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Utilization of polymeric carriers for (*E*)-1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one as anticancer agent

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Abstract

In the current work, 4-nitroaniline **(1)** reacted with sodium azide to produce 4-nitrophenyl azide **(2)** and converted into the corresponding 1,2,3-triazole derivative **(3)**. The obtained 1,2,3-triazole derivative **(3)** converted into (*E*)-1-[5-methyl-1-(4-nitrophenyl)triazol-4-yl]-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **(4)** through Claisen-Schmidt condensation with 3,4,5-trihydroxybenzaldehyde in ethanolic NaOH. Compound **4** was then characterized with the suitable spectroscopic techniques and investigated for its anticancer activity. Blends of **4** with Chitosan (CS) and carboxymethylcellulose (CMC) were prepared by uploading it into the polymer matrices through physical mixing. The release behavior of **4** from the polymer blends into an aqueous medium at room temperature was investigated and discussed.

Keywords: 1,2,3-triazole chalcone hybrid**,** anticancer activity, chitosan; CMC, controlled release

1. Introduction

Chitosan (CS) can be considered as a famous natural polysaccharide after cellulose. It is a polycationic linear polysaccharide derived from chitin through partial deacetylation **[1]**. Hence, it is consisted of random copolymer of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine **[2,3]**. CS can form electrostatic multilayer structures or complexes with other negatively charged polymers due to its cationic nature **[4]**. Biodegradability, low allergy, non-toxicity, and biocompatibility in addition to biological characteristics such as antimicrobial, antioxidant, and antitumor activities are unique properties of CS which make it suitable for several applications **[5,6]**. Carboxymethylcellulose (CMC) is a non-toxic; hydrophilic, synthetic biocompatible polymer used in wound dressing and controlled drug delivery systems **[7]**.

Aromatic azides could be obtained early using various methods in which the different preparation routes of aromatic azides mainly from different aromatic amines in addition to other starting materials. Formation of 1,4-disubstituted-1,2,3-triazoles through Huisgen 1,3-dipolar cycloaddition of azides with terminal alkynes under copper-catalyzed conditions is an example of a click reaction **[8]**. 1,4,5-Trisubstituted-1,2,3-triazoles were also obtained *via* 1,3-dipolar cycloaddition of azides and 1,3-dicarbonyl compounds **[9]**. The synthesis of 1,2,3-triazole-chalcone hybrids were reported *via* Claisen-Schmidt condensation between different aldehydes and ketones in presence of NaOH/EtOH **[10-12]**, KOH/EtOH **[13]**, piperidine/EtOH **[14]**, NaOH/MeOH **[15]** or NaOH/acetone **[16]**. Novel 1,2,3-triazole-chalcone hybrids were also prepared *via* green approach using DBU/(PEG-400) **[17]**, grinding with aqueous NaOH **[18]**, or microwave-assisted synthesis **[14,19]**. Problems associated with poor solubility of Chalcones can lead to lower bioavailability and hence lower drug delivery. About 40% of the approved drugs on the market are poorly water-soluble, so it was reformulated using polymers as drug carrier mediators to improve their efficacy. Hence, these drugs were coated with micelles to allow their formulation in a watery medium, where they are offering the greatest advantage in solubilization capacity, lowest critical micelle concentration, and greatest tolerability. Such properties encourage scientists to use these substances in biomedical fields **[20]**.

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In the current work, an aryl azide **(2)** was prepared, converted into 1,2,3-triazole derivative **(3),** and yielded (*E*)-1-[5-methyl-1-(4-nitrophenyl)triazol-4-yl]-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **(4)** through Claisen-Schmidt condensation with 3,4,5-trihydroxybenzaldehyde in ethanolic NaOH. The anticancer activity of **4** was investigated. The prepared **4** was uploaded into a polymer matrix using Chitosan (CS) and carboxymethylcellulose (CMC) through physical mixing and converted into the corresponding polymer blends. After that, the release of **4** from the polymer blend into an aqueous medium at room temperature was investigated and the obtained results were summarized and discussed.

2. Experimental Part

2.1. Materials:

Chitosan (CS) of 85% degree of deacetylation was obtained from Alfa Aesar. Carboxymethylcellulose (CMC) of MW 41 kDa was purchased from Alpha Chemika, India. All other chemicals and solvents were purchased from Sigma-Aldrich, USA without further purification unless otherwise mentioned.

