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In Sight into Innovative Cancer Therapeutic Approach Based on Nanotechnology: Chitosan/Polyvinyl alcohol Films loaded with Synthesized Bisthiazole Derivative for Cancer Treatment

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Abstract

Acetyl thiazole thiosemicarbazone **3** was used as key intermediate for preparation of novel series of thiazolylhydrazono thiazole derivatives via reaction of **3** with two types of hydrazonoyl halides. Also, compound **3** reacted with α-haloketones and α-haloester in ethanol or in acetic acid containing anhydrous sodium acetate under reflux to give another new thiazole based compounds. Addionally, the key intermediate **3** was utilized for synthesis of functionalized thiazolehydrazonothiazolones *via* its reaction with a variety of compounds containing activated double or triple bonds vis maleic anhydride and dimethyl acetylene dicarboxylate. All spectroscopic techniques (IR,[1] HNMR and C^{13}NMR, Mass Spectrometry) and elemental analysis were used for confirmation of all the newly synthesized compounds. Moreover, the synthetic mechanism of most of the newly developed compounds was discussed. The as-prepared compounds were screened for their in vitro antiproliferative potential towards different human cell lines, including: colon (**LoVo**), and liver (**HEPG2**) and breast (**MCF7**) cancer cell lines. Results revealed that compounds **1, 6a, 6b** and **6e** were the most potent against tested cancer cell lines. Moreover, compound **6e** 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 and genotoxic investigation compared to Doxorubicin. colon (**LoVo**) cells were treated with various concentrations from the selected compound **6e** loaded in CS/PVA drug delivery in comparison to doxorubicin to evaluate its anticancer activity and then analyzed via comet assay to study genotoxicity.

Keywords: Thiazoles; bisthiazoles, thiosemicarbazones, hydrazonoyl halides; drug delivery; anticancer activity

Introduction

Cancer is a major leading cause of death globally, accounting for an estimated 10 million deaths in 2020 [1]. However, the usually used medicines for treatment of cancer are now not sufficient. So, investigation and development of novel active agents with promising therapeutic is the main topics that concerned recently [2]. Moreover, drug delivery systems were used to improve the efficiency of currently and newly synthesized anticancer drug with the hope of minimize their serious side effects. Genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, the alteration can have direct or indirect effects on the DNA, however, cells prevent expression of the genotoxic mutation by either DNA repair or apoptosis. Recently, regulatory authorities all over the world have require
data on the genotoxic potential of new drugs, as part of the safety evaluation process. The pre-clinical studies are generally conducted to obtain the basic toxicological profile of new chemical entities (NCE). Genotoxicity assays have become an integral component of regulatory requirement[3–11]. The Single-cell gel electrophoresis assay (comet assay) is a rapid sensitive method for detecting genotoxic and

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geno-protective effects of both chemical and nonchemical compounds and is accepted as a valid technique for determining DNA damage in individual cells [12–16].

Thiazoles tethered by heterocyclic compounds have a prominent role in medicinal chemistry due to their wide range of activities in the field of drug design and discovery. Literature reports indicate that thiazoles have a broad spectrum of biological activities like antibacterial, antifungal, antiprotozoal, antiviral, anticancer, anti-inflammatory, antioxidant, analgesic, anticonvulsant, antidiabetic, and antihypertensive activities [17–24].

Pyrazolylthiazoles have revealed significant *in vitro* antiproliferative activity against MCF-7 [25–29]. Also, *N*-pyridinyl-2-(6-phenylimidazo[2,1-*b*]thiazol-3-yl)acetamides have demonstrated inhibitory activity against VEGFR2 kinase [30]. Moreover, 5-benzylidene- 2,4-thiazolidine diones have been evaluated as VEGFR-2 kinase inhibitors and revealed anti-angiogenesis activity [31]. Recently, the utility of thiazolylhyrazono-thiazoles has been endorsed as potential anticancer drugs [29,32,33]. In addition, azolylthiazoles have been handled in several clinically available anticancer drugs, such as ixabepilone, dabrafenib [3,34,35] and dasatinib [36] (Figure 1). Otherwise, conjugated hydrazone system has been widespread employed in pharmacological research as antitumor[37] agents (Figure 1).

Thiosemicarbazones are a huge heterocyclic compounds group, with promising medical and pharmaceutical applications against parasites and pathogenic microbes [38–40]. They are considered also as one of the most interesting efficient antitumor agents. The usage of the current cancer medicines decreases the body immunity which makes the human body not ready to threat any microbial infections.

The main objective of the current work is to estimate the cytotoxicity and in vitro anticancer activity of a series of novel dithiazole derivatives. The antiproleferative potency of these compounds is assessed towards human liver (HEGP2), colon (LoVo) and breast (MCF7) cancer cell lines as compared to doxorubicin as a standard anticancer drug. Furthermore, the most potent compound (6e) 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 and genotoxic investigation compared to doxorubicin. Colon (LoVo) cells were treated with various concentrations from the selected compound (6e) loaded in CS/PVA drug delivery in comparison to doxorubicin to evaluate its anticancer activity and then analysed via comet assay in liver tissue to study genotoxicity.

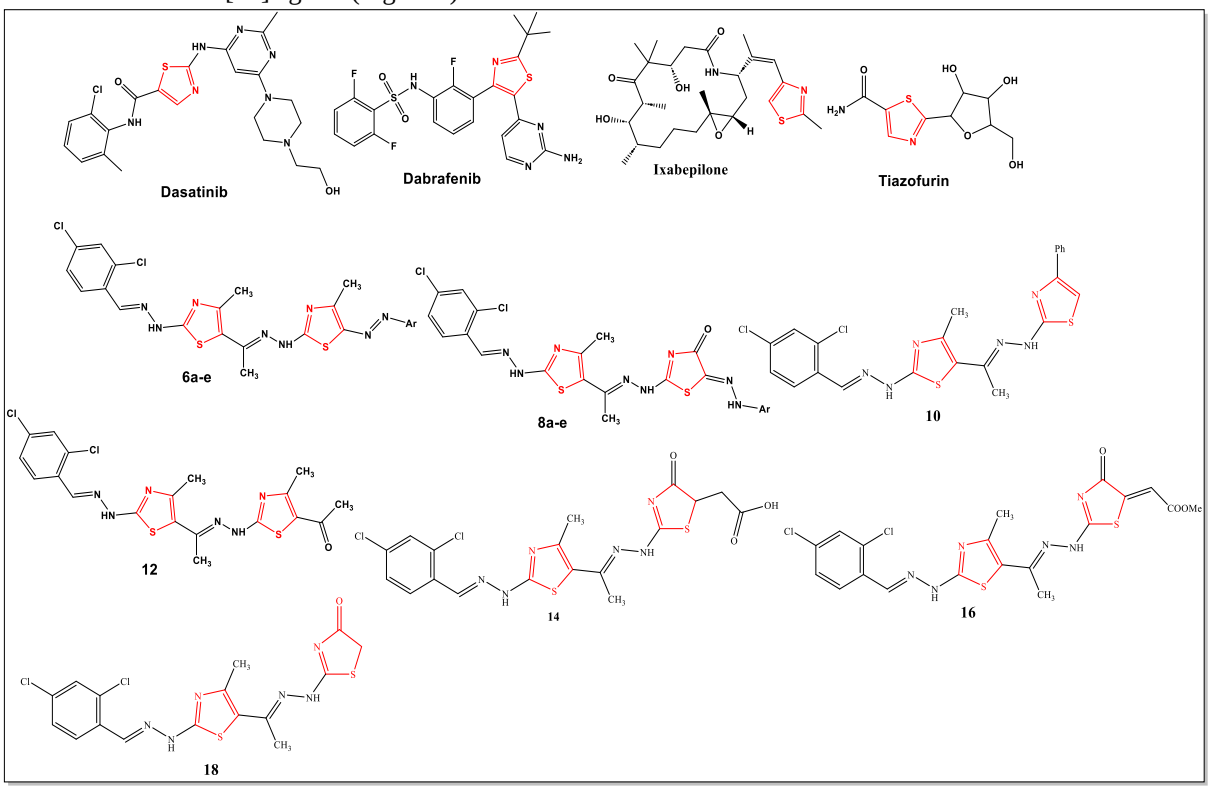


Figure 1. thiazole derivatives as anticancer drugs, as well as targeted compounds for cancer treatment

