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Novel Sono-synthesized Triazole Derivatives Conjugated with Selenium Nanoparticles for Cancer Treatment

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Abstract

Green chemistry is among the important applied aspects to synthesize novel bioactive compounds. We aim to provide triazole derivatives using eco-friendly methods such as sonication technology. This procedure was performed by using natural catalysts such as natural citrus squeezes. Ultrasonic irradiation was employed in the presence of natural juices such as lemon or/and orange juice to synthesize triazole derivatives. The synthesized compounds were investigated using FTIR, 1H-NMR, 13C-NMR, mass spectroscopy and elemental analysis. The cytotoxicity and in vitro anticancer evaluation of the synthesized triazole derivatives and the derivatives selenium nanoparticles have been assessed towards human liver cancer cell line (HEGP2) in addition to BALB/3T3 (murine fibroblast) normal cell line. Some compounds showed an active selectivity towards the human liver cancer cell line (HEGP2). However, they have no activity for the BALB/3T3 (murine fibroblast) normal cell line. Meanwhile, after evaluating the cytotoxicity of the synthesized compounds and in vitro anticancer activities, compounds **12a**, **12b**, **13a**, **13b** were selected to contribute in synthesizing in-situ selenium nanoparticles (SeNPs). The results showed a significant in vitro antiproliferative activity of the synthesized compounds.

Keywords: Green Chemistry; Triazoles; Selenium nanoparticles; Ultrasonic Technology; liver cancer cell line (HEPG2) ; Normal cell line (BALB/3T3).

1. Introduction

Cancer is considered among the major causes of death for humans. Millions of new cancer cases and deaths are recorded annually [1]. However, medical treatment of malignant tumors is not sufficient for such purpose. Hence, developing novel agents with potent therapeutics are the key topics which are currently concerned [2–7]. Selenium nanoparticles (SeNPs) [8, 9] are considered as a standout amongst the most encouraging explorations for oncotherapy [10–23]. By the beginning of the twenty-first century, the public became much more aware of the hazardous substances that are used and produced during the chemical reactions. Consequently, the 'concept of green and sustainable chemistry' has been evolved. The main aim of this concept is to develop smooth and nonpolluting pathways. Moreover, it is important to find creative ways for reduce the use of toxic reagents, solvents, harsh reaction conditions and expensive catalysts [24–28]. The concept of "Green Chemistry" has been widely accepted to be among the basic scientific challenges of protecting human health and environment [24, 29, 30]. In order to complete these needs, chemical reactions are proceeds in solvent-free, water as a solvent, ionic liquids, bio-based chemicals and supercritical fluids as green solvents. The increasing interest to employ fruit juice in biocatalysts and in the synthesis of heterocyclic was achieved [31–38]. Recently, more attention has been attracted on targeting anticancer drugs disrupting tumor-specific cell signaling, cell

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division, energy metabolism, gene expression, drug resistance and so on. The structural modification of natural compounds for developing new antitumor drugs with an elevated selectivity and low toxicity is desired. For example, the 1,2,3-triazole derivatives either isolated from natural products or synthesized in laboratory conditions possess diverse pharmacological properties such as antibacterial, anti-tubercular anti-cancer and anti-malarial activities. These expectations force scientific communities to define or develop new cancer treatment methods [39–42]. The multicomponent reactions are essential in pharmaceutical preparations and organic conversions. Furthermore, nucleosides have various pharmacological and biological applications like antiviral [43], anticancer [44] and antimicrobial activities [45]. Selenium (Se) is an essential trace element. It is crucial for many cellular functions through incorporating selenoproteins [46]. The importance of selenium nanoparticles (SeNPs) comes from their low toxicity and bioavailability. Moreover, they are biocompatible and can interact with proteins [47, 48]. SeNPs are known as potent anticancer agents at high dosages [49]. Besides, they are employed in photoelectric and semiconductor applications [50]. They have a wide variety of applications including biology and medicine [51]. Biomedical applications of SeNPs include drug and targeted gene delivery, anticancer activity, antibacterial activities, anti-inflammatory activities and biosensors [52]. Many reports explained the synthesis of SeNPs. They are prepared by various methods comprising laser ablation, microwave-assisted, chemical reduction, electro-deposition, solvo-thermal and green synthesis. However, some strict synthetic conditions are required such as harsh chemicals, acidic pH and high temperature. The latter restricts their ability to be used in some biomedical applications [53–55]. The aim of this work is to synthesize novel heterocyclic compounds of triazole moieties using ecofriendly methods to act as selective anticancer agents. In this approach, we are focusing on employing sono-synthesized selenium nanoparticles and natural catalysts to contribute in this application