2.2. Instruments and Techniques:

Melting points (ºC) were recorded using a *Stuart* melting point apparatus and are uncorrected. FT-IR spectra (KBr) were recorded on ThermoFisher SCIENTIFIC Nicolet IS10 Spectrometer (υ in cm^{-1}) at the Faculty of Science, Mansoura University, Egypt. ^{1}H-NMR spectra were obtained in DMSO-d_6 using JEOL RESONANCE (500 MHz) at NMR unit, Faculty of Science, Mansoura University, Egypt, using TMS as internal standard (chemical shifts in ppm, δ units). ^{13}C-NMR spectra were obtained in DMSO-d_6 using Brucker (100 MHz) at NMR unit, Faculty of Pharmacy, Mansoura University, Egypt, using TMS as internal standard (chemical shifts in ppm, δ units). The reactions were monitored by TLC plates, Silica gel 60 F_{254} pre-coated (Merck) using UV (366 nm) to visualize spots. CH_2Cl_2:MeOH (20:1) and pet. ether: EtOAc (3:1) was used as elution solvent. Anticancer screening (GI%) of the prepared compound against a panel of 60 cancer cell lines was performed at the National Cancer Institute (NCI), USA. Electron Scanning Micrographs were imaged on JOEL JSM-6510 Scanning Electron Microscope in the Faculty of Agriculture, Mansoura University, Egypt. The average particle diameter and consequently particle size were determined from SEM images using ImageJ software developed at the National Institute of Health (NIH) **[21]**.

2.3. Synthesis of 1-azido-4-nitrobenzene (2):

About 6.9 g, 0.05 mol of 4-nitroaniline **(1)** was added to 640 ml of 17% HCl solution while stirring at room temperature until a suspension was formed. EtOH was then added dropwise until the solution became clear, then the solution was cooled to 0 ºC and 5.18 g, 0.075 mol of $NaNO_2$ in 10 ml water was added and stirred for about 20 min, then 4.88 g, 0.075 mol of NaN_3 was added in the hood. The yellow precipitate formed after continuous stirring was filtered, washed with water, and dried. A yellow solid of **2** (7.88 g) was obtained in 96% yield and of m.p. 65-66 ºC. ^{1}H-NMR (500 MHz, DMSO-d_6) and FT-IR (KBr, υ, cm^{-1}) data are matched with that reported **[22,23]**.

2.4. Synthesis of 1-[5-methyl-1-(4-nitrophenyl)triazol-4-yl]ethan-1-one (3):

To a solution of 2.3 g, 0.1 mol Na metal in 100 ml anhydrous MeOH, 10 g, 0.1 mol acetylacetone was added. A solution of 16.4 g, 0.1 mol of **2** in 20 ml anhydrous MeOH was added. The mixture was stirred at room temperature for 24 h and the resulting suspension was filtered. The obtained solid material was washed with MeOH and dried to yield 22.7 g, 92% of **3** with m.p. of 146-148 ºC; ^{1}H-NMR (500 MHz, DMSO-d_6) and FT-IR (KBr, υ, cm^{-1}) spectral data are matched with that reported literature **[22,23]**.

2.5. Synthesis of (E)-1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (4):

To a suspension of 0.615 g, 2.5 mmol of **3** in 20 ml, 95% EtOH, 0.49 g, 2.5 mmol of 3,4,5-trimethoxy-benzaldehyde was added while stirring in an ice bath. A solution of 3 g, 75 mmol NaOH in 5 ml, 95% EtOH was added while stirring for 30 min. The reaction mixture was stirred for 2-3 days at room temperature. The reaction was monitored by TLC, diluted with 40 ml ice-cold distilled water, and neutralized with an HCl solution. The obtained material was filtered, washed with cold water, and dried. About 0.56 g of pale-yellow solid **(4)** of 53 % yield and m.p. of 195-197 ºC was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6); ^{13}C-NMR (100 MHz, DMSO-d_6) and FT-IR (KBr, υ, cm^{-1}) spectral data were recorded.