2. Results and Discussion

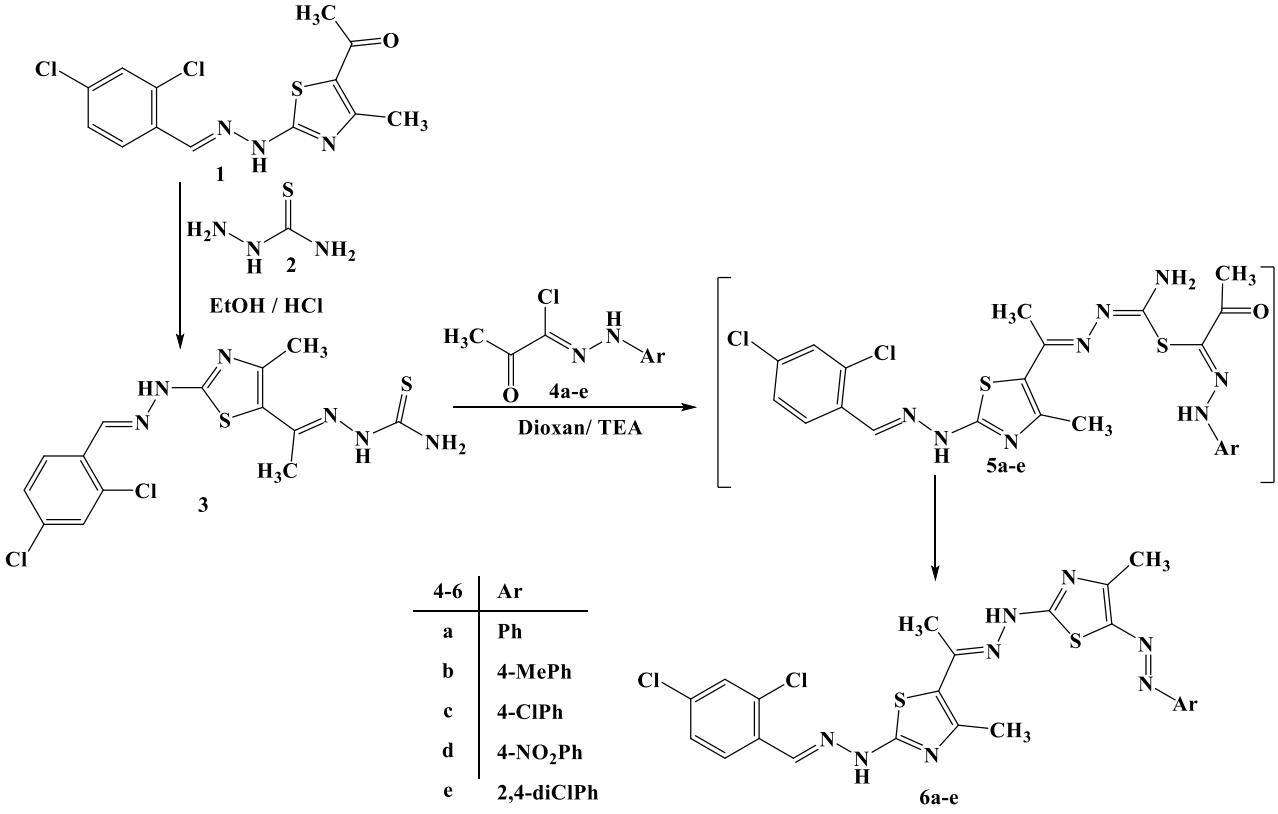
2.1. Chemistry

Thiazoles are biologically and pharmaceutically very active compounds[41,42,51,43–50], this finding prompted us to synthesize some novel compounds that contain two thiazole rings linked via hydrazone group hoping to increase their biological activity. To achieve this objective, we prepared the new thiazole thiosemicarbazone **3** by reaction of acetylthiazole **1** with thiosemicarbazide **2** in ethanol at reflux in the presence of few drops of HCl for (2-4 h) monitored by TLC (**Scheme 1**). The chemical structure of compound **3** was evidenced by elemental analysis and spectral (IR, ^{1}HNMR, Mass) data. The IR revealed the presence of an absorption band at ν 3434-3152 cm^{-1} for the 2NH and NH_2 group, in addition to the other absorption bands at ν 3019, 2920 cm^{-1} that assigned for the aromatic and aliphatic C-H stretching. ^{1}HNMR of compound **3**, showed two singlet signals at δ 2.36 and 2,41 ppm at the two methyl groups, a singlet at δ 8.41 for the CH =N proton, four singlet signals at δ 8.18, 10.31, 11.65 and 12.23 ppm assigned for the NH_2 and 2NH protons, in addition to the signals of the aromatic protons (see experimental). In addition, the mass spectrum of **3** showed a molecular ion peak at 400 which is consistent with the molecular weight of **3** (molecular formula $C_{14}H_{14}Cl_2N_6S_2$).

As compound **3** contained the carbothioamide group, it can be utilized as a building block for construction of another thiazole ring. Thus, reaction of thiazole thiosemicarbazone derivative **3** with N-aryl 2-OxO propane hydrazonyl chloride **4** in dioxane under reflux in the presence of triethylamine for 4-6 h, led to formation of products **6** (**Scheme 1**).

The latter products were formed by elimination of one molecule of water from the non-isolable intermediate **5**. The mechanism of formation of products **6** that outlined in **Scheme 1** was supported by previously reported research work from our laboratory and others[52].

The structure assigned for products 6 was confirmed based on elemental analysis and spectral data (IR, ^{1}HNMR, MS). For example, the IR spectra showed in each case two absorption bands at ν 3422, 3173 attributed to the NH's of the two hydrazone groups; in addition to the bands of the aromatic and aliphatic C-H stretching and C=C and C=N groups (see Experimental). ^{1}HNMR of products **6** revealed three singlet signals at ν 2.42, 2.51, 2.62 assigned for the three methyl groups, singlet signal at δ 8.34 for CH=N and two singlets at δ 10.56 and 10.78 ppm for the 2NH protons of hydrazone groups. The mass spectra revealed in each case a molecular ion peak which agreed with the expected structure.



