of compound **17** showed three singlet signals at δ 2.46, 2.50, 2.73 ppm for the three methyl groups, a singlet signal at δ 6.52 ppm for the NH_2 group and a multiplet signal at δ 7.37-7.92 ppm for the four aryl protons. The mass spectrum showed a molecular ion peak at $m/z=418$ (Mol. Formula $\text{C}_{20}\text{H}_{18}\text{N}_8\text{OS}$), which is in accordance with its molecular weight.

Treatment of triazolopyrimidine derivative **17** with hydrazine hydrate in ethanol led to formation of pyrazolo[4,3-*c*]pyridine derivative **18** (**Scheme 4**).

The structure assigned for compound **18** was inferred by spectral (IR, ^1H NMR, Mass) and elemental analyses data (see experimental). The ^1H NMR spectrum of compound **18** revealed three signals at δ 2.28, 2.50, 2.72 ppm assigned for the three methyl groups, other signals at δ 5.31, 6.53, 10.19 for the NH_2 and NH groups, in addition to multiplet signal for the aromatic protons. The mass spectrum of compound **18** displayed a molecular ion peak at $m/z=402$ which is consistent with its molecular weight (see experimental section).



Scheme 4. Synthesis of pyrazolopyridine derivative **18**

2.2. Cytotoxic activity

2.2.1. Antiproliferative activity of the tested compounds

The antiproliferative activities were expressed by median growth inhibitory concentration (IC_{50}). As shown in **Table 1**, *in vitro* antiproliferative activity towards human colon (**LoVo**), liver (**HEPG2**) and breast (**MCF7**) cancer cell lines, were evaluated using SRB assay, in comparison with doxorubicin as standard drug.

The results revealed that most compounds showed variable activity against tested cancer cell lines. The tumor cell line showed normal growth in our culture system and DMSO did not seem to have any noticeable effect on cellular growth. A gradual decrease in viability of cancer cells was observed with increasing concentration of the tested compounds, in a dose-dependent inhibitory effect.

Evaluation of the antitumor effect of the tested compounds towards human breast (**MCF7**) and human liver (**HEPG2**) cancer cell lines revealed that: in **Group 2 (1, 3, 5a, 5b, 5c, 5d, 5e, 5f, 5g, 5k, 5i, 5j, 9, 13 and 17)** most compounds showed nonsignificant to weak potency. On the other hand, the antitumor effect of tested compounds showed variable antiproliferative activity towards colon cancer cell line (**LoVo**) as compounds **9, 13 and 17** had no effect on this cell line, compounds **1, 3, 5a,**

5e, 5g, 5k, 5i and 5j showed weak to moderate potency, while compounds **5b, 5c and 5f** were found to be good potent derivatives towards colon cancer cell line (**LoVo**) compared to doxorubicin the standard anticancer drug, with IC_{50} values 19.4 ± 3.7 , **5c** 26.2 ± 3.8 and **5f** 37.6 ± 4.5 $\mu\text{g/ml}$ versus 4.8 ± 0.6 $\mu\text{g/ml}$ for doxorubicin. Moreover, compound **5d** showed the strongest potency towards (**LoVo**) cell line with IC_{50} value 9.5 ± 0.7 $\mu\text{g/ml}$ versus 4.8 ± 0.6 $\mu\text{g/ml}$ for doxorubicin.

2.3. Drug loading on CS/PVA as a drug delivery system

Compound **5d** was selected to be loaded on a polymeric carrier CS/PVA to be used as a drug delivery system. The chitosan (CS) / polyvinyl alcohol (PVA) film particles with and without **5d** loading was further evaluated using DLS. The samples were well dispersed in water and held in the instrument for about 16 runs. The obtained values are the average of 16 runs (**Figure 3 a, b**). It is clearly seen that the chitosan (CS) / polyvinyl alcohol (PVA) film particles exhibited an average size around 19.31 nm. The size was increased to be around 107.9 nm when these film particles were loaded with the drug candidate **5d**.

Table 1: *In vitro* cytotoxic activity of the newly synthesized compounds towards human colon (**LoVo**), liver cancer (**HEPG2**) and Breast (**MCF7**) cell lines.