2. Materials and methods

2.1. Materials

5-Methyl-2-furaldehyde, malononitrile, 2-cyanoacetamide, ethyl 2-cyanoacetate were provided from Sigma. Solvents such as Ethanol, DMF, DMSO, triethylamine,…. etc were supplied from aldrich. All reactions were followed up by thin layer chromatography (TLC). Aluminum sheets were used recoated with UV fluorescent silica gel (Merck Kieselgel 60 F_{245}). It was visualized using UV lamp and iodine vapor. All melting points are uncorrected. They were measured by using an electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalyses were carried out at the Micro analytical center (Faculty of Science, Cairo University, Egypt). IR spectra were carried out on JASCO FT/IR 6100 Japan spectrometer (National Research Centre, Egypt) using KBr discs. ^{1}H-NMR spectra were measured in DMSO, $CDCl_3$ or $CDCl_3/CF_3COOD$ using JEOL EX-270 run for ^{1}H-NMR at 270 MHz; JEOL ECA-500 run for ^{1}H-NMR at 500 MHz and run for ^{13}C-NMR at 125 MHz spectrometer. Chemical shifts were expressed in part per million (δ *ppm*) against tetramethylsilane (TMS) as an internal standard. The coupling constant *J* is expressed in Hz. Mass spectra were recorded on GCMS Finnigan mat SSQ 7000 spectrometer. UV-Vis was recorded using (*Shimadzu spectrophotometer*). TEM was recorded by using High Resolution Transmission Electron Microscopy (HRTEM) JEOL (JEM-2100 TEM). Scanning electron microscopy (SEM) was designated to investigate the surface morphology of the prepared specimens. It was carried out by means of (SEM) (QUANTA FEG 250 ESEM). To inspect the elemental content in the investigated samples, energy dispersive X-ray spectroscopy (EDX) was utilized. The elemental analyzer (EDAX AMETEK Inc.; Mahwah, NJ, USA) was operated with 15 kV acceleration voltages. Fine chemicals were of analytical grade Selenious acid (H_2SeO_3) (Aldrich), ascorbic acid (99%, Aldrich).

2.2. Conventional method for the synthesis of compounds 4a-c

An equimolecular quantity of 3-amino-1,2,4-triazole (**1**) (0.083g, 0.001mol), 5-methyl furfuraldehyde (**2**) (0.011g, 0.001 mol) and (**3**) ethyl cyanoacetate or ethyl cyanoacetamide or malononitrile in ethanol (25 ml) was refluxed for 5 days. However, no reaction occurred after the intermediate stage. Then the reaction continued after addition of 4-5 drops of lemon juice, immediately a color changed to yellow, TLC (eluent DCM/Ethyl Acetate 5-15%) monitored progress. The reaction

mixture was kept overnight at room temperature. The resulting precipitate was filtered, washed with ethanol, dried and recrystallized from ethanol. **4a,** yield 75 %; mp 160-162 ˚C, **4b,** yield 70%; mp 168-170 ˚C, **4c**, yield 70 %; mp 163-165 ˚C. This method is followed in the synthesis of the other compounds as shown in Fig. 1.

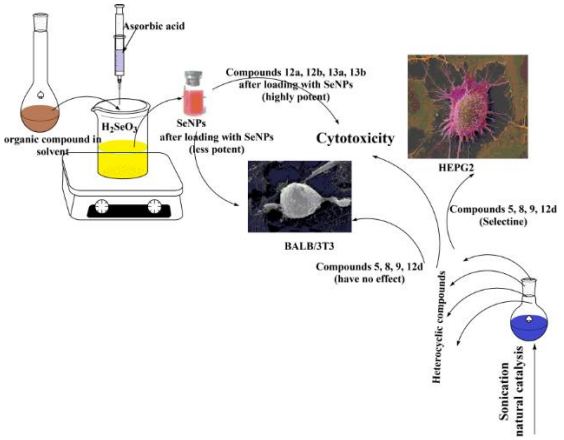

Fig. 1. A proposed route for the synthesis process of the heterocyclic compounds; loading onto SeNPs through sonication**.**

2.3. Ultrasonic radiation method for the synthesis of compounds 4a-c

An equimolecular amounts of 3-amino-1,2,4-triazole (**1**) (0.083g, 0.001mol), 5-methyl furfuraldehyde (**2**) (0.011g, 0.001 mol) and (**3**) ethyl cyanoacetate or ethyl cyanoacetamide or malononitrile, were added in a conical flask with few drops of lemon juice and water (10 ml). The mixture introduced under ultrasonic waves using an ultrasonic bath (operating at 230 V generating 33 KHz output frequencies) for 3 h; at 25 ºC. The product started to separate out during the course of reaction. The crystalline solid filtered and found pure on TLC (eluent DCM/Ethyl Acetate 5-15%) with no need of further recrystallization. **4a,** yield 77 %; mp 160-162 ˚C, **4b,** yield 73 %; mp 168-170 ˚C**, 4c,** yield 72 %; mp163-165 ˚C.