4-6	Ar
a	Ph
b	4-MePh
c	4-ClPh
d	4-NO_2Ph
e	2,4-diClPh

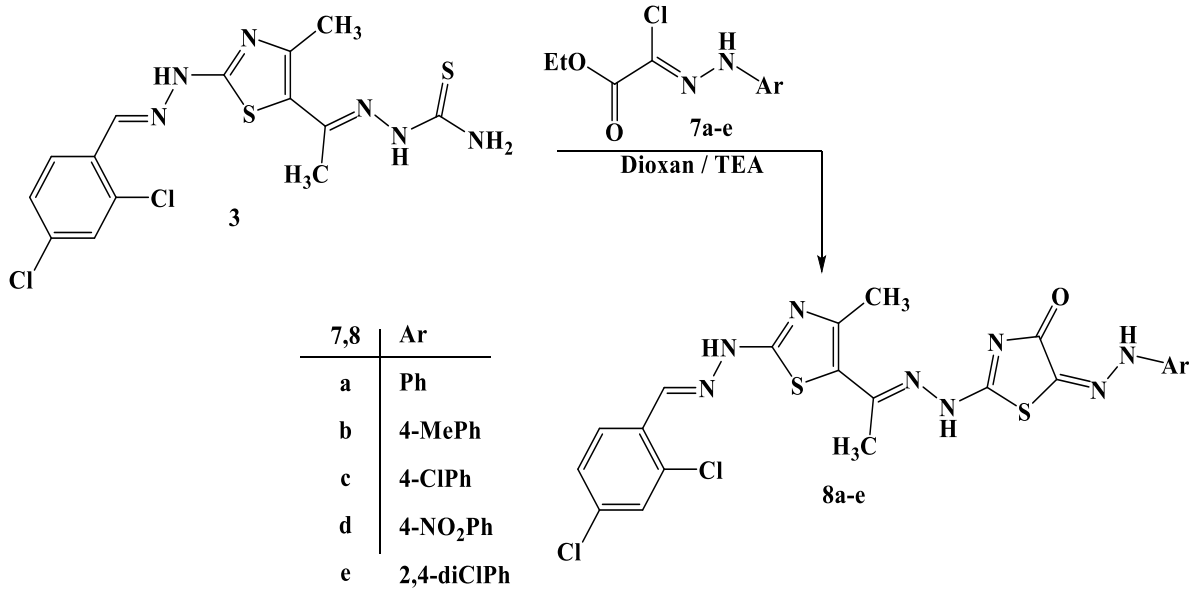
Scheme 1. Synthesis of thiazoles **6a-e**

Similarly, the reaction of thiazolethiosemicarbazone **3** with ethylarylhydrazonochloro acetate **7** gave the final products **8** (**Scheme 2**).

The structure of compounds **8** which contain thiazolone ring in addition to the thiazole ring was established by elemental analysis and spectral (IR, ^{1}HNMR and Mass) data. For example, the IR of compounds **8a** showed absorption bands at ν 3431-3181 cm^{-1} corresponding to the NH say hydrazone groups and a characteristic band at ν 1685 cm^{-1} attributed to the C=O group. The ^{1}HNMR spectrum of **8a** displayed two singlet signals at δ 2.42 and 2.51 ppm assigned for the two methyl groups, one singlet signal at δ 8.39 for CH=N proton, three singlet signals at δ 8.69, 10.53 and 10.82 ppm corresponding to the three NH protons, in addition to the signals of the aromatic protons. The mass spectrum of **8a** showed a molecular ion peak at m/z = 544.

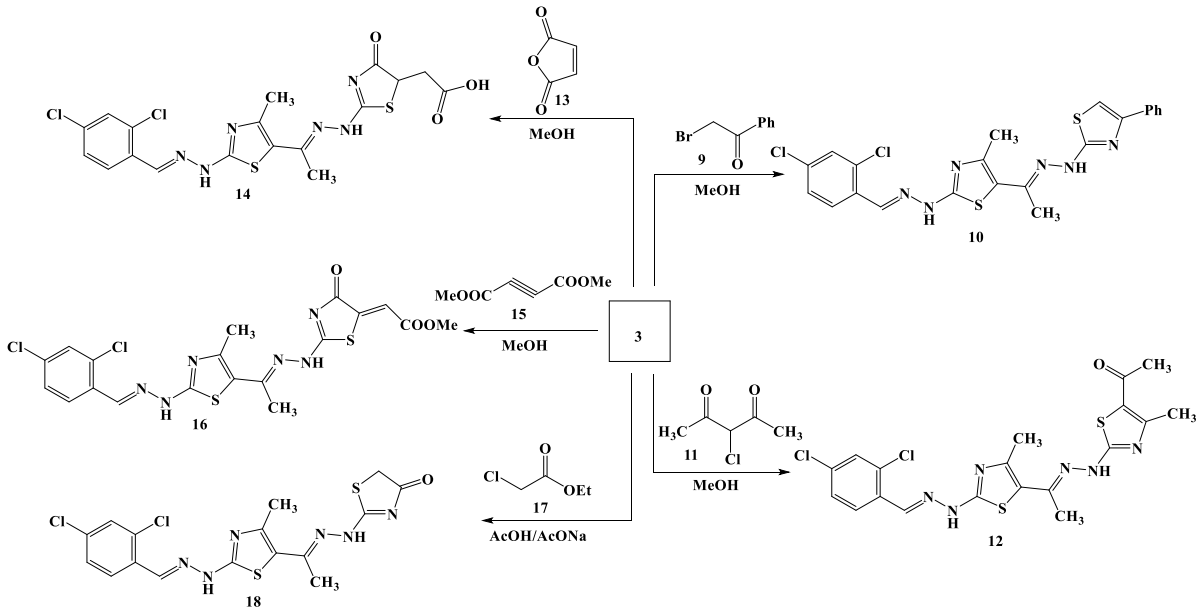
Our study was extended to prepare another thiazolylthiazole compounds by reaction of thiazole thiosemicarbazone 3 with α-haloketones and α-haloesters. For example, reaction of compound **3** with 2-bromo 1-phenylethane **9** in ethanol at reflux for 2-4 h led to formation of product **10** (**scheme 3**). By the same way, the reaction of compound 3 with 3-chloro-2,4-pentanedione **11** gave product **12** (**Scheme 3**) whose structure containing acetylthiazole ring in addition to the thiazole ring. The confirmation of structures **10** and **12** were based on IR, ^{1}HNMR and Mass spectral data (See Experimental).

Treatment of compound **3** with ethyl chloroacetate **13** in glacial acetic acid under reflux containing anhydrazone sodium acetate afforded a product whose spectra (IR, ^{1}HNMR and mass spectrometry) together with elemental analysis data are consistent with Structure **14** (**Scheme 3**) (see Experimental). Moreover, the chemical reaction of compound **3** towards unsaturated compounds was studied. Thus, reaction of thiazole thiosemicarbazone **3** with maleic anhydride **15** in dry methanol yielded a product whose spectra (IR, ^{1}HNMR and MS) and elemental analysis data are in agreement with the proposed structure **16** (**Scheme 3**). In addition, reaction of compound **3** with dimethyl acetylene dicarboxylate **17** in dry methanol at reflux for 2-4 h (**Scheme 3**) afforded product **18**. The structure of **18** was evidenced by elemental analysis and spectral (IR, ^{1}HNMR and Mass spectroscopy) data (see Experimental). The reaction of formation of product **18** was proposed to proceed *via* Michael addition of the -SH group of thiosemicarbazone derivative **3** to the triple bond of **17** followed by direct elimination of one molecule of water to give the final cyclized product (**Scheme 3**).