Compounds	Colon (LoVo) IC_{50} [$\mu\text{g/ml}$]	liver (HEPG2) IC_{50} [$\mu\text{g/ml}$]	Breast (MCF7) IC_{50} [$\mu\text{g/ml}$]
1	72.1 ± 9.3	N.A.	86.1 ± 14.8
3	56.2 ± 7.5	85.4 ± 17.1	N.A.
5a	81.3 ± 12.6	91.3 ± 19.6	95.1 ± 18.4
5b	19.4 ± 3.7	76.5 ± 11.8	80.9 ± 16.5
5c	26.2 ± 3.8	74.2 ± 9.5	78.3 ± 15.7
5d	9.5 ± 0.7	55.2 ± 7.9	61.4 ± 9.6
5e	66.7 ± 9.4	96.3 ± 14.8	N.A.
5f	37.6 ± 4.5	70.1 ± 12.5	75.7 ± 11.3
5g	68.9 ± 7.1	79.4 ± 15.2	87.9 ± 14.6
5k	77.4 ± 10.3	80.7 ± 14.2	90.1 ± 18.7
5i	95.2 ± 11.7	N.A.	N.A.
5j	81.3 ± 9.8	89.6 ± 16.4	92.5 ± 18.1
9	N.A.	N.A.	N.A.
13	N.A.	N.A.	N.A.
17	N.A.	N.A.	N.A.
Dox	4.8 ± 0.6	2.7 ± 0.06	5.03 ± 0.7
DMSO	N.A.	N.A.	N.A.

Data were expressed as Mean \pm SD of three independent experiments.
 IC_{50} ($\mu\text{g/ml}$): 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak).
 DOX: Doxorubicin is the drug reference.
 N.A.: is no activity

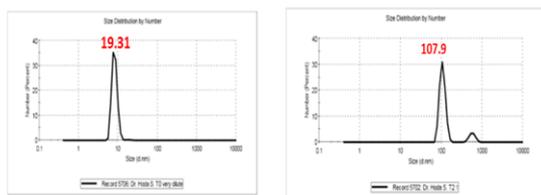


Figure 3a,b: Particle size of the chitosan (CS) / polyvinyl alcohol (PVA) film particles (a) without the drug candiadte **5d** and (b) with the candidate **5d**

Moreover, the particle size and surface morphology of the developed chitosan (CS) / polyvinyl alcohol (PVA) film particles (**Figure 4a-c**), and drug like compound **5d** loaded chitosan (CS) / polyvinyl alcohol (PVA) film particles were further examined using TEM. As observed from TEM, the sample of the chitosan (CS) / polyvinyl alcohol (PVA) film particles was assessed at different magnifications to clarify the nature of its particles. It was particles were formed with cavities. These cavities are available to be encapsulated with any of the model drugs or other organic compounds. The micrograph shows random shape and their particles in the Nano grade with diameter ranges from 20- 28 nm. Additionally, and by checking the particle feature of chitosan (CS) / polyvinyl alcohol (PVA) film particles loaded with **5d** (**Figure 5a-c**), it was depicted that the cavities were formed with black color which suggested that they are filled with the drug (**5d**) signifying that, **5d** was successfully encapsulated inside the cavities of the chitosan (CS) / polyvinyl alcohol (PVA) film particles. The micrograph shows spherical shape and their particles in the Nano size with diameter ranges from 77-117 nm.

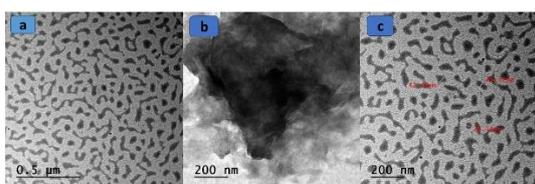


Figure 4a-c: TEM images of the chitosan (CS) / polyvinyl alcohol (PVA) film particles

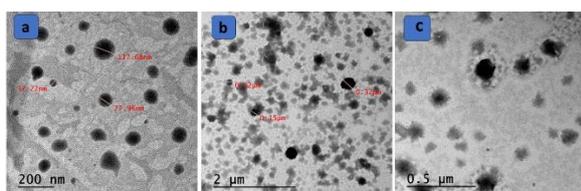


Figure 5a-c: TEM images of the chitosan (CS) / polyvinyl alcohol (PVA) film particles with **5d** loading

On the other hand, the morphology of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **5d** was estimated using the field-emission scanning electron microscopy. The image revealed that the surface texture of the chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **5d** is smooth and homogeneous and had a good distribution of all ingredients, also there are no pores and have no interface layer with no evidence of aggregations (**Figure 6**). The films also exhibit flat smooth surface in general indicates the uniform distribution of (CS) and (PVA) molecules throughout the films. The formation of homogeneous films was mostly caused by the interaction of hydrogen bonds between the functional groups of the film components. (**Figure 6**)

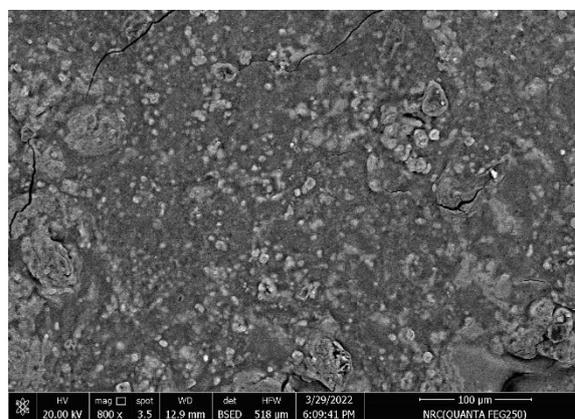


Figure 6: SEM micrographs of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **5d**.