2.6. Investigation of anticancer activity of 4:

The anticancer activity of **4** was investigated by National Cancer Institute (NCI), USA against 9 types of cancer, namely, Leukemia, Non-Small Cell Lung Cancer, Colon Cancer, CNS Cancer, Melanoma, Ovarian Cancer, Renal Cancer, Prostate Cancer, and Breast Cancer. Cell lines were allowed to grow under sterilized conditions in RPMI 1640 media (Gibco, NY, USA*)* with 10% fetal bovine serum (Biocell, CA, USA*)*, 5×10^5 cell/ml. This medium was utilized for testing the growth inhibitory activity of an arbitrary concentration of 10 μM of **4** in phosphate buffer saline. After that, 0.2 ml was introduced to separate well of a

microliter tray in duplicate. Cell culture (1.8 ml) in a cell population of 6×10^4 cells/ml was also introduced to each well. Control solutions were prepared adopting the same procedure but containing only phosphate buffer. The prepared cell cultures were incubated in a humidified incubator at 37°C, supplied with 5% CO_2 atmosphere. After 48 h, dilution of each well ten times with saline and cells were counted *via* coulter counter **[24,25]**. The results are summarized in **section 3.5.**

2.7. Preparation of carboxymethylcellulose (CMC) and chitosan (CS) membranes

A Solution of CMC and CS was prepared by dissolving 3 g (0.01 mole) CMC and CS each in 300 ml distilled water while continuously stirring till a homogeneous viscous liquid was formed. In the case of CMC only, an amount of 0.15 g (0.8 mmole) citric acid was added as a crosslinker (8% crosslinking ratio) of while continuous stirring till dissolved. The obtained solutions were cast on plastic Petri dishes and dried overnight in an oven at 50 °C. The obtained membranes were kept until use and characterization.

2.8. Preparation of CS and CMC blends with 4

A solution of CS was prepared by dissolving 3 g (0.01 mole) CS in a mixture of 300 ml distilled water and 5 ml acetone while continuously stirring till a homogeneous viscous liquid was formed. A solution of 0.2 g (0.5 mmole) of **4** in the least amount of ethyl acetate was added to the previously prepared solution of CS. In the case of the CMC blend with **4**, an amount of 0.15 g (0.8 mmole) citric acid was added as a crosslinker to the mixture of CMC and **4**. After constant stirring for 2 h, the mixtures were poured into glass Petri dishes and kept overnight in an oven at 50 °C. The obtained membranes were kept under vacuum in a desiccator before use and characterization.

2.9. Scanning Electron Microscopy (SEM) of the blend samples

Blend samples were micro-graphed by SEM to determine the average particle of the chalcone within the blend matrix. The average particle size could be calculated after image processing with ImageJ Software. The data derived from the image analysis was summarized in Table 1.

Table 1: Average particle size calculated by SEM images analysis by ImageJ Software

Sample	Magnification (x)	Average Size (nm)
CS-4	**10000**	19.584
CMC-4	**10000**	12.744

2.10. Release Experiments

The release behavior of **4** from its blends with CMC and CS into an aqueous medium at room temperature was investigated. Table 2 shows the composition of the investigated blends considering that CMC blends were crosslinked with citric acid to avoid solubility during the release process.

About 0.5 g each of **CMC-4** and **CS-4** were immersed separately in 100 ml of distilled water at room temperature (~25 °C). After certain time intervals, about 1 ml of the soaking solution was picked out, diluted to 5 ml with distilled water, and kept for UV analysis to determine the Wt. % of the released **4**. The release time and the corresponding Wt. % of the released **4** are shown in Table 3.

Table 2: Composition of carboxymethylcellulose (CMC) and chitosan (CS) blends with **4**

Sample	CMC (g)	CS (g)	4 (g)
CMC-4*	3	-	0.2
CS-4	-	3	0.2

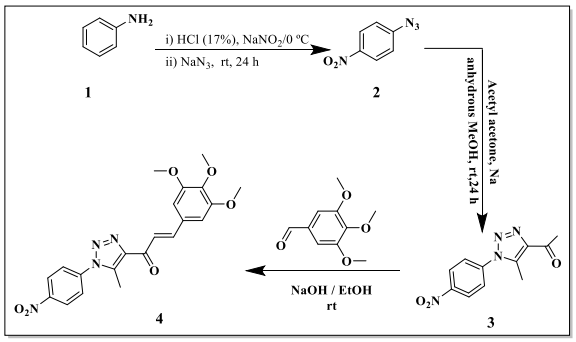
*: Samples were treated with a certain amount of citric acid to achieve crosslinking.

Table 3: Time dependence of the amount of **4** released from **CMC-4** and **CS-4** blends.