7,8	Ar
a	Ph
b	4-MePh
c	4-ClPh
d	4-NO_2Ph
e	2,4-diClPh

Scheme 2. Synthesis of thiazoles **8a-e**



Scheme 3. Synthesis of thiazole derivatives **10, 12, 14, 16** and **18**

2.2. Antiproliferative activity of the tested compounds

The antiproliferative activity of the new compounds toward different selected cell lines named "human colon (**LoVo**), and liver (**HEPG2**) and breast (**MCF7**) cancer cell lines" was performed by SRB assay, in comparison with doxorubicin as standard drug and the results obtained were expressed by IC_{50} as mentioned in **Table 1.**

Evaluation of the antitumor effect of the tested compounds towards human breast (**MCF7**) and human liver (**HEPG2**) cancer cell lines revealed that most of the tested compounds showed nonsignificant to weak potency. On the other hand, the antitumor effect of the tested compounds showed variable antiproliferative activity towards colon cancer cell line (LoVo) as compounds **8d**, **12**, **14**, **16** and **18** had no effect on this cell line, compounds **6c**, **6d, 10, 8a, 8b, 8c and 8e** showed weak to moderate potency, while compounds **6a, 6c** and **6b and 1** were found to be the most potent derivatives towards colon cancer cell line (LoVo) compared to doxorubicin the standard anticancer drug, with IC_{50} **values 0.041, 0.067, 0.071, and 0.076 µmol/l** versus **0.008µmol/l** for doxorubicin. Moreover, compound **6e** showed the strongest activity towards (LoVo) cell line with IC_{50} value **0.016 µmol/l versus 0.008 µmol/l** for doxorubicin. So, **6e** was selected to be loaded on polymeric carrier prepared from chitosan and polyvinyl alcohol CS/PVA to be used as a drug delivery system.

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

Compound **6e** was selected for loading on a polymeric carrier CS/PVA to be used as a drug delivery system. The average diameter of the as prepared chitosan (CS) / polyvinyl alcohol (PVA) film particles with and without **6e** loading was further evaluated using DLS. The samples were well dispersed in water and held in the instrument for about 16 run. The obtained values are the average of 16 run (**Figure 2 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 91.06 nm when these film particles were loaded with the drug like compound **6e**.

Additionally, the particle size and surface morphology of the developed chitosan (CS) / polyvinyl alcohol (PVA) film particles (**Figure 3 a-c**), and drug like compound **6e** loaded chitosan (CS) / polyvinyl alcohol (PVA) film particles (**Figure 4 a-c**) were further examined using TEM. As observed from TEM (**Figure 3 a-c**) 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 depicted that these particles were formed with cavities. These cavities are available to be encapsulated with any of the model drugs or other organic compounds. Additionally, and by checking the particle feature of chitosan (CS) / polyvinyl alcohol (PVA) film particles loaded with **6e** (**Figure 4a-c**), it was depicted that the cavities were formed with black color which suggested that they are filled with the drug (**6e**) signifying that, **6e** was successfully encapsulated inside the cavities of the chitosan (CS) / polyvinyl alcohol (PVA) film particles.

On the other hand, the morphology of the developed chitosan (CS) / polyvinyl alcohol (PVA)

film loaded with **6e** 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 **6e** is homogeneous and had a good distribution of all ingredients, also smooth with no evidence of aggregations (**Figure 5**)

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

Compound ID	Colon (LoVo) IC50 [µg/ml]	Colon (LoVo) IC50 µmol/l	liver (HEPG2) IC50 [µg/ml]	liver (HEPG2) IC50 µmol/l	Breast (MCF7) IC50 [µg/ml]	Breast (MCF7) IC50 µmol/l
1	24.3 ± 3.02	0.076	82.5 ± 14.6	0.250	76.3 ± 7.1	0.232
3	76.3 ± 7.1	0.19	92.2 ± 4.6	0.230	N.A.	N.A.
6a	21.9 ± 2.5	0.041	61.7 ± 9.5	0.112	85.3 ± 6.2	0.156
6b	40.1 ± 8.2	0.071	86.3 ± 9.1	0.154	76.3 ± 7.1	0.136
6c	38.7 ± 5.2	0.067	71.4 ± 6.3	0.123	93.7 ± 5.7	0.161
6d	85.3 ± 6.	0.144	41.5 ± 4.3	0.069	N.A.	N.A.
6e	10.3 ± 0.8	0.016	50.3 ± 5.1	0.082	59.14 ± 6.3	0.096
8a	71.4 ± 6.3	0.130	N.A.	N.A.	N.A.	N.A.
8b	60.3 ± 5.1	0.102	71.4 ± 13.2	0.127	87.4 ± 11.3	0.155
8c	67.5 ± 8.03	0.115	86.3 ± 9.1	0.148	N.A.	N.A.
8d	N.A.	N.A.	93.7 ± 5.7	0.159	N.A.	N.A.
8e	41.5 ± 4.3	0.067	79.5 ± 14.3	0.131	85.3 ± 6.2	0.076
10	71.4 ± 6.3	0.142	82.2 ± 11.6	0.164	96.3 ± 13.1	0.076
12	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
14	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
16	N.A.	N.A.	86.3 ± 9.1	0.168	N.A.	N.A.
18	N.A.	N.A.	93.7 ± 5.7	0.213	N.A.	N.A.
DOX	4.8 ± 0.6	0.008	2.7 ± 0.06	0.005	5.03 ± 0.7	0.008
DMSO	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Data were expressed as Mean ± SD of three independent experiments.
IC50 (µg/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

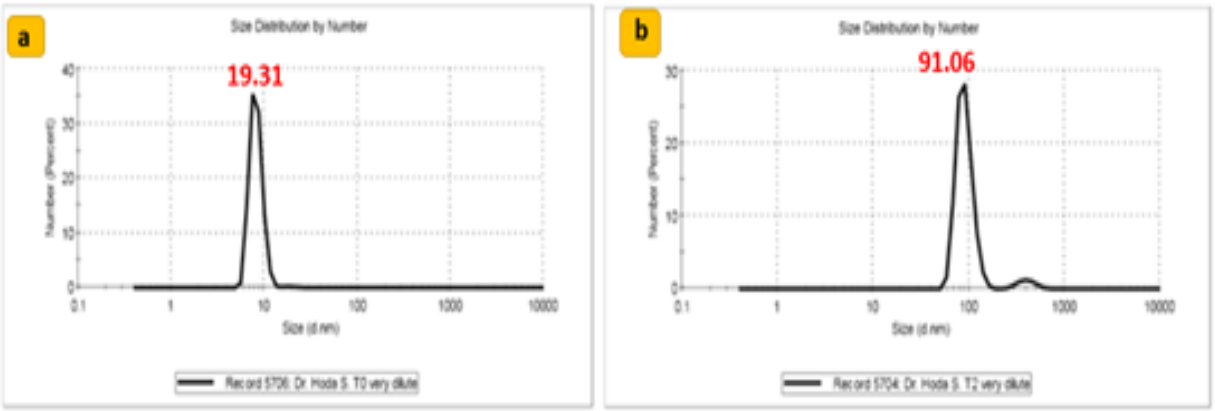


Figure 2: particle size of the chitosan (CS) / polyvinyl alcohol (PVA) film particles (a) without the drug like compound **6e** and (b) with the drug like compound **6e**.