2.4. In vitro cytotoxic evaluation of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **5d**

The antiproliferative potency of the selected compound **5d** loaded in CS/PVA drug delivery system was evaluated towards human colon cancer cell line (LoVo) according to the method described before. The results revealed that this loaded compound (**5d**) indicated a better strong potency towards (LoVo) cell line in this drug delivery form than in its drug like compound with IC_{50} value $9.5 \pm 0.7 \mu\text{mol/l}$ versus $0.008 \mu\text{mol/l}$ for doxorubicin.

3. Experimental section

3.1. Chemistry

3.1.1. General

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded in potassium bromide discs on PyeUnicam SP 3300 and Shimadzu

FTIR 8101 PC infrared spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz ($^1\text{H-NMR}$) and run in deuterated dimethylsulfoxide (DMSO- d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were measured by using a German made Elementarvario LIII CHNS analyzer.

3.2. Synthesis of 2-cyano- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acetohydrazide (3).

A solution was prepared by combining 1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethan-1-one **1** (2.15 g, 10 mmol) and 2-cyanoacetohydrazide **2** (0.99 g, 10 mmol) in 50 mL of ethanol with catalytic amount of hydrochloric acid. The mixture was then refluxed for 4 hours, with monitoring by TLC. The resulting hydrazone product was obtained as a precipitate, which was subsequently filtered, washed using ethanol, and subjected to recrystallization in acetic acid. This process yielded compound **3** in a pure, white solid form with a 78% yield; mp. 199-201 °C; IR (KBr): $\nu = 3429$ (NH), 2920 (C-H's), 2218 (CN), 1612 (C=O), 1562, 1514 (C=C) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.49 (s, 3H, CH₃), 2.72 (s, 3H, CH₃), 3.30 (s, 2H, CH₂), 7.51-8.03 (m, 4H, Ar-H), 10.60 (s, br, 1H, NH); MS m/z (%): 296 (M^+ , 13); Anal. Calcd. for C₁₅H₁₆N₆O (296.33): C, 60.80; H, 5.44; N, 28.36. Found: C, 60.72; H, 5.35; N, 28.29%.

3.3. General method for synthesis of 2-cyano- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-3-arylacrylohydrazide (5a-k).

An equimolecular mixture of 2-cyano- N' -[1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene]acetohydrazide (**3**) (0.296 g, 1 mmol) and the corresponding aldehyde **4a-k** (1 mmol) was combined in absolute ethanol (10 mL) with catalytic amount of piperidine (0.3 mL). The mixture was refluxed for 4-6 hours, as monitored by TLC. Afterward, the reaction mixture was cooled and introduced to cold water. The resulting solid was filtered, dried, and then purified through recrystallization using an appropriate solvent, yielding chalcones compounds **5a-k** in 70-85% yield. Below, you'll find the compounds **5a-k** along with their respective physical properties and spectral data.

3.3.1. 2-Cyano- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-3-phenylacrylohydrazide (5a).

Yellow solid; mp. 100-102 °C (EtOH); IR (KBr) $\nu = 3452$ (NH); 3048, 2921 (C-H's); 2281 (CN), 1685 (C=O); 1516 (C=N) cm^{-1} ; $^1\text{H NMR}$ (300

MHz, DMSO- d_6) δ $^1\text{H-NMR}$ (DMSO- d_6): δ 2.42 (s, 3H, CH₃); 2.48 (s, 3H, CH₃); 2.65 (s, 3H, CH₃); 7.42-7.48 (m, 10H, Ar-H and CH=); 7.50 (s, 1H, NH) ppm; MS, m/z (%) 384 (M^+ , 25); Anal. Calcd. for C₂₂H₂₀N₆O (384.44): C, 68.73; H, 5.24; N, 21.86. Found: C, 68.66; H, 5.13; N, 21.70%.

3.3.2. 2-Cyano- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-3-(*p*-tolyl)acrylohydrazide (5b).