All other compounds were prepared using the same procedure as in section 2.3 and their characterizations were illustrated in supplementary data

2.4. Synthesis of in-situ Selenium Nanoparticles (SeNPs) using synthesized heterocyclic compounds 12a, 12b, 13a, 13b

Selenious acid (H_2SeO_3, 0.013 gm., 0.01 mmol.) was dissolved in deionized water (80 ml.). Compounds ***12a, 12b, 13a, 13b*** (0.01 g) were placed separately in DMSO (10 ml) then introduced to the solution of selenious acid. The mixture under continuous was maintained stirring at 60 °C for 1 h. 200 µL of 40 mM ascorbic acid was added as a catalyst. The ruby red SeNPs were suspended. The formation of selenium nanoparticles was confirmed and characterized by UV-Spectrophotometer, particle size, SEM and TEM.

2.5. Biological activity

2.5.1. Cells

Cell lines: human liver cancer cell line (HEPG2) was obtained from American Type Culture Collection (Rockville, Maryland, USA). They were maintained in the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (Wrocław, Poland). Cells were cultured in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 8 ug/mL of insulin and 1% mem non-essential amino acid solution 100x (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Murine fibroblast normal cell line (BALB/3T3) was cultured in DMEM (Gibco, UK) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, UT, USA). All culture media were also supplemented with antibiotics: 100 µg/ml streptomycin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 100 units/ml penicillin (Polfa Tarchomin SA, Warsaw, Poland). All cell lines were grown at 37 °C with 5% CO_2 humidified atmosphere.

2.5.2. Compounds

Prior to usage, the compounds were dissolved in DMSO (stock solution 10 mg/ml) and culture medium (1:9) to the concentration of 1 mg/ml. Subsequently, they were diluted in culture medium to reach the required concentrations (ranging from 100 to 0,1 µg/ml. One compound 3a was the only tested one in different ranges of concentrations from 10 to 0,01 µg/ml.

2.5.3. An anti-proliferative assay in vitro

24 hours before addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at density of $1x10^4$ cells per well. The assay was performed after 72 h; exposure to varying concentrations of the tested compounds. The in vitro cytotoxic effect of all compounds was examined using the SRB assay.

2.5.4. Cytotoxic test SRB

The details of this technique were described by Skehan et al (1991) [56]. The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich

Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h. They were then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 minutes. Unbounded dye was removed by rinsing (5 times) in 1% acetic acid. The protein-bound dye was extracted with 10 m Mun buffered Tris base (POCH, Gliwice, Poland). This step is to determine the optical density (λ = 540 nm) in Synergy H4 multi-mode microplate reader (BioTek Instruments USA).

The relationship between surviving fraction and drug concentration is plotted to obtain the survival curve for each cell line after the specified time. The required concentration for 50% inhibition of cell viability (IC_{50}) was calculated. The results are given in Table 1. These results were compared to the antiproliferative effects of the reference control doxorubicin [57].

2.5.5. Statistical analysis

The results are reported as Mean ± Standard error (S.E.) for at least 3 times experiments.

3. Result and Discussion

As to synthesize privileged class molecules [58–60] in an aqueous medium for heterocyclic synthesis, we herein report a convenient and rapid one ultrasound promoted, economic, ecofriendly, methodology for the synthesis of triazolopyrimidines [61]. This process was carried out through an addition of an equimolecular mixture of amino triazole **1**, carbonyl compounds **2** and active methylene compounds **3** in an aqueous medium.

To optimize the method, the reaction was studied under different reaction conditions to find the best results. Initially, we examined the reaction in ethanol with triethylamine catalyst under the conventional method. It was observed that the desired product was formed in low yield. Interestingly, no product was formed when the reaction was carried out in ethanol in the absence of catalyst under the conventional method. Furthermore, all our attempts to improve the yield at elevated temperature and longer reaction times were unsuccessful. To increase the efficiency, we decided to perform the reaction under mild conditions in water. It was noticed that the reaction proceeded. It did not form the desired product in good to excellent yields. For improving the procedure, the reaction was carried out in an ultrasonic bath in presence of lemon juice as biocatalyst. Fruit juice plays an important role as a biocatalyst in many of the chemical reactions. This biocatalyst follows all the parameters of green chemistry. The structure of the newly synthesized compounds was confirmed from their analytical and spectral data as shown in Fig. 2.

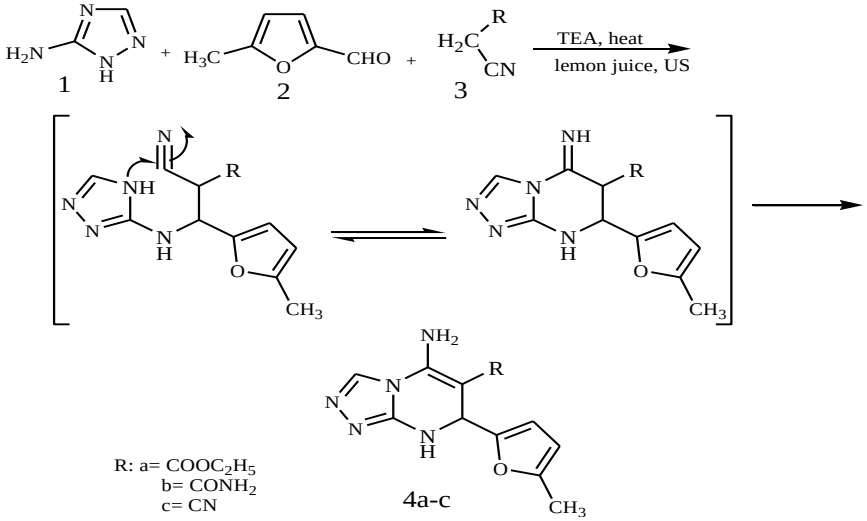

Fig. 2. A schematic diagram for the synthesized compounds using biocatalyst.