Time, min	Wt.% of released 4	
	CMC-4	CS-4
5	10.7	13.2
10	21.4	22.1
15	29.3	28.7
30	41.1	42.3
45	46.7	47.4
60	51.7	52.8
120	63.4	64.7
180	73.6	75.8
240	77.3	79.5
300	82.1	83.4
360	85.3	86.2
720	91.4	93.2
1440	96.2	97.8

3. Results and Discussion

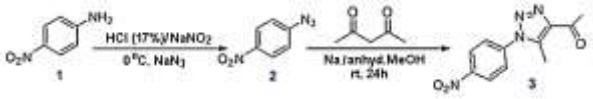
4-Nitroaniline **(1)** was converted into 4-nitrophenyl azide **(2)**, then was converted into the 1,2,3-triazole derivative **(3)** and finally converted into the target chalcone derivative **(4)** as represented in **Scheme 1**.



Scheme 1: Synthetic mainframe for chalcone derivative **(4)**.

3.2. Synthesis of 1-[5-methyl-1-(4-nitrophenyl)triazol-4-yl]ethan-1-one (3):

4-Nitroaniline **(1)** was converted into 4-nitrophenyl azides **(2)**, then **2** was converted into 1-[5-methyl-1-(4-nitrophenyl)triazol-4-yl]ethan-1-one **(3)** according to **Equation 1**. Structure of **2** was confirmed spectroscopically where FT-IR (KBr, υ, cm^{-1}) in Figure 1 showed azide absorption bands at 3449, 3109, 3070, 2126 (azide), 1595, 1517 (asym. NO_2), 1491, 1346 (sym. NO_2), 1287, 1109, 848, 747 cm^{-1}. The data matched with that reported **[22]**.



Eq. 1

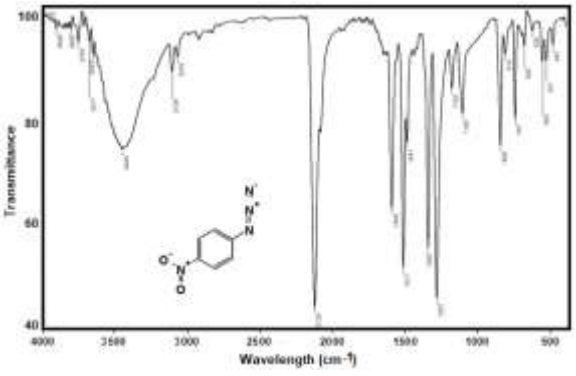


Figure 1: FT-IR spectrum of **2**

^{1}H-NMR spectrum of **2** in Figure 2 showed two doublet signals at 8.25 and 7.35 ppm attributed to aryl protons of phenyl ring. 4-Nitrophenyl azide **(2)** was obtained in 96% yield *via* diazotization of 4-nitroaniline **(1)** using $NaNO_2$/HCl followed by azidation using NaN_3 **[22]**.

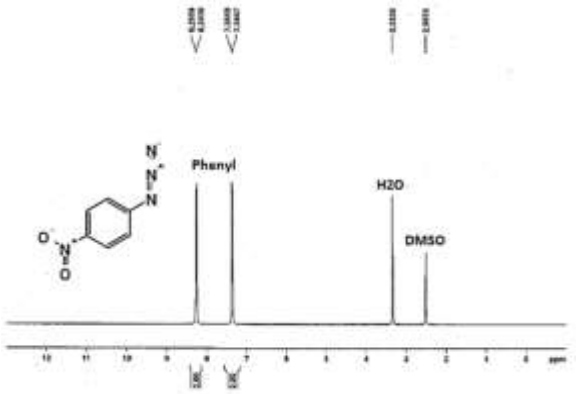


Figure 2: ^{1}H-NMR spectrum (DMSO-d_6) of **2.**

The obtained compound **3** was approved spectroscopically where FT-IR (KBr, υ, cm^{-1}): 3447, 3078, 2929, 1680 (C=O ketone), 1652, 1599, 1558, 1522 (asym. NO_2), 1424, 1343 (sym. NO_2), 856, 651. FT-IR spectra in Figure 3 showed disappearance of azide absorption bands around 2100 cm^{-1} and the appearance of carbonyl absorption band at 1687 and 1680 cm^{-1}. Figure 4a,b showed ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 8.48 (d, *J* = 8.1 Hz, 2H, ArH), 7.98 (d, *J* = 8.1 Hz, 2H, ArH), 2.65 (s, 3H, CH_3), 2.6 (s, 3H, $COCH_3$). Yield = 92%; m.p. = 146-148 ºC as reported and the spectral data matched with that reported as well **[22,23]**.