Yellow solid; mp. 122-124 °C (EtOH); IR (KBr) $\nu = 3407$ (NH); 3026, 2921 (C-H's); 2283 (CN); 1601 (C=O), 1514 (C=C) (C=N) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 2.36 (s, 3H, CH₃); 2.40 (s, 3H, CH₃); 2.63 (s, 3H, CH₃); 2.75 (s, 3H, CH₃); 7.29 (s, 1H, CH=); 7.32-7.54 (m, 4H, Ar-H), 7.74-7.85 (m, 4H, Ar-H), 8.65 (s, 1H, NH) ppm; MS, m/z (%) 398 (M^+ , 42); Anal. Calcd. for C₂₃H₂₂N₆O (398.47): C, 69.33; H, 5.57; N, 21.09. Found: C, 69.14; H, 5.51; N, 21.02%.

3.3.3. 2-Cyano-3-(4-methoxyphenyl)- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5c).

Yellow solid; mp 117-119 °C (EtOH); IR (KBr) $\nu = 3186$ (N-H), 2925 (C-H's), 2283 (CN), 1674 (C=O), 1595 (C=N), 1514 (C=C) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 2.41 (s, 3H, CH₃); 2.48 (s, 3H, CH₃); 2.74 (s, 3H, CH₃); 3.85 (s, 3H, OCH₃); 7.11 (s, 1H, CH=); 7.45-7.58 (m, 4H, ArH); 7.78-7.80 (m, 4H, Ar-H), 7.84 (s, 1H, NH) ppm MS, m/z (%) 414 (M^+ , 21); Anal. Calcd. for C₂₃H₂₂N₆O₂ (414.47): C, 66.65; H, 5.35; N, 20.28. Found: C, 66.57; H, 5.21; N, 20.15%.

3.3.4. 2-Cyano-3-(4-(dimethylamino)phenyl)- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5d).

Yellow solid; mp 110-112 °C (DMF); IR (KBr) $\nu = 3425$ (N-H); 3047, 2919 (C-H's); 2036 (CN); 1687 (C=O), 1516 (C=C) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 2.46 (s, 3H, CH₃), 2.64 (s, 3H, CH₃), 2.74 (s, 3H, CH₃), 7.31-8.12 (m, 9H, Ar-H and NH₂), 10.77 (s, br, 1H, NH); MS, m/z (%) 427 (M^+ , 61); Calcd. for C₂₄H₂₅N₇O (427.51): C, 67.43; H, 5.89; N, 22.93. Found: C, 67.36; H, 5.92; N, 22.80%.

3.3.5. 2-Cyano-3-(2-hydroxyphenyl)- N' -(1-(5-methyl-1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5e).

Yellow solid; mp 177-179 °C (EtOH); IR (KBr) $\nu = 3420$ (N-H); 3054, 2922 (C-H's); 2010 (CN); 1691 (C=O), 1610 (C=N); 1552, 1514 (C=C) cm^{-1} ; $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 2.42 (s, 3H, CH₃); 2.48 (s, 3H, CH₃); 2.62 (s, 3H, CH₃); 6.96 (s, 1H, CH=); 7.37-7.50 (m, 8H, Ar-H); 7.52 (s, 1H,

NH); 8.99 (s, 1H, OH) ppm; MS, m/z (%) 400 (M^+ , 75); Anal. calcd for $C_{22}H_{20}N_6O_2$ (400.44): C, 65.99; H, 5.03; N, 20.99. Found: C, 65.82; H, 4.92; N, 20.78%.

3.3.6. 2-Cyano-3-(4-hydroxyphenyl)-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5f).

yellow solid; mp 175-177 °C (EtOH); IR (KBr) ν = 3199 (N-H); 2921 (C-H's); 2379 (CN); 1594 (C=N) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.38 (s, 3H, CH₃); 2.45 (s, 3H, CH₃); 2.48 (s, 3H, CH₃); 6.90 (s, 1H, CH=); 7.41-7.70 (m, 8H, Ar-H), 7.81 (s, 1H, NH); 10.00 (s, 1H, OH) ppm; Anal. calcd for: $C_{22}H_{20}N_6O_2$ (400.44): C, 65.99; H, 5.03; N, 20.99. Found: C, 65.75; H, 5.01; N, 20.85%.

3.3.7. 3-(2-Chlorophenyl)-2-cyano-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5g).

Brown solid; mp 135-137 °C (DMF); IR (KBr) ν = 3408 (NH); 3062, 2920 (C-H's); 1922 (CN); 1675 (C=O); 1613 (C=N); 1516 (C=C) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.42 (s, 3H, CH₃); 2.48 (s, 3H, CH₃); 2.62 (s, 3H, CH₃); 7.42 (s, 1H, CH=); 7.45-7.59 (m, 4H, Ar-H); 7.62-8.17 (m, 4H, Ar-H); 8.97 (s, 1H, NH) ppm; MS, m/z (%) 418 (M^+ , 27); Anal. calcd for $C_{22}H_{19}ClN_6O$ (418.89): C, 63.08; H, 4.57; N, 20.06. Found: C, 63.01; H, 4.44; N, 19.93%.