To gain more potent and selective anticancer To gain more potent and selective anticancer compounds, we designed and synthesized a series of pyrimidine derivatives based on, 1,2,4-triazols. For the preparation of complex molecules, several attempts were carried out for the synthetic manipulation of triazolopyrimidines. As a result, a number of reports have appeared requiring drastic conditions. They comprise long reaction times and complex synthetic pathways and often react in

organic solvents [12-16]. The latter are not desired commercially. Thus, developing efficient, selective and ecofriendly synthetic methods for applications in complex organic preparations are the ultimate objectives. In addition, environmental concerns and restrictions towards utilizing hazardous organic solvents are considered. It is significant to focus on developing reactions in water. It is an environmentally benign protocol. The one pot tandem synthesis of triazolopyrimidines in an aqueous medium has not been studied [17]. Therefore, compound **4a** underwent cyclization reaction with formamide affording **5**. This reaction has been prepared via heating of **4a** with formamide and/or under ultrasound irradiation. The structure of the formed compound was confirmed from its correct values in elemental analyses as illustrated in Fig. 3. It is supported by the agreeable spectral data IR (KBr, ν, cm^{-1}), ^{1}H-NMR (DMSO-d_6, δ ppm).

[1,2,4]triazolo[1,5-*a*]pyrimidin-6 (7*H*)-one derivatives (**7a-d**) were achieved upon heating or ultrasound irradiation of **4a** with acetic anhydride forming compound **6**. This was followed by the reaction of the obtained products with appropriate amine (hydrazine hydrate, m-Anisidine,2-Chloroaniline and 2-Bromoaniline) respectively (Fig. 3). The structures of the resulting products were confirmed from their correct values in elemental analyses and their agreeable spectral data. Triazolo[1,5-*a*]pyrimidin-7-yl)formamide **8** derivative was prepared *via* heating or sonicated in the presence of formic acid. The structure of the products (Fig. 3) were confirmed from their correct values in elemental analyses and their agreeable spectral data. ^{1}H-NMR (DMSO-d_6, δ ppm) of compound **8** showed significant resonances at 8.11 (s, 1H, -CH), and 10.73 (br s, 1H, -NH exchangeable with D_2O) with the disappearance of resonance for NH_2 group δ 3.35. The formamidotriazolo derivative **9** was prepared *via* addition elimination reaction of compound **8** with hydrazine hydrate. ^{1}H-NMR (DMSO-d_6, δ ppm) of compound **9** showed expected resonances δ 3.59 corresponding to NH_2 group and two resonance δ 9.50 and 10.51 corresponding to -NH groups. Triazolo[1,5-*a*]pyrimidin-6(7*H*)-one **10** was prepared through different routes. Firstly; heating of **4a** with trimethylorthoformate, followed by cyclization with hydrazine hydrate. Secondly: Ultrasonic radiation of **4a** with trimethylorthoformate then ultrasonic radiation with hydrazine hydrateto achieve compounds **11** (Fig. 3).