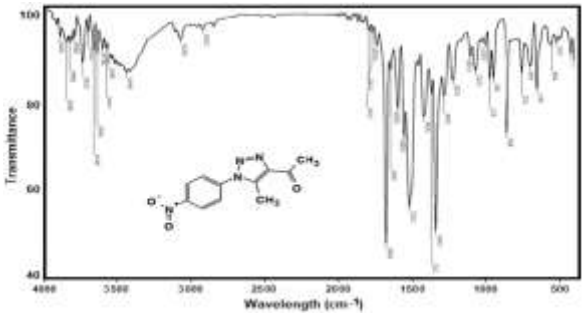


Figure 3: FT-IR spectrum of **3.**

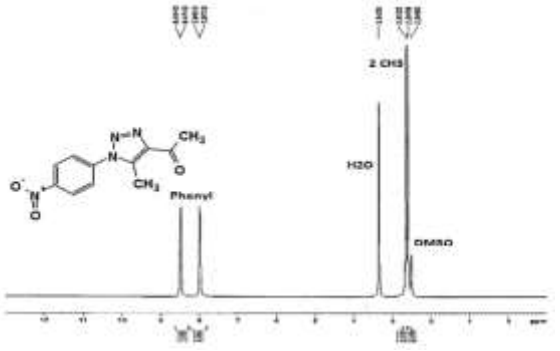


Figure 4a: ^{1}H-NMR spectrum (DMSO-d_6) for compound **3.**

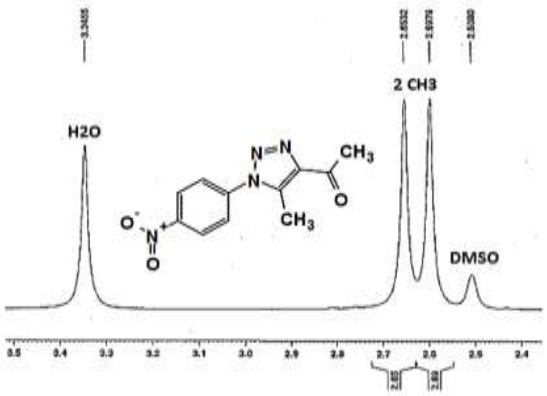
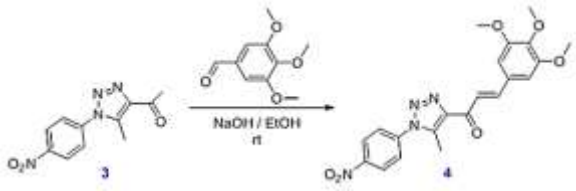


Figure 4b: Magnified ^{1}H-NMR spectrum (DMSO-d_6) of **3.**

3.4. Synthesis of (*E*)-1-[5-methyl-1-(4-nitrophenyl)triazol-4-yl]ethan-1-one (4):



Eq. 2.

Claisen-Schmidt condensation of **3** with 3,4,5-trimethoxybenzaldehyde in ethanolic NaOH gave the chalcone (*E*)-1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one **(4)** in 92% yield according to **Equation 2**. Structure of **4** was proved spectroscopically where Figure 5 showed FT-IR (KBr, υ, cm^{-1}): 3448, 3071, 1690 (C=O α,β-unsaturated), 1590, 1498, 1422, 1363, 1227, 1187, 1159, 1028, 999, 977, 833. Figure 6a,b showed ^{1}H-NMR (500 MHz, DMSO-d$_6$): δ = 8.50 (d, *J* = 8.8 Hz, 2H, ArH), 8.02 (d, *J* = 8.9 Hz, 2H, ArH), 7.98 (d, *J* = 15.7 Hz, 1H, CH olefinic), 7.83 (d, *J* = 15.8 Hz, 1H, CH olefinic), 7.18 (s, 2H, ArH), 3.87 (s, 6H, 2OCH$_3$), 3.72 (s, 3H, OCH$_3$), 2.68 (s, 3H, CH$_3$). Figure 7 showed ^{13}C-NMR (100 MHz, DMSO-d6): δ = 183.4, 153.2 (2C), 148, 144, 143.4, 139.9, 139.9, 139.5, 129.9, 126.6 (2C), 125.2 (2C), 122, 106.4 (2C), 60.2, 56.1 (2C), 10.1. Yield = 92%; m.p. = 195 - 197 °C.