3.3.8. 3-(4-Chlorophenyl)-2-cyano-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5h).

Yellow solid; mp 303-305 °C (DMF); IR (KBr) ν = 3151 (N-H), 2924 (C-H's), 1593 (C=N), 1514 (C=C) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.41 (s, 3H, CH₃); 2.43 (s, 3H, CH₃); 2.61 (s, 3H, CH₃); 7.44 (s, 1H, CH=); 7.47-7.61 (m, 4H, Ar-H); 7.81-7.90 (m, 4H, Ar-H); 8.70 (s, 1H, NH) ppm; Anal. Calcd. for $C_{22}H_{19}ClN_6O$ (418.89): C, 63.08; H, 4.57; N, 20.06. Found: C, 63.01; H, 4.46; N, 20.01%.

3.3.9. 2-Cyano-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-3-(3-nitrophenyl)acrylohydrazide (5i).

Brown solid; mp 105-107 °C (DMF); IR (KBr) ν = 3413 (NH); 3072, 2920 (C-H's); 2294 (CN); 1685 (C=O); 1627 (C=N); 1597 (C=C) 1519 (C=C) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.49 (s, 3H, CH₃), 2.94 (s, 3H, CH₃), 7.13-7.83 (m, 17H, Ar-H and NH₂), 10.81 (s, br, 1H, NH), 11.15 (s, br, 1H, NH); Anal. Calcd. for $C_{22}H_{19}N_7O_3$ (429.44): C, 61.53; H, 4.46; N, 22.83. Found: C, 61.42; H, 4.37; N, 22.70%.

2-Cyano-3-(4-formylphenyl)-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-

yl)ethylidene)acrylohydrazide (5j). Yellow solid; mp 275-277 °C (DMF); IR (KBr) ν = 3435, 3269 (N-H), 3040, 2924 (C-H's), 2212 (CN), 1687 (C=O), 1606 (C=N), 1516 (C=C) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.49 (s, 3H, CH₃), 2.94 (s, 3H, CH₃), 7.13-7.83 (m, 17H, Ar-H and NH₂), 10.81 (s, br, 1H, NH), 11.15 (s, br, 1H, NH); MS, m/z (%) 412 (M^+ , 73); Anal. calcd for $C_{23}H_{20}N_6O_2$ (412.45): C, 66.98; H, 4.89; N, 20.38. Found: C, 66.74; H, 4.81; N, 20.29%.

3.3.10. 2-Cyano-3-(2,4-dichlorophenyl)-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acrylohydrazide (5k).

Pale brown solid; mp 130-132 °C (DMF); IR (KBr) ν = 3417 (N-H), 2924 (C-H's), 2513 (CN), 1682 (C=O), 1592 (C=N), 1517 (C=C) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.42 (s, 3H, CH₃); 2.48 (s, 3H, CH₃); 2.58 (s, 3H, CH₃); 7.42 (s, 1H, CH=); 7.45-7.54 (m, 3H, Ar-H); 7.58-7.80 (m, 4H, Ar-H); 8.13 (s, 1H, NH) ppm; MS, m/z (%) 453 (M^+ , 73); Anal. Calcd. for $C_{22}H_{18}Cl_2N_6O$ (453.33): C, 58.29; H, 4.00; N, 18.54. Found: C, 58.15; H, 3.91; N, 18.44%.

3.4. Synthesis of azolopyrimidines 9 and 13:

An equimolecular mixture consisting of 2-cyano-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-3-phenylacrylohydrazide (**5a**) (0.384g, 1 mmol) and the corresponding heterocyclic amine, specifically, 4H-1,2,4-triazol-3-amine (**6**) and 1H-tetrazol-5-amine (**12**) (1 mmol), was refluxed in absolute ethanol (10 mL) for a duration of 10 hours (monitored by TLC). Subsequently, the mixture was allowed to cool to room temperature. The resulting solid product was separated by filtration, rinsed with ethanol, and subjected to recrystallization from DMF, yielding the azolopyrimidine derivatives **9** and **13**, respectively.

3.4.1. 7-Amino-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbohydrazide (9).

White solid; mp 290-292 °C (EtOH); IR (KBr) ν = 3413, 3058 (NH₂, NH), 1659 (C=O), 1592 (C=N), 1518 (C=C) cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6) δ 2.43 (s, 3H, CH₃); 2.62 (s, 3H, CH₃); 2.75 (s, 3H, CH₃); 7.44 (s, 2H, NH₂), 7.74-7.56 (m, 5H, Ar-H), 7.79-7.81 (m, 4H, Ar-H), 7.87 (s, 1H, NH) ppm; Anal. Calcd for $C_{24}H_{22}N_{10}O$ (466.51): C, 61.79; H, 4.75; N, 30.03. Found C, 61.67; H, 4.66; N, 29.86%.