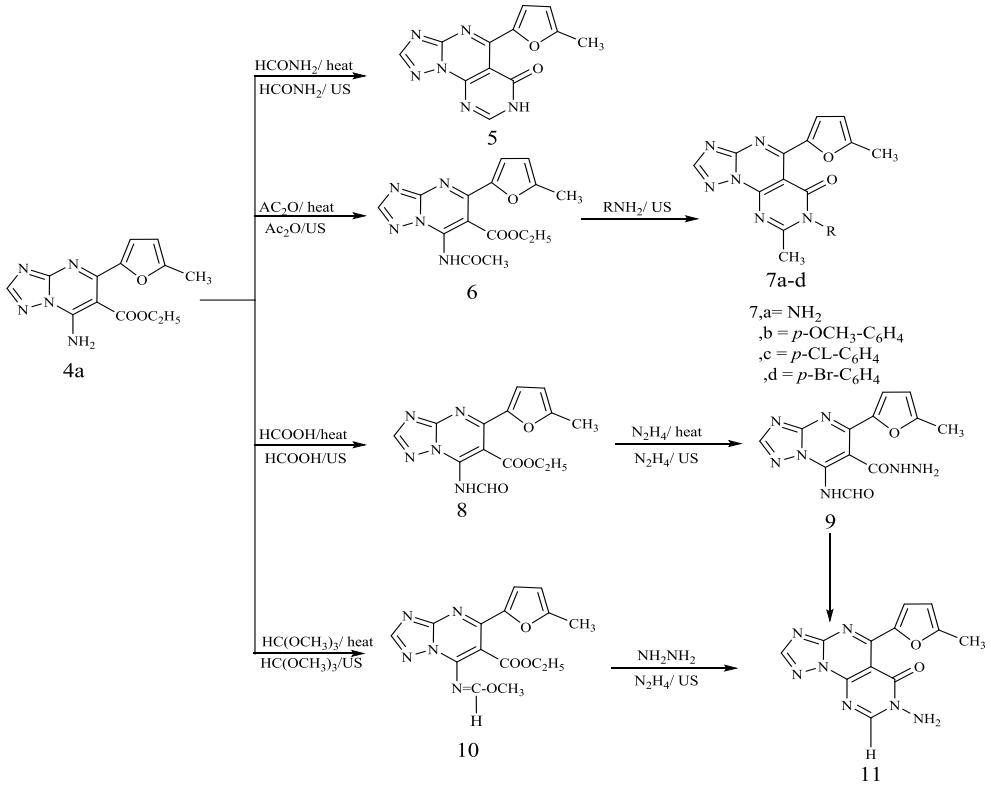

Fig. 3. A schematic diagram for the synthesis of pyrimidine derivatives based on, 1,2,4-triazols.

New acyclic *C*-nucleoside derivatives were purposed. Thus, heating under reflux for compound New acyclic *C*-nucleoside derivatives were purposed. Thus, heating under reflux for compound **7a, 11** with D-glucose and D-xylosein was carried out in acidic medium. The acyclic *C*-nucleosides **12a-d** were obtained in satisfactory yields (62-68%). ^{1}H-NMR (DMSO-d_6, δ ppm) for compound **12a** showed doublet at 7.33-7.34 ppm corresponding to the azomethine -N=CH proton. The Schiff base of compound **7a, 11** was formed upon heating it with an appropriate aldehyde in the presence of acetic acid. To enhance this procedure, the reaction was carried out in an ultrasonic bath in presence of lemon juice as biocatalyst. A green approach was applied to improve the products. Thus, the reactions were carried out in an ultrasonic bath in the presence of lemon juice as biocatalyst as shown in Fig. 4. The assignment of the structure for the formed products was based on their correct values in elemental analysis and agreeable spectral data.

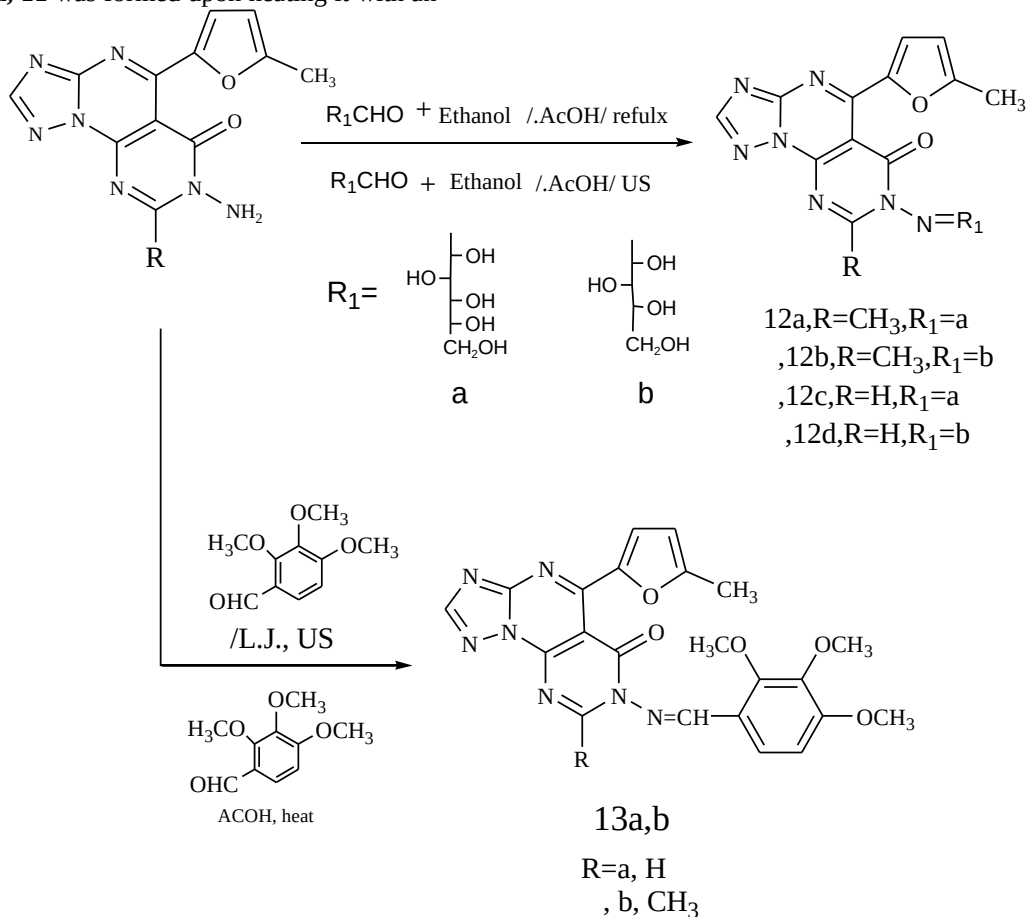

Fig. 4. A schematic diagram for the synthesis of *C*-nucleoside derivatives.