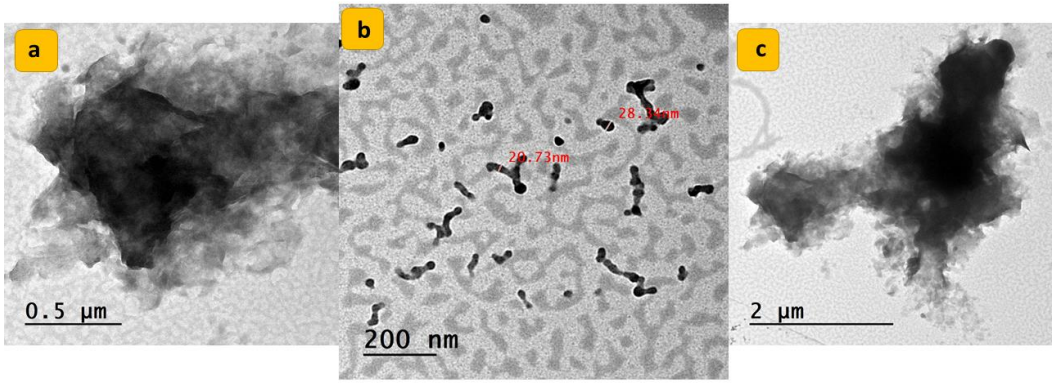


Figure 3 (a-c): TEM images of the chitosan (CS) / polyvinyl alcohol (PVA) film particles without **6e** at different magnifications

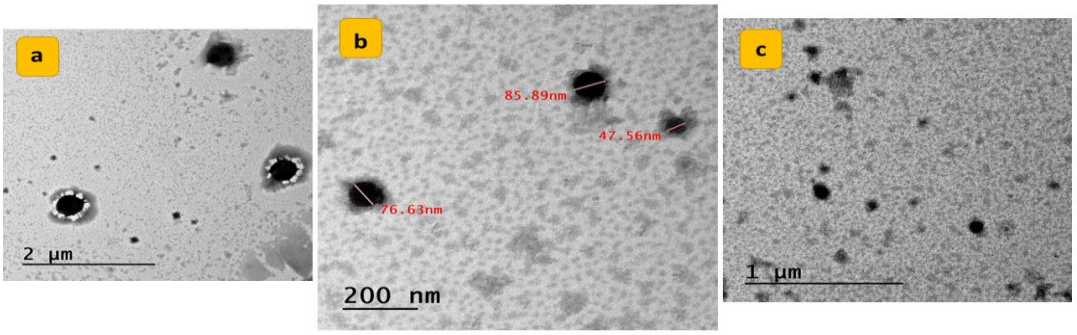


Figure 4 (a-c): TEM images of the chitosan (CS) / polyvinyl alcohol (PVA) film particles with **6e** loading at different magnifications

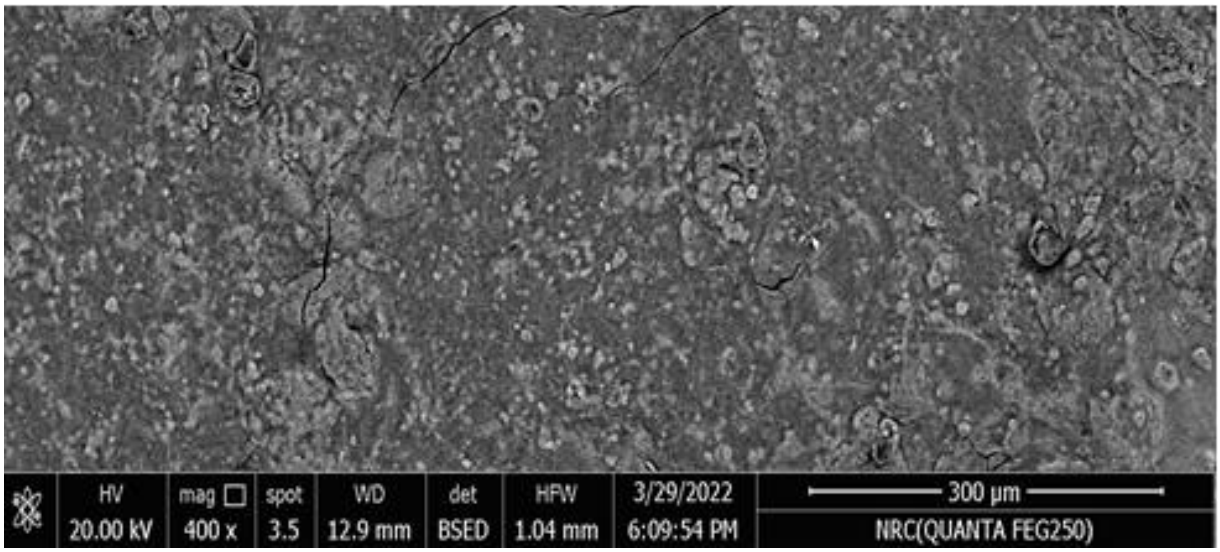


Figure 5: SEM micrographs of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **6e**.

2.4. In vitro cytotoxic evaluation of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with 6e

The antiproleferative potency of the selected compound (**6e**) loaded in CS/PVA drug delivery system was evaluated towards human colon cancer cell line (LoVo) according to the method described before. Results revealed that this loaded compound (**6e**) indicated a better strong potency towards (**LoVo**) cell line in this drug delivery form than in its drug like compound with IC50 value 8.4 ± 0.7 µg/ml versus 4.8 ± 0.6 µg/ml for doxorubicin.

2.5. Genotoxic activities of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with 6e in comparison to doxorubicin: The effect of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with 6e, on the rate of DNA damage in lymphocyte cells using comet assay

The genotoxic effect of the developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **6e** in comparison to doxorubicin was determined in human lymphocyte cells using the comet assay.

The data obtained in **table 2** revealed that doxorubicin intoxication produced a significant elevation in tail moment reaching 15 folds of the control group. On the other hand, treatment with chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **6e** showed a significantly reduced tail moment as compared to doxorubicin -intoxicated group reaching only 8 folds of the control group. It is obvious that the chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **6e** produced the most marked reduction in tail moment as compared to doxorubicin intoxicated group, which may indicate fewer side effects than resulted from the treatment with doxorubicin.

Figure 6: **a** and **b**, showed examples of visual score for DNA damage as classes: 0, 1, 2 and 3 using comet assay in lymphocyte cells.

Table 2: Effect of developed chitosan (CS) / polyvinyl alcohol (PVA) film loaded with **6e** on the rate of DNA damage in lymphocyte cells using comet assay in comparison to doxorubicin

Treatment Groups of cells	No. of cells*		Class¥ of comet				DNA damaged cells (mean ± SEM)
	Analyzed	Total comets	0	1	2	3	
Control	500	12	488	8	4	0	2.4 ± 0.08
Doxorubicin	500	156	344	36	49	71	31.2 ± 0.63
6e on CS/PVA	500	87	413	37	31	19	17.4 ± 0.2

¥: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.
(*): No of cells analyzed were 100 per treatment

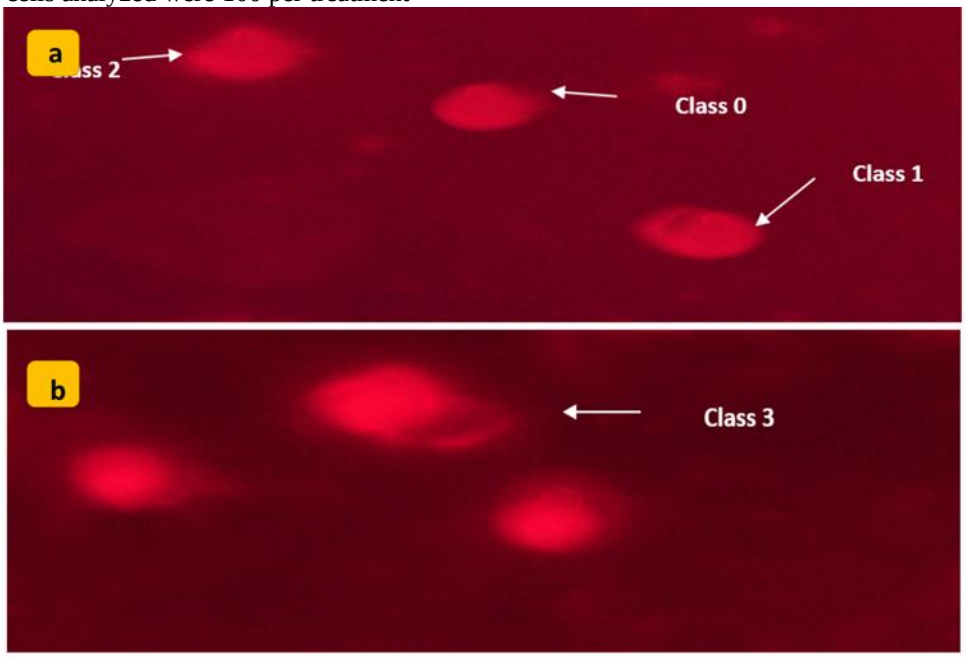


Figure 6: Visual score of DNA damage using comet assay in lymphocyte cells
A: classes 0, 1 and 2. B: class 3.