3.4.2. 7-Amino-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)-5-phenyltetrazolo[1,5-a]pyrimidine-6-carbohydrazide (13).

White solid; mp 279-281°C (EtOH); IR (KBr) ν = 3411, 3050 (NH₂, NH), 2921 (C-H's), 1657 (C=O), 1591 (C=N), 1517 (C=C) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 2.38 (s, 3H, CH₃); 2.41 (s, 3H, CH₃); 2.62 (s, 3H, CH₃); 7.32-7.40 (m, 5H, Ar-H), 7.44 (s, 2H, NH₂); 7.46-7.54 (m, 4H, Ar-H), 7.86 (s, 1H, NH) ppm; Anal.Calcd for C₂₃H₂₁N₁₁O (467.50): C, 59.09; H, 4.53; N, 32.96. Found C, 59.01; H, 4.45; N, 32.80%.

3.4.3. Synthesis of 6-amino-1-((1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)amino)-4-(methylthio)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (17).

A mixture consisting of 2-cyano-N'-(1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)acetohydrazide (**3**) (2.96 g, 10 mmol) and 2-(bis(methylthio)methylene)malononitrile (**15**) (1.7 g, 10 mmol) in 30 mL of dioxane, containing KOH (1 g) as a catalyst, was agitated at room temperature for 24 hours. The resulting solid was isolated through filtration, dried, and then purified by recrystallization in DMF, yielding compound **17** in the form of a yellow solid; mp 237-239 °C (DMF); IR (KBr) ν = 3426 (NH₂), 3057, 2930 (C-H), 2190 (CN), 1642 (C=O), 1606 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 2.46 (s, 3H, CH₃), 2.50 (s, 3H, CH₃), 2.73 (s, 3H, CH₃), 6.52 (s, br, 2H, NH₂), 7.37-7.92 (m, 4H, Ar-H); Anal.Calcd for C₂₀H₁₈N₈O₂S (418.48): C, 57.40; H, 4.34; N, 26.78. Found C, 57.31; H, 4.29; N, 26.64%.

3.4.4. Reaction of 17 with hydrazine hydrate to produce 3,4-diamino-5-((1-(5-methyl-1-(p-tolyl)-1H-1,2,3-triazol-4-yl)ethylidene)amino)-6-oxo-5,6-dihydro-1H-pyrazolo[4,3-c]pyridine-7-carbonitrile (18).

A mixture of compound **17** (0.418 g, 1 mmol) and hydrazine hydrate (5 mL) in dioxane (30 mL) underwent agitation at room temperature for 4 hours. The resulting precipitate was filtered, dried, and subjected to recrystallization from DMF, yielding compound **18** in the form of a yellow solid.; mp 206-208 °C (EtOH); IR (KBr) ν 3434 (NH), 3045, 2924 (C-H), 2229 (CN), 1644 (C=O), 1600 (C=N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 2.28 (s, 3H, CH₃), 2.50 (s, 3H, CH₃), 2.72 (s, 3H, CH₃), 5.31(s, 2H, NH₂), 6.53(s, 2H, NH₂), 7.29-7.63 (m, 4H, Ar-H), 10.19 (s, br, 1H, NH); Anal.Calcd for C₁₉H₁₈N₁₀O (402.42): C, 56.71; H, 4.51; N, 34.81. Found C, 56.63; H, 4.42; N, 34.69%.

3.5. Antiproliferative activity of the tested compounds

Preparation of the cells

LoVo, HEPG2 and MCF7 cell lines were obtained from the American Type Culture collection (Rockville, Maryland, USA).

3.5.1. In vitro anti-proliferative assay and samples preparation

The tested compounds were prepared as previously mentioned and examined using SRB assay[6], [24].

3.5.2. SRB (Cytotoxic test)

The details of this technique were described by Skehan et al[25]

3.6. Statistical data

The results obtained were stated as Mean \pm Standard error (S.E.) and each experiment was repeated for at least 6 times.