3.1. Synthesis of in-situ selenium nanoparticles by using heterocyclic compounds *12a, 12b, 13a, 13b* (HET-SeNPs)

It was required to innovate selenium nanoparticles (SeNPs) by employing the synthesized compounds **12a, 12b, 13a, 13b.** The aforementioned compounds exhibit appropriate low reducing behavior. However, they provide high stabilizing characteristics during the preparation process of SeNPs. Hence, ascorbic acid was used as a catalyst. Reducing Se^+ into Se^0 has been carried out by introducing a disaccharide with the ascorbic acid (catalyst). It acts as an aldehyde to form SeNPs and stabilize the nanostructure of the formed nanoparticles. Meanwhile, the glucosinolates have been oxidized to gluconic acid [47, 62–64]. The reduction of Selenium cation can be made by some organic compounds. They possess reductive groups such as -OH, -SH and -NH. Fig. 5 represents the structure of the synthesized compounds.

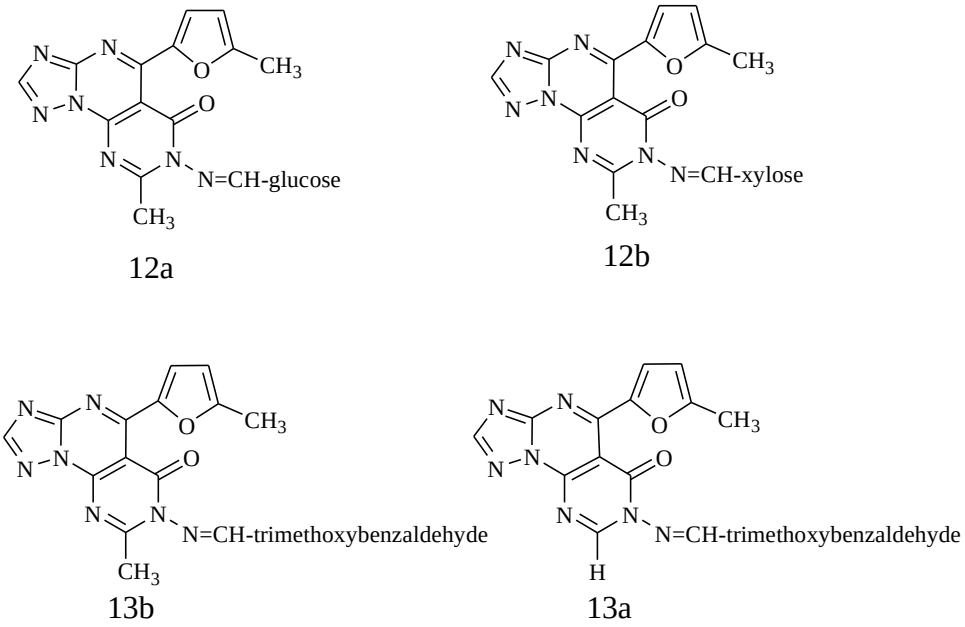

Fig. 5. Chemical structures of synthesized heterocyclic compounds.

The synthesized heterocyclic compounds **12a, 12b, 13a, 13b** were dissolved in 10 ml DMSO. However, after adding H_2SeO_3 and stirring for 1hr. at 60ºC, the solution has turned to red color as shown in Fig. 6a indicating the nanoparticles formation. The formation of SeNPs was monitored by UV-Vis spectroscopy. UV-Vis absorption spectrum of selenium nanoparticles is displayed in Fig. 6b. Selenium colloidal solution shows an absorption peak at wavelength 530 nm, according to the surface Plasmon resonance peaks.

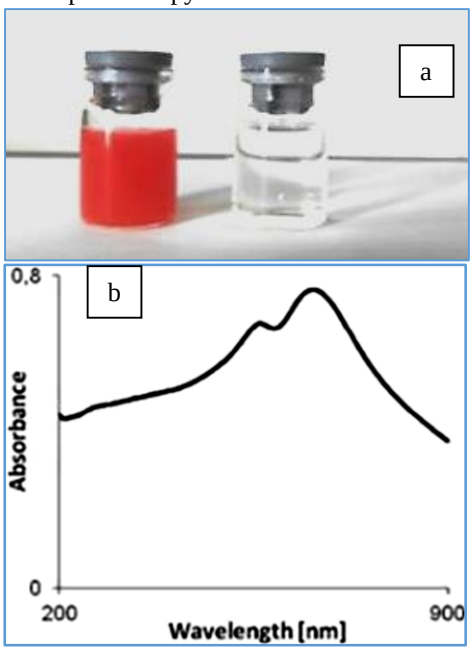

Fig. 6. a) A photograph for the formed selenium nanoparticles. b) UV-Vis spectrum of selenium nanoparticles.