3. Experimental

3.1. Chemistry

3.1.1. Materials

Poly vinyl alcohol granules (PVA, Mw = 19,800–24,200)
with 88% alcoholysis degree were purchased from Shanghai Yingjia Industrial Development Co., Ltd. Chitosan (CS; Its degree of deacetylation is greater than >75%), acetic acid and dimethyl sulphoxide (DMSO) were provided from Aldrich company, Germany.

3.1.2. Experimental instrumentation

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 a BRUKER NMR spectrometer operating at 400 MHz (^{1}H-NMR) and run in deuterated dimethylsulfoxide (DMSO-*d6*). Chemical shifts were related to that of the solvent. ^{13}C-NMR was recorded on a BRUKER spectrometer at 100 MHz. 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.

3.1.1. Synthesis

1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethan-1-one (1). A mixture of 2-(2,4-dichlorobenzylidene)hydrazine-1-carbothioamide (2.48 g, 10 mmol) and 3-chloropentane-2,4-dione (1.34 g, 10 mmol) in 50 mL of ethanol was refluxed for 3h and then was cooled to room temperature. The desired product precipitated from reaction mixture was filtered, washed with ethanol and recrystallized from ethanol to give pure product of compound **1** as Yellow solid (82% yield); m.p. 279-281°C (m.p. 240-242);IR (KBr) ν 3437 (NH), 3056, 2931 (C-H), 1689 (C=O), 1612 (C=N), 1588 (C=C Ar), 735 (C-S-C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.50-7.53 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.94-7.96 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.41(s, 1H, CH=N), 11.63 (s, br, 1H, NH) ppm; MS, *m/z* (%) 327 (M$^+$, 39), 248 (100), 329 (78), 73 (68), 64 (70). Anal. calcd for $C_{14}H_{11}Cl_2N_3OS$ (327): C, 47.57; H, 3.38; N, 12.80. Found: C, 47.36; H, 3.25; N, 12.69%.

2-(1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazine-1-carbothioamide (3)

A mixture of acetylthiazole derivative **1** (3.27 g, 10 mmol) and thiosemicarbazide **2** (0.91 g, 10 mmol) in 20 mL EtOH and HCl (2 drops) for 2–4 h (monitored by TLC). The formed solid product was filtered and crystallized from ethanol to afford thiosemicarbazone derivative **3** as yellow solid, 80% yield, m.p. 180-182°C; IR (KBr) ν = 3434–3152 (2NH + NH_2), 3019, 2920 (C-H), 1614 (C=N), 1588 (C=C Ar) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.53-7.55 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.94-7.96 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.18 (s, br, 1H, NH), 8.41(s, 1H, CH=N), 10.31 (s, br, 1H, NH), 11.65 (s, br, 1H, NH), 12.23 (s, br, 1H, NH) ppm; MS m/z (%): 402(M$^+$+2, 27), 400 (M$^+$, 30), Anal. Calcd for $C_{14}H_{14}Cl_2N_6S_2$ (400.01): C, 41.90; H, 3.52; N, 20.94. Found: C, 41.75; H, 3.39; N, 20.88%.

General method for synthesis of thiazoles 6a-e and 8a-e.

A mixture of thiosemicarbazone **3** (0.4 g, 1 mmol) and the appropriate hydrazonoyl halides **4** or **7** (1 mmol) in dioxane (20 mL) containing TEA (0.07 mL) was refluxed for 4-6 hr (monitored by TLC). The hot solution was allowed to cool and the solid formed was filtered off, washed with EtOH, dried and recrystallized from the proper solvent to give the corresponding thiazoles **6a-e** and **8a-e**, respectively. The physical constants and analytical information for the synthesized products **6a-e** and **8a-e** are listed below.

2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methyl-5-(1-(2-(4-methyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazineylidene)ethyl)thiazole (6a). Yellow solid; mp:200-202 °C (EtOH); IR (KBr) ν = 3422, 3173 (2 NH), 3048, 2916 (C-H), 1593 (C=N), 1558 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.35-7.38 (m, 5H, Ar-H),7.50-7.52 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.94-7.96 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.34(s, 1H, CH=N), 10.56 (s, br, 1H, NH), 10.78 (s, br, 1H, NH) ppm; MS, *m/z* (%) 544(M$^+$+2, 8)542 (M$^+$, 15). Anal. calcd for $C_{23}H_{20}Cl_2N_8S_2$ (542.06): C, 50.83; H, 3.71; N, 20.62. Found: C, 50.71; H, 3.59; N, 20.48%.

2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methyl-5-(1-(2-(4-methyl-5-(p-tolyldiazenyl)thiazol-2-yl)hydrazineylidene)ethyl)thiazole (6b). Yellow solid; mp 162-164 °C (EtOH); IR (KBr) ν = 3436, 3217 (2 NH), 3042, 2916 (C-H), 1609 (C=N), 1579 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.13-7.15 (d, J = 8.2 *Hz*, 2H, Ar-H), 7.25-7.27 (d, J = 8.2 *Hz*, 2H, Ar-H), 7.56-7.58 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.94-7.96 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.40 (s, 1H, CH=N), 10.50 (s, br, 1H, NH), 10.74 (s, br, 1H, NH) ppm; ^{13}C-NMR (DMSO-d_6): δ 10.6, 16.6, 17.3, 20.8 (CH_3), 114.6, 127.0, 127.2, 128.1, 128.4, 129.8, 130.1, 130.2, 130.4, 131.0, 131.3, 133.2, 133.5, 134.9, 138.0, 141.7, 160.6, 169.6 (Ar-C and C=N) ppm; MS, *m/z* (%) 558(M$^+$+2, 14), 556 (M$^+$, 22). Anal. calcd for $C_{24}H_{22}Cl_2N_8S_2$ (556.08): C, 51.71; H, 3.98; N, 20.10. Found: C, 51.55; H, 3.78; N, 20.03%.

5-((4-Chlorophenyl)diazenyl)-2-(2-(1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-4-methylthiazole (6c). Yellow solid; mp:181-183 (AcOH); IR (KBr) ν = 3430, 3219 (2 NH), 3040, 2921 (C-H), 1594 (C=N), 1563 (C=C Ar) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.37-7.39 (d, *J* = 8.4 *Hz*, 2H, Ar-H), 7.56-7.58 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.63-7.65 (d, *J* = 8.4 *Hz*, 2H, Ar-H), 7.73 (s, 1H, Ar-H), 7.94-7.96 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.41 (s, 1H, CH=N), 10.63 (s, br, 1H, NH), 10.84 (s, br, 1H, NH) ppm; MS, *m/z* (%) 576 (M^+, 37). Anal. calcd for $C_{23}H_{19}Cl_3N_8S_2$ (576.02): C, 47.80; H, 3.31; N, 19.39. Found: C, 47.70; H, 3.25; N, 19.27%.