3.7. Chitosan (CS) / polyvinyl alcohol (PVA) films with and without 5d loading preparation

The films of the chitosan / polyvinyl alcohol were fabricated as mentioned previously[26], [27]

4. References

- [1]. H. R. M. Rashdan, S. M. Nasr, H. A. El-Refai, and M. S. Abdel-Aziz, "A novel approach of potent antioxidant and antimicrobial agents containing coumarin moiety accompanied with cytotoxicity studies on the newly synthesized derivatives," *J. Appl. Pharm. Sci.*, vol. 7, no. 7, 2017, doi: 10.7324/JAPS.2017.70727.
- [2]. H. R. M. Rashdan, M. El-Naggar, and A. H. Abdelmonsef, "Synthesis, Molecular Docking Studies and In Silico ADMET Screening of New Heterocycles Linked Thiazole Conjugates as Potent Anti-Hepatic Cancer Agents," *Molecules*, vol. 26, no. 6, p. 1705, 2021.
- [3]. I. A. Shehadi, M. T. Abdelrahman, M. Abdelraof, and H. R. M. Rashdan, "Solvent-Free Synthesis, In Vitro and In Silico Studies of Novel Potential 1, 3, 4-Thiadiazole-Based Molecules against Microbial Pathogens," *Molecules*, vol. 27, no. 2, p. 342, 2022.
- [4]. M. El-Naggar, A. S. Abd El-All, S. I. A. El-Naem, M. M. Abdalla, and H. R. M. Rashdan, "New potent 5 α - Reductase and aromatase inhibitors derived from 1,2,3-triazole derivative," *Molecules*, vol. 25, no. 3, 2020, doi: 10.3390/molecules25030672.
- [5]. A. Shaik, R. R. Bhandare, K. Palleapati, S. Nissankararao, V. Kancharlapalli, and S. Shaik, "Antimicrobial, antioxidant, and anticancer