Selenium nanoparticles have been formed and dispersed in the aqueous solution without aggregations [61]. SeNPs were characterized by Transmission electron microscopy (TEM) as shown in Fig. 7. (TEM) confirmed the formation of selenium nanoparticles either solely or loaded onto the synthesized heterocyclic compounds. In Fig. 7a, selenium nanoparticles are observed in spherical shapes. They exhibit minor aggregates. The size of these nanoparticles is lie in the range of 12-35 nm. Upon loading selenium nanoparticles onto the investigated compounds (**12a**) and (**12b**), it is noticed that selenium nanoparticles spread homogeneously

through both samples as shown in Fig. (7b and 7c). The synthesized compounded contributed in preventing such agglomerates that may arise in selenium nanoparticles. It seems to be a stabilizing effect for the nitrogen based compounds [62,63] contributed in the existing of Se nanoparticles apart from each other with a convenient distribution.

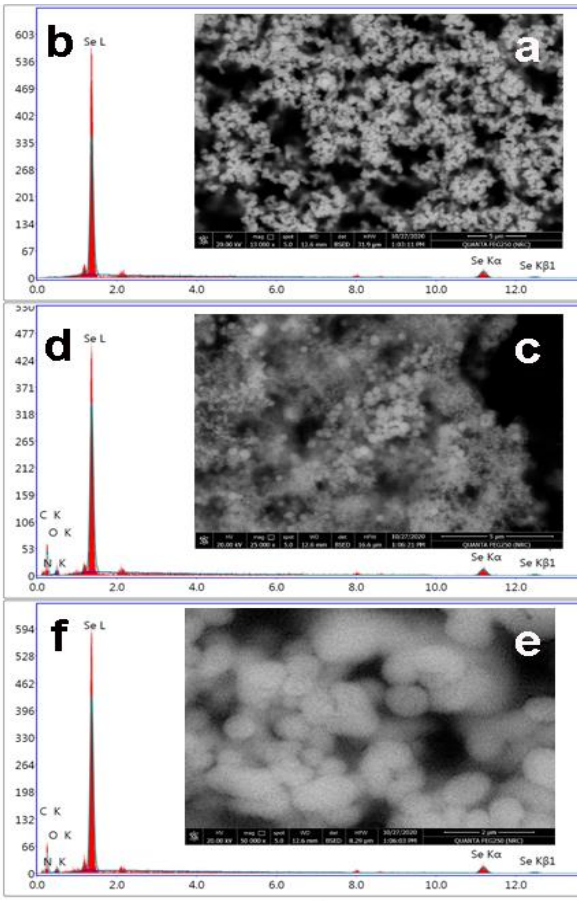

Fig. 7.: a) TEM of SeNPs; b and c) TEM of SeNPs loaded onto (**12a** and **12b**) respectively.

To investigate the morphology of the synthesized selenium nanoparticles (SeNPs), different techniques were equipped for such purpose. Scanning electron microscopy (SEM) was used to describe the surface of the synthesized nanoparticles. In Fig. 8a, selenium nanoparticles (SeNPs) exist as spheres. Their appearances do not show aggregates. Figures 8c and 8e depict the same globular structure for SeNPs after being loaded onto the synthesized heterocyclic compounds (**12a** and **12b**) respectively. These organic compounds may pose a hazy appearance around SeNPs. Meanwhile, these organic compounds contribute in stabilizing SeNPs with avoiding aggregation. Fig. 8b demonstrates energy dispersive X-ray spectroscopy (EDX) spectrum for SeNPs. EDX technique is employed to confirm the change from ionic to elemental state for the tested samples [64,65]. A sharp characteristic peak for nanostructured selenium appears at 1.3 keV. Besides, complementary peaks seem around 11 and 12 keV confirming the formation of elemental selenium. Upon loading SeNPs on the aforementioned heterocyclic compounds, the characteristic peaks of selenium appear with new peaks for carbon, oxygen and nitrogen peaks as main elements composing the synthesized organic compounds in the corresponding spectra .Fig. 8d and 8f.

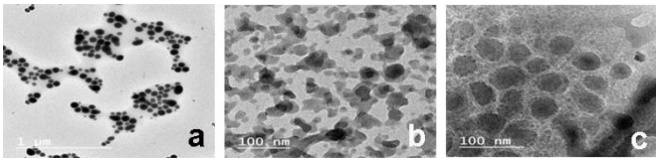

Fig. 8. a) SEM of SeNPs c and e) SEM of SeNPs loaded onto (**12a** and **12b**) b) EDX spectrum of SeNPs d and f) EDX spectra of SeNPs loaded onto (**12a** and **12b**) respectively.