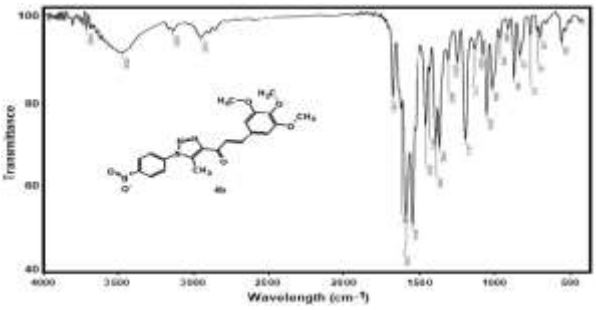


Figure 5: FT-IR Spectrum of **4**

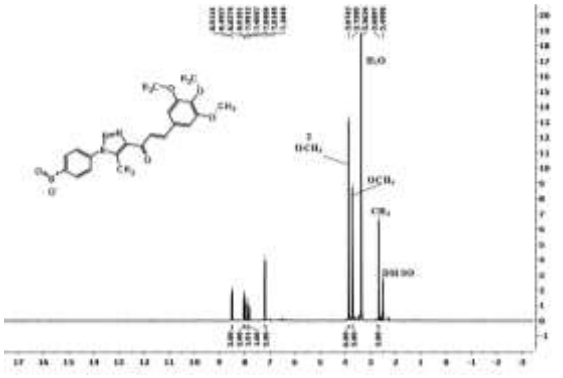


Figure 6a: ^{1}H-NMR spectrum (DMSO-d_6) of **4.**

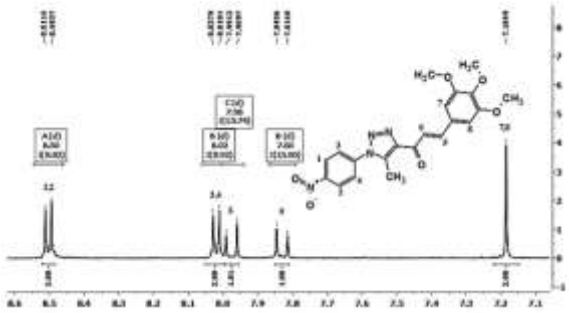


Figure 6b: Magnified ^{1}H-NMR spectrum (DMSO-d_6) of **4.**

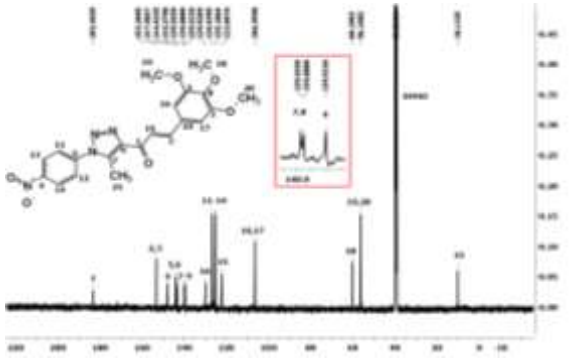


Figure 7: ^{13}C-NMR spectrum (DMSO-d_6) of **4**.

3.5. Investigation of anticancer activity of 4:

(E)-1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **(4)** was submitted to the National Cancer Institute (NCI) for *in-vitro* screening of their anti-proliferative activity against nine tumor subpanels (60 cell lines) at one dose (10 μM) of **4** was investigated in the light of their anticancer activity against different 9 types of cancer, namely, Leukemia, Non-Small Cell Lung Cancer, Colon Cancer, CNS Cancer, Melanoma, Ovarian Cancer, Renal Cancer, Prostate Cancer, and Breast Cancer. The results are calculated as the mean-graph of the percent growth of the treated cell lines and are reported as percentage growth inhibition (GI %) caused by the test compounds (**Table 4**). Among the tested cell lines panels, the most potent with a remarkable broad-spectrum anticancer activity is

showed a GI% of 86.69 % towards Ovarian cancer NCI/ADR-RES, breast cancer MDA-MB-468 with a GI% of 78.21, Leukemia K-562, Leukemia SR, Leukemia CCRF-CEM, Leukemia RPMI-8226 and Leukemia MOLT-4 with a GI% of 53.00, 58.62, 45.98, 36.55 and 34.83, respectively. CNS Cancer SNB-75 with a GI% of 52.35. Compound **4** showed anticancer potency towards most of cell lines. Whereas cell lines Colon Cancer COLO 205, Melanoma SK-MEL-2, Ovarian Cancer IGROV1, OVCAR-3, OVCAR-5, SK-OV-3, Renal Cancer 786-0, A498, TK-10 and Breast Cancer BT-549 proved to be the non-growth inhibition in this study (**Table 4**).