- activities of some novel isoxazole ring containing chalcone and dihydropyrazole derivatives,” *Molecules*, vol. 25, no. 5, 2020, doi: 10.3390/molecules25051047.
- [6]. hanaa F. roaiah, H. R. Rashdan, abdelmohsen soliman, Z. Muhammed, J. Wietrzyk, and M. Milczarek, “Design, efficient synthesis, mechanism of reaction and antiproliferative activity against cancer and normal cell lines of a novel class of fused pyrimidine derivatives,” *Acta Pol. Pharm. Res.*, vol. 75, no. 3, 2018.
- [7]. S. A. Osman, M. A. Abdallah, H. Bassiony, and H. R. M. Rashdan, “Insight into the Synthesis, Biological Impact and Convenient Routes of Hydrazoneoyl Halides for Synthesis of Novel Bioactive Heterocycles: Mini-Review,” *Egypt. J. Chem.*, vol. 66, no. 13, pp. 2371–2378, Dec. 2023, doi: 10.21608/EJCHEM.2024.249272.8883.
- [8]. A. H. Abdelmonsef, M. Omar, H. R. M. Rashdan, M. M. Taha, and A. M. Abobakr, “Design, synthetic approach, in silico molecular docking and antibacterial activity of quinazolin-2,4-dione hybrids bearing bioactive scaffolds,” *RSC Adv.*, vol. 13, no. 1, pp. 292–308, 2023, doi: 10.1039/D2RA06527D.
- [9]. A. H. Abdelmonsef *et al.*, “A search for antiinflammatory therapies: Synthesis, in silico investigation of the mode of action, and in vitro analyses of new quinazolin-2, 4-dione derivatives targeting phosphodiesterase-4 enzyme,” *J. Heterocycl. Chem.*, vol. 59, no. 3, pp. 474–492, 2022.
- [10]. H. R. M. Rashdan, H. M. F. Roaiah, Z. A. Muhammad, J. Wietrzyk, M. Milczarek, and A. M. M. Soliman, “Design, efficient synthesis, mechanism of reaction and antiproliferative activity against cancer and normal cell lines of a novel class of fused pyrimidine derivatives,” *Acta Pol. Pharm. - Drug Res.*, vol. 75, no. 3, 2018.
- [11]. S. M. M. Lopes *et al.*, “Hetero-Diels-Alder reactions of novel 3-triazolyl-nitrosoalkenes as an approach to functionalized 1, 2, 3-triazoles with antibacterial profile,” *Eur. J. Med. Chem.*, vol. 143, pp. 1010–1020, 2018.
- [12]. K. P. McDonald, Y. Hua, and A. H. Flood, “1, 2, 3-Triazoles and the expanding utility of charge neutral CH \cdots anion interactions,” in *Anion recognition in supramolecular chemistry*, Springer, 2010, pp. 341–366.
- [13]. H. R. M. Rashdan, S. M. Gomha, M. S. El-Gendey, M. A. El-Hashash, and A. M. M. Soliman, “Eco-friendly one-pot synthesis of some new pyrazolo[1,2-b]phthalazinediones with antiproliferative efficacy on human hepatic cancer cell lines,” *Green Chem. Lett. Rev.*, vol. 11, no. 3, 2018, doi: 10.1080/17518253.2018.1474270.
- [14]. J. Totobenazara and A. J. Burke, “New click-chemistry methods for 1, 2, 3-triazoles synthesis: Recent advances and applications,” *Tetrahedron Lett.*, vol. 56, no. 22, pp. 2853–2859, 2015.
- [15]. H. R. M. Rashdan, I. A. Shehadi, and A. H. Abdelmonsef, “Synthesis, anticancer evaluation, computer-aided docking studies, and ADMET prediction of 1,2,3-triazolyl-pyridine hybrids as human aurora B kinase inhibitors,” *ACS Omega*, vol. 6, no. 2, pp. 1445–1455, 2021, doi: 10.1021/ACSOMEGA.0C05116/ASSET/IMAGE S/LARGE/AOOC05116_0002.JPEG.
- [16]. H. R. M. Rashdan, M. T. Abdelrahman, A. C. De Luca, and M. Mangini, “Towards a New Generation of Hormone Therapies: Design, Synthesis and Biological Evaluation of Novel 1,2,3-Triazoles as Estrogen-Positive Breast Cancer Therapeutics and Non-Steroidal Aromatase Inhibitors,” *Pharm. 2024, Vol. 17, Page 88*, vol. 17, no. 1, p. 88, Jan. 2024, doi: 10.3390/PH17010088.
- [17]. H. R. M. Rashdan, A. H. Abdelmonsef, I. A. Shehadi, S. M. Gomha, A. M. M. Soliman, and H. K. Mahmoud, “Synthesis, Molecular Docking Screening and Anti-Proliferative Potency Evaluation of Some New Imidazo[2,1-b]Thiazole Linked Thiadiazole Conjugates,” *Mol. 2020, Vol. 25, Page 4997*, vol. 25, no. 21, p. 4997, Oct. 2020, doi: 10.3390/MOLECULES25214997.
- [18]. S. C. Holm and B. F. Straub, “Synthesis of N-Substituted 1,2,4-Triazoles. A Review,” *Org. Prep. Proced. Int.*, vol. 43, no. 4, pp. 319–347, 2011, doi: 10.1080/00304948.2011.593999.
- [19]. H. R. M. Rashdan, E. K. Radwan, A. A. Koryam, G. S. El-Sayyad, and R. M. Fathy, “Insights into promising basic red 18 dye removal and water disinfection utilizing novel sulfone biscompound-based chalcone derivative,” *J. Water Process Eng.*, vol. 54, Aug. 2023, doi: 10.1016/j.jwpe.2023.104036.
- [20]. S. A. Al Kiey, H. R. M. Rashdan, and M. E. El-Naggar, “Insight into mitigation of corrosion behavior of novel Chalcone derivative for AZ91 Mg alloy in saline solution: Synthesis, Characterization, Electrochemical and adsorption studies,” *J. Electroanal. Chem.*, p. 117304, 2023.
- [21]. Insight into the Synthesis, Biological Impact and Convenient Routes of Hydrazoneoyl Halides for Synthesis of Novel Bioactive Heterocycles: Mini-Review.” Accessed: Feb. 23, 2024. [Online]. Available: https://journals.ekb.eg/article_334091.html
- [22]. E. K. Radwan, H. R. M. Rashdan, B. A. Hemdan, A. A. Koryam, and M. E. El-Naggar, “A dual-functional sulfone biscompound containing 1,2,3-triazole moiety for decolorization and disinfection of contaminated water,” *Environ. Sci. Pollut. Res.*, vol. 29, no. 51, pp. 77238–77252, Nov. 2022, doi: 10.1007/S11356-022-20932-5.
- [23]. Facial Synthesis of Some New Pyrazolopyridine, Barbituric and Thiobarbituric Acid Derivatives with Antimicrobial Activities,” 2014.
- [24]. A. Abdel-Aziem, H. R. M. Rashdan, E. Mohamed Ahmed, and S. N. Shabaan, “Synthesis and cytotoxic activity of some novel benzocoumarin derivatives under solvent free conditions,” *Green Chem. Lett. Rev.*, vol. 12, no. 1, pp. 9–18, 2019.
- [25]. P. Skehan *et al.*, “New colorimetric cytotoxicity assay for anticancer-drug screening,” *JNCI J. Natl. Cancer Inst.*, vol. 82, no. 13, pp. 1107–1112, 1990.

- [26]. Y. Liu, R. Song, X. Zhang, and D. Zhang, "Enhanced antimicrobial activity and pH-responsive sustained release of chitosan/poly (vinyl alcohol)/graphene oxide nanofibrous membrane loading with allicin," *Int. J. Biol. Macromol.*, vol. 161, pp. 1405–1413, 2020.
- W. Lan, S. Wang, M. Chen, D. E. Sameen, K. Lee, and Y. Liu, "Developing poly (vinyl alcohol)/chitosan films incorporate with d-limonene: Study of structural, antibacterial, and fruit preservation properties," *Int. J. Biol. Macromol.*, vol. 145, pp. 722–732, 2020.