3.2. Antiproliferative activity of the tested compounds

The antiproliferative activities were expressed by median growth inhibitory concentration (IC_{50}). As shown in table 1, the in vitro antiprolefrative activity towards human liver cancer cell line (HEPG2) in addition to BALB/3T3 (murine fibroblast) normal cell line was evaluated using SRB assay, in comparison with doxorubicin as reference drug. The results revealed that most of the compounds showed variable activity against human liver cancer cell line (HEPG2). The tumor cell line showed a normal growth in our culture system. DMSO did not seem to have any noticeable effect on cellular growth. A gradual decrease in the viability of cancer cells was observed with increasing concentration of the tested compounds, in a dose-dependent inhibitory effect.

Evaluating the antitumor effect of the tested compounds towards human liver cancer (HEPG2) cells revealed that while compounds **10, 7a, 11** and **12c** had no effect on these cancer cells. Meanwhile, compound **13a** was found to be the highest potent derivative compared to doxorubicin the standard anticancer drug, with IC_{50} value 3.8 ± 0.03 µg/ml versus 2.7 ± 0.06 µg/ml for doxorubicin. Moreover, compounds **13b**, **12a** and **12b** showed good activity with IC_{50} values (IC_{50}: 4.7 ± 0.1, 7.9 ± 0.4 and 10.8 ± 0.5 µg/ml respectively versus 2.7 ± 0.06 µg/ml for doxorubicin). Compounds (**5, 8, 9**, **4a,** and **12d**) were showed slight or moderate activities. However, all tested compounds showed slight or no activity towards normal cell line BALB/3T3 except

compound **13a** when compared to doxorubicin. It shows an IC_{50} value 14.9 ± 0.9 µg/ml versus 5.03 ± 0.7 µg/ml for doxorubicin. Moreover, results indicated that the synthesis of in-situ selenium nanoparticles by using the highly potent compounds (**13a, 13b, 12a and 12b)** increased their antiprolefrative efficacy towards liver cancer cell line (HEPG2). Meanwhile they exert a lower effect on normal cell line BALB/3T3.

In conclusion, the tested compounds exert anti-proliferative activity on human liver cancer cell line (HEPG2) through reducing cell proliferation. It resulted in a significant growth inhibitory effect. Although compounds **13a, 13b**, **12a** and **12b** showed cytotoxicity and growth inhibitor activity on liver cancer cell line with IC_{50} values near to the standard drug, compounds **13a and 13b** were found to be the most potent on this type of cell line. Moreover, the present study reveals that human liver cancer cells (HEPG2) are more sensitive to the tested compounds than the normal cells (BALB/3T3).

Also, the present investigation declared that the selenium nanoformation of the most active compounds indicated higher potency towards (HEPG2) cancer cells, while they showed lower efficiency towards (BALB/3T3) normal cells.

Table 1: In vitro cytotoxic activity of the newly synthesized compounds towards human liver cancer cell line (**HEPG2**) and normal cell line (**BALB/3T3)** is expressed as IC_{50} values.

Compounds	**Liver (HEPG2)** IC_{50} [µg/ml]	**BALB/3T3** IC_{50} [µg/ml]
4a	36.5 ± 0.6	96.45 ± 10.3
5	69.20 ± 9.37	N.A.
7a	N.A.	51.54 ± 6.35
8	25.9 ± 3.2	N.A.
9	22.8 ± 2.9	N.A.
10	N.A.	N.A.
11	N.A.	N.A.
12a	7.9 ± 0.4	52.8 ± 0.6
12b	10.8 ± 0.5	34.09 ± 12.03
12c	N.A.	76.72 ± 4.14
12d	64.09 ± 12.00	N.A.
13a	3.8 ± 0.03	14.9 ± 0.9
13b	4.7 ± 0.1	53.24 ± 8.65
12a-SeNPs	6.5 ± 0.2	49.2 ± 2.9
12b-SeNPS	8.4 ± 0.7	41.6 ± 8.2
13a-SeNPS	3.1 ± 0.08	21.3 ± 1.3
13b-SeNPS	4.1 ± 0.09	61.7 ± 4.5
Doxorubicin	2.7 ± 0.06	5.03 ± 0.7
DMSO	N.A.	N.A.

Data were expressed as Mean ± SD of 3 independent experiments.
N.A.: No activity.

4. Conclusions

Increasing the yield of the synthesized compounds with reducing the consumed time in a green chemical processes is one of the most important outcomes we have obtained from applying ultrasonic technology and natural catalytic juices. These synthesized compounds demonstrated variable antiproleferative activity towards human liver cancer cells (HEPG2). Compounds **5, 8, 9,** and **12d** demonstrated a selective activity against liver cancer cells (HEPG2). It showed no effect on the normal cell line (BALB/3T3). Moreover, the most potent compounds **12a, 12b, 13a, 13b** were selected to be loaded with selenium nanoparticles. Their antiproleferative activity on both cell lines was studied. It was discovered that they possess a higher selectivity towards malignant growth cells. Meanwhile, they demonstrated a lower efficiency towards normal cells which might be support in depressing the side effects of these chemical agents.

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Conflicts of Interest
The authors declare no conflict of interest.

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