Table 4: Growth Inhibition % (GI%) induced by (E)-1-(5-methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**4**), at 10 µM concentration on NCI-60 cell line panel.

Subpanel tumor cell lines	GI%*
Leukemia	
CCRF-CEM	45.98
HL-60(TB)	26.33
K-562	53.00
MOLT-4	34.83
RPMI-8226	36.55
SR	58.62
Non-Small Cell Lung Cancer	
A549/ATCC	3.95
EKVX	13.18
HOP-62	5.25
HOP-92	21.15
NCI-H226	10.48
NCI-H23	8.02
NCI-H322M	8.62
NCI-H460	12.12
NCI-H522	13.22
Colon Cancer	
COLO 205	NI
HCC-2998	3.38
HCT-116	16.98
HCT-15	28.12
HT29	5.73
KM12	11.21
CNS Cancer	
SF-268	9.66
SF-295	10.15
SF-539	10.75
SNB-19	10.11
SNB-75	52.35
U251	9.3
Melanoma	
MALME-3M	21.35
M14	11.19
MDA-MB-435	19.29
SK-MEL-2	NI
SK-MEL-28	5.37
SK-MEL-5	10.94
UACC-257	15.88
UACC-62	12.34
Ovarian Cancer	
IGROV1	NI
OVCAR-3	NI
OVCAR-4	14.55
OVCAR-5	NI
OVCAR-8	4.39
NCI/ADR-RES	86.69
SK-OV-3	NI
Renal Cancer	
786-0	NI
A498	NI
ACHN	7.14
CAKI-1	0.21
RXF 393	2.76
SN12C	22.14
TK-10	NI
UO-31	8.9
Prostate Cancer	
PC-3	8.63
DU-145	18.37
Breast Cancer	
MCF7	31.29
MDA-MB-231/ATCC	14.62
BT-549	NI
T-47D	20.7
MDA-MB-468	78.21
Mean	87.63
Delta	46.25
Range	78.78

***** The bold red and blue figures indicate compound strong (GI% >70%), and moderate (GI% 70%-30%) anticancer activity, respectively, NI, no inhibition.

3.6. Scanning Electron Microscopic (SEM) characterization of CMC-4 and CS-4 blends:

Blend samples were micro-graphed by SEM to determine the average particle size of **4** within the blend which could be calculated after image processing with ImageJ Software. The data derived from the manual image analysis was summarized in Table 3. The average particle diameter could be calculated from the average particle size in Table 5 according to Equation 3. Figure 8 shows the SEM micrographs of the CS-4 and CMC-4 blends where the surface of samples seems to be smooth except some random dissimilarities known to be present in most hydrogels due to swelling/drying processes.

$$\text{Particle Diameter } (\mu m) = 2[(21*\text{Average Size}/88)]^{1/3} \quad \text{Eq. 3}$$

Table 5: Average particle size calculated by SEM images analysis by ImageJ software.

Sample	Average Size, (nm)	Particles Diameter, (nm)
CS-4	19.584	1.67
CMC-4	12.744	1.45

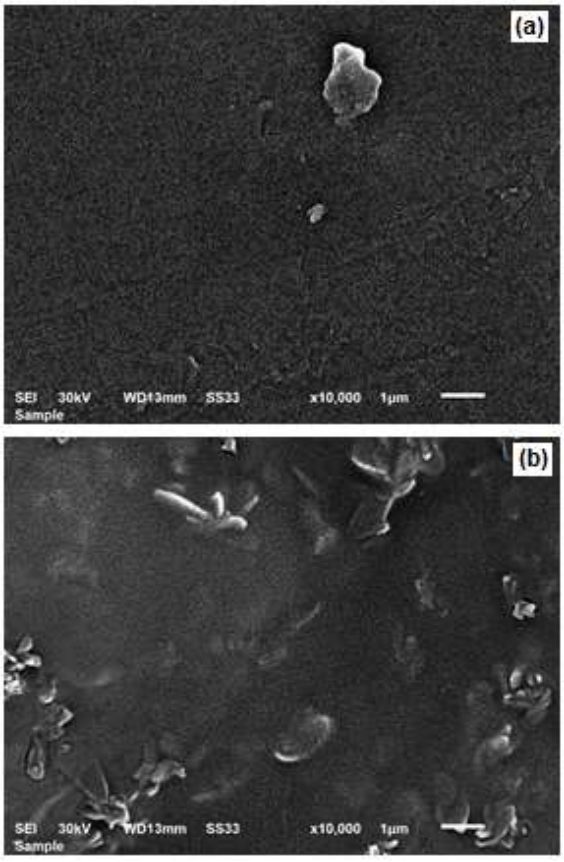


Figure 8: SEM micrograph of a) **CS-4** blend and b) **CMC-4** blend in x10000 magnification