2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methyl-5-(1-(2-(4-methyl-5-((4-nitrophenyl)diazenyl)thiazol-2-yl)hydrazineylidene)ethyl)thiazole (6d). Yellow solid; mp 222-224°C (AcOH); IR (KBr) ν = 3436, 3252 (2 NH), 3065, 2925 (C-H), 1592 (C=N), 1555 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.37-7.39 (d, *J* = 8.5 *Hz*, 2H, Ar-H), 7.54-7.56 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.93-7.95 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.18-8.20 (d, *J* = 8.5 *Hz*, 2H, Ar-H), 8.37 (s, 1H, CH=N), 10.31 (s, br, 1H, NH), 11.08 (s, br, 1H, NH) ppm; MS, *m/z* (%) 587 (M^+), Anal. calcd for $C_{23}H_{19}Cl_2N_9O_2S_2$ (587.05): C, 46.94; H, 3.25; N, 21.42. Found: C, 46.75; H, 3.15; N, 21.22%.

2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-5-(1-(2-(5-((2,4-dichlorophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazineylidene)ethyl)-4-methylthiazole (6e). Yellowish orange solid; mp 184-186 °C (AcOH); IR (KBr) ν = 3395, 3262 (2 NH), 3053, 2911 (C-H), 1603 (C=N), 1581 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 7.44-7.46 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.53-7.55 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.93-7.95 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.00-8.02 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.34 (s, 1H, CH=N), 10.31 (s, br, 1H, NH), 12.50 (s, br, 1H, NH) ppm; MS, *m/z* (%) 609 (M^+, 32). Anal. calcd for $C_{23}H_{18}C_{l4}N_8S_2$ (609.98): C, 45.11; H, 2.96; N, 18.30. Found: C, 45.04; H, 2.83; N, 18.18%.

2-(2-(1-(2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-5-(2-phenylhydrazineylidene)thiazol-4(5H)-one (8a). Yellow solid; mp 171-173 °C (AcOH); IR (KBr) ν = 3431-3181 (3 NH), 3043, 2917 (C-H), 1685 (C=O), 1623 (C=N), 1553 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.26-7.39 (m, 5H, Ar-H),7.49-7.51 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.93-7.95 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.39 (s, 1H, CH=N), 8.69 (s, br, 1H, NH), 10.53 (s, br, 1H, NH), 10.82 (s, br, 1H, NH) ppm; MS, *m/z* (%) 544 (M^+, 51) Anal. calcd for $C_{22}H_{18}Cl_2N_8OS_2$ (544.04): C, 48.44; H, 3.33; N, 20.54. Found: C, 48.25; H, 3.20; N, 20.48%.

2-(2-(1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-5-(2-(p-tolyl)hydrazineylidene)thiazol-4(5H)-one (8b). Yellow solid; mp 202-204 °C (EtOH); IR (KBr) ν = 3419-3177 (3 NH), 3040, 2911 (C-H), 1682 (C=O), 1625 (C=N), 1543 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.16-7.18 (d, 2H, Ar-H), 7.25-7.28 (d, 2H, Ar-H), 7.51-7.53 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.94-7.96 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.40 (s, 1H, CH=N), 8.69 (s, br, 1H, NH), 10.47 (s, br, 1H, NH), 10.79 (s, br, 1H, NH) ppm; ^{13}C-NMR (DMSO-d_6): δ 14.5, 18.0, 20.8 (CH_3), 114.4, 115.0, 118.9, 128.2, 128.5, 129.0, 129.8, 130.2, 130.5, 131.1, 132.2, 133.7, 135.0, 135.2, 136.6, 140.6, 141.7 (Ar-C and C=N), 169.4 (C=O) ppm; MS, *m/z* (%) 558 (M^+, 70). Anal. calcd for $C_{23}H_{20}Cl_2N_8OS_2$ (558.06): C, 49.38; H, 3.60; N, 20.03. Found: C, 49.26; H, 3.48; N, 20.00%.

5-(2-(4-Chlorophenyl)hydrazineylidene)-2-(2-(1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)thiazol-4(5H)-one (8c). Brown solid; mp 180-182 °C (EtOH); IR (KBr) ν = 3428-3171 (3 NH), 3043, 2919 (C-H), 1691 (C=O), 1621 (C=N), 1550 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.25-7.28 (d, 2H, Ar-H), 7.34-7.37 (d, 2H, Ar-H), 7.49-7.51 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.70 (s, 1H, Ar-H), 7.93-7.95 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.39 (s, 1H, CH=N), 8.68 (s, br, 1H, NH), 10.61 (s, br, 1H, NH), 11.89 (s, br, 1H, NH) ppm; MS, *m/z* (%) 578 (M^+, 12). Anal. calcd for $C_{22}H_{17}Cl_3N_8OS_2$ (578.00): C, 45.57; H, 2.95; N, 19.32. Found: C, 45.44; H, 2.74; N, 19.17%.

2-(2-(1-(2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-5-(2-(4-nitrophenyl)hydrazineylidene)thiazol-4(5H)-one

(8d). Orange solid; mp 242-244 °C (AcOH); IR (KBr) ν = 3425-3172 (3 NH), 3060, 2922 (C-H), 1708 (C=O), 1617 (C=N), 1589 (C=C Ar) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.38-7.41 (d, 2H, Ar-H), 7.51-7.53 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.93-7.95 (d, *J* = 8.8 *Hz*, 1H, Ar-H), 8.20-8.22 (d, 2H, Ar-H), 8.39 (s, 1H, CH=N), 8.69 (s, br, 1H, NH), 11.16 (s, br, 1H, NH), 11.63 (s, br, 1H, NH) ppm; MS, *m/z* (%) 591 (M^++2, 14), 589 (M^+, 14). Anal.

calcd for $C_{22}H_{17}Cl_2N_9O_3S_2$ (589.03): C, 44.75; H, 2.90; N, 21.35. Found: C, 44.63; H, 2.77; N, 21.19%.

2-(2-(1-(2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-5-(2-(2,4-dichlorophenyl)hydrazineylidene)thiazol-4(5H)-one

(8e). Yellow solid; mp 156-158 °C (EtOH); IR (KBr) ν = 3427-3175 (3 NH), 3059, 2921 (C-H), 1704 (C=O), 1613 (C=N), 1582 (C=C Ar) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 7.45-7.51 (m, 2H, Ar-H), 7.68 (s, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.93-7.95 (d, J = 8.8 *Hz*, 2H, Ar-H), 8.38 (s, 1H, CH=N), 8.68 (s, br, 1H, NH), 10.66 (s, br, 1H, NH), 12.05 (s, br, 1H, NH) ppm; MS, *m/z* (%) 613(M^++2, 21), 611 (M^+, 24). Anal. calcd for $C_{22}H_{16}Cl_4N_8OS_2$ (611.96): C, 43.01; H, 2.63; N, 18.24. Found: C, 42.91; H, 2.52; N, 18.18%.

Synthesis of thiazoles 10, 12, 14 and 16.

To a solution of thiosemicarbazone **3** (0.4 g, 1 mmol) in dry methanol (20 mL) was added 2-bromo-1-phenylethan-1-one **9** or 3-chloropentane-2,4-dione **11** or maleic anhydride **15** or dimethyl acetylene dicarboxylate **17** or (1 mmol). The solution was refluxed for 2-4 h. The precipitate was filtered, washed with methanol, and recrystallized from the proper solvent to give thiazoles **10, 12, 14** and **16,** respectively**.** The products **10, 12, 14** and **16** together with their physical constants are listed below.

2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methyl-5-(1-(2-(4-phenylthiazol-2-yl)hydrazineylidene)ethyl)thiazole (10). Yellow solid; mp 231-233 °C (AcOH); IR (KBr) ν = 3414, 3164 (2 NH), 3044, 2912 (C-H), 1590 (C=N) cm^{-1};^1H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 7.29-7.43 (m, 6H, Ar-H and thiazole-H5),7.51-7.53 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.70 (s, 1H, Ar-H), 7.92-7.94 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.39 (s, 1H, CH=N), 10.90 (s, br, 1H, NH), 12.50 (s, br, 1H, NH) ppm; MS, *m/z* (%) 500 (M^+, 51), 498 (46). Anal.Calcd for $C_{22}H_{18}Cl_2N_6S_2$ (500.04): C, 52.70; H, 3.62; N, 16.76. Found C, 52.69; H, 3.56; N, 16.69%.

1-(2-(2-(1-(2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-4-methylthiazol-5-yl)ethan-1-one (12). Yellow solid; mp 251-253 °C (AcOH); IR (KBr) ν = 3423, 3167 (2 NH), 3046, 2911 (C-H), 1694 (C=O), 1590 (C=N), 1570 (C=C Ar) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.48-7.50 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 7.92-7.94 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.38 (s, 1H, CH=N), 10.61 (s, br, 1H, NH), 12.36 (s, br, 1H, NH) ppm; MS, *m/z* (%) 482(M^++2, 40), 480 (M^+, 45). Anal.Calcd for $C_{19}H_{18}Cl_2N_6OS_2$ (480.04): C, 47.40; H, 3.77; N, 17.46. Found C, 47.29; H, 3.71; N, 17.38%.

2-(2-(2-(1-(2-(2-(2,4-Dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-4-oxo-4,5-dihydrothiazol-5-yl)acetic acid (14). Yellow solid; mp 230-232 °C (AcOH); IR (KBr) ν = 3436, 3232, 3145 (2 NH and OH), 3033, 2961 (C-H), 1720, 1681 (2C=O), 1592 (C=N), 1573 (C=C Ar) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.85 (dd, 1H, CH_2), 2.94 (dd, 1H, CH_2), 4.57 (dd, 1H, CH), 7.47-7.49 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.91-7.93 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.40 (s, 1H, CH=N), 10.27 (s, br, 1H, NH), 11.72 (s, br, 1H, NH), 12.68 (s, br, 1H, OH) ppm; MS, *m/z* (%) 500 (M^++2, 23), 537 (M^+, 15). Anal.Calcd for $C_{18}H_{16}Cl_2N_6O_3S_2$ (498.01): C, 43.29; H, 3.23; N, 16.83. Found C, 43.17; H, 3.09; N, 16.65%.

Methyl 2-(2-(2-(1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)-4-oxothiazol-5(4H)-ylidene)acetate (16). Orange solid; mp 281-283 °C (DMF); IR (KBr) ν = 3439, 3164 (2 NH), 3041, 2943 (C-H), 1702, 1689 (2 C=O), 1590 (C=N), 1554 (C=C Ar) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.80 (s, 3H, $COOCH_3$), 6.68 (s, 1H, CH=C),7.49-7.51 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.92-7.94 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.37 (s, 1H, CH=N), 8.67 (s, br, 1H, NH), 12.72 (s, br, 1H, NH) ppm; ^{13}C-NMR (DMSO-d_6): δ 18.4, 29.9 (CH_3), 52.8 (OCH_3), 125.3, 128.2, 128.5, 128.6, 129.8, 130.0, 130.4, 130.7, 133.7, 135.2, 136.8, 138.0, 143.1, 149.0 (Ar-C and C=N), 166.2, 169.4 (2 C=O) ppm; MS, *m/z* (%) 512 (M^++2, 6), 510 (M^+, 21). Anal.Calcd for $C_{19}H_{16}Cl_2N_6O_3S_2$ (510.01): C, 44.62; H, 3.15; N, 16.43. Found C, 44.50; H, 3.11; N, 16.29%.

Synthesis of 2-(2-(1-(2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)hydrazineyl)thiazol-4(5*H*)-one (18).

To a mixture of thiosemicarbazone **3** (0.4 g, 1 mmol) in glacial acetic acid (10 mL) containing anhydrous sodium acetate (0.123g, 1.5 mmol), ethyl chloroacetate **17** (0.122 g, l mmol) was added. The mixture was refluxed for 6 hr (monitored by TLC), then left to cool. The solid product was filtered off, washed with water and recrystalized from DMF to afford the thiazolone derivative **18** as Beige solid (82% yield); mp 161-163 °C (AcOH); IR (KBr) ν = 3429, 3177 (2 NH), 3054, 2915 (C-H), 1681 (C=O), 1590 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.37 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.61 (s, 2H, CH_2), 7.48-7.50 (d, J = 8.8 *Hz*, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.93-7.95 (d, J = 8.8 *Hz*, 1H, Ar-H), 8.37 (s, 1H, CH=N), 10.72 (s, br, 1H, NH), 12.44 (s, br, 1H, NH) ppm; MS, *m/z* (%) 442(M^++2, 7), 440 (M^+,

15). Anal.Calcd for $C_{16}H_{14}Cl_2N_6OS_2$ (440.00): C, 43.54; H, 3.20; N, 19.04. Found C, 43.38; H, 3.08; N, 18.96%.

3.2. Antiproliferative activity of the tested compounds

3.2.1. Cells

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

3.2.2. Compounds preparation and anti-proliferative in vitro assay.

The tested compounds were prepared as previously mentioned and examined using SRB assay[17,39].

3.2.4. Cytotoxic test SRB

The details of this technique were described by Skehan et al[53].

3.2.5. Statistical analysis

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

3.2. Preparation of chitosan (CS) / polyvinyl alcohol (PVA) films with and without 6e loading.

The chitosan (CS) / polyvinyl alcohol (PVA) films were prepared as previously mentioned.[54,55]

3.3. Determination of percent of DNA damage by comet assay in lymphocyte cells:

The Comet assay was performed according to Blasiak et al. protocol [56–59]

4. Conclusion

In conclusion, some of the tested compounds (**1, 6a, 6b** and **6e**), exerted significant anti-proliferative potency towards colon cancer cell line (**LoVo**), while showed a moderate activity on human liver cancer cell line (**HEPG2**) through reducing cell proliferation and resulted in reasonable significant growth inhibitory effect. On the other hands, tested compounds gave no or slight activity towards breast **(MCF7)** cell line. So, the present study revealed that human colon (**LoVo**) and liver (**HEPG2**) cancer cell lines were more sensitive to the tested compounds than the breast **(MCF7)** cell line which indicated a specific anti-proliferative potency of these newly synthesized compounds toward different cancer cell lines. The results showed that the in vitro antiproliferative potency of the newly synthesized compounds was moderately significant and may be investigated for further in vivo and pharmacokinetic studies. Moreover, the genotoxicity study of the most potent compound (**6e**) after chitosan (CS) / polyvinyl alcohol (PVA) films loading showed a significantly reduced DNA damage as compared to doxorubicin, which may indicate lower side effects.

5. References

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