3.7. Release of 4 from CMC-4 and CS-4 blends:

The release of **4** from **CMC-4** and **CS-4** blends in an aqueous medium at room temperature was investigated. Figure 9 shows the release behavior of **4** from both **CMC-4** and **CS-4** blends. The released Wt.% of **4** starting with increasing trend until reaching its peak time at about 6 h. After that the released Wt. % of **4** was almost constant with a maximum of about 95-97%. This may be attributed to the high ability of both matrices to swell good in aqueous medium. It means that the hydration force within the matrix give the opportunity to be released easily from the matrix. Although the release behavior is almost similar for **CMC-4** and **CS-4** blends, the release process is relatively slow in the case of **CMC-4** in comparison with that for **CS-4**. It may be attributed to the nature of **CMC-4** blend matrix where crosslinks are already involved by the effect of citric acid used in the preparation of the blend.

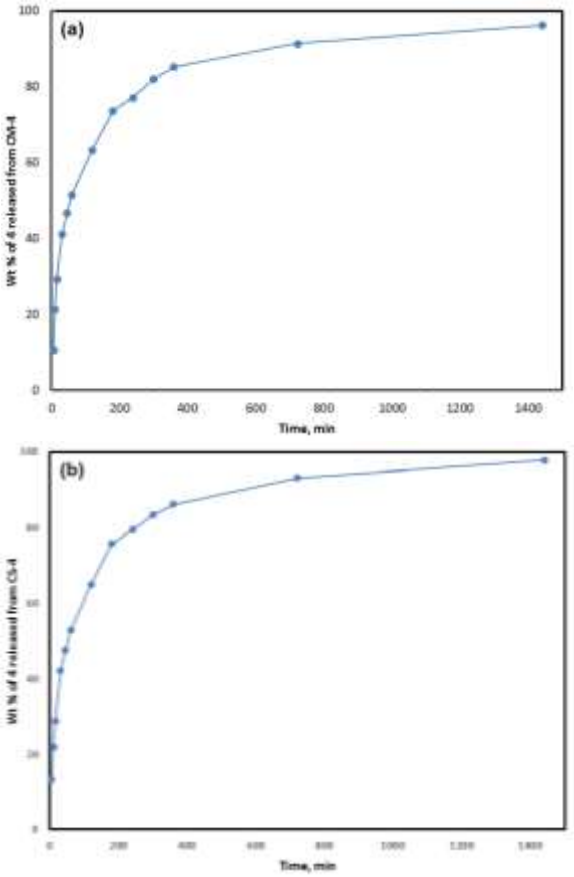


Figure 9: Time dependence of Wt. % of the released **4** from **(a) CMC-4** and **(b) CS-4** blends in distilled water at room temperature

4. Conclusion

It can be concluded that aryl azides can be prepared easily from arylamines and converted into triazole derivatives which is easily converted into triazole chalcone hybrid material. Such hybrid materials showed a promising anticancer activity against several types of cancer cells. Triazole chalcone hybrid tends to be released easily from hydrogel matrix which can be controlled through the change of the main features of the polymer matrix such as crosslinking density and hydrophilic/hydrophobic nature.

5. Data Availability:

All data generated or analyzed during this study are included in this published article.

6. Conflict of interest

The authors declared there is no conflict of interest

7. Funding

Authors receive no funding

8. Contribution of authors

A.A. Mohamed: Literature survey, performing the experiments, collecting results, writing the manuscript draft, and submitting to the Journal.

D.M. Ayad: Raising the idea of the research point, following up on the experiments, and discussing the results.

H. Al-Subbagh: Raising the idea of the research point, following up on the experiments, collecting data, discussing the results, writing the draft, and revising the final manuscript.

M.Y. Abdelaal: Raising the idea of the research point, following up on the experiments, discussing the results, revising the manuscript, and following up on the publishing process.

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