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SYNTHESIS OF NEW CEPHEMS AND SELENACEPHEMS BASED ON 6H-1, 3-THIAZINES AND 6H-1, 3-SELENAZINES AND THERE BIOCHEMICAL STUDY

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Abstract

Several new and known 6-(4-substituted-phenyl)-4-(4-substituted-phenyl)-2-phenyl-6H-1,3-thiazine (or selenazine) (Z4B7, Z4D5, Z4B7 and Z4D5) were prepared by US Pat 1,4-Michael addition reaction of chalcone derivatives with thiobenzamide or phenylselenocarboxamide in basic medium (the chalcones being formed by Claisen-Schimidt condensation of aromatic aldehydes with 4-substituted acetophenone in the presence of sodium hydroxide). These 6H-1,3-thia or selenazines were used for a new series of cephem and selenacephem compounds (i.e. 7-chloro-4-(4-substituted phenyl)-2-(4-substituted phenyl)-6- Phenyl-5-thia (or 5-selena)-1-azabicyclo[4.2.0]oct-2-en-8-one; AZ4B7, AZ4D5, AZ4B7 and AZ4D5). All new compound derivatives were characterized by IR, ^{1} H NMR, ^{13} C NMR, mass spectroscopic techniques and elemental analysis. The toxicity of new compounds was assessed by determining their LD50 value using the Dixon up and down method. The antibacterial activity of cephem and selenacephem compounds was tested in vitro against Staphylococcus aureus, Bacillus, Escherichia coli and Pseudomonas aeruginosa. In addition, the antioxidant, anticancer, and DNA cleavage efficiencies of compounds were evaluated.

Keywords: Acute toxicity, Antioxidant, Antibacterial, Anticancer activity, Cephem, DNA cleavage, Selenacephem, 1,3-Selenazine, 1,3-Thiazine

INTRODUCTION

1,3-Thiazines or 1,3-selenazines are a class of six-membered heterocyclic organic compounds having one nitrogen and one sulfur or selenium atom in a 1,3-position. 1, 3-Thiazines and 1,3-Selenazines are weak bases, and there are three types whose names differ according to the position of the double bond in the ring. Because of nitrogen, thiazines are chemically basic, 1,3-thiazines are of great importance as they form part of the backbone of cephem derivatives. Characterized by the presence of the unit (S-C-N or Se-C-N) in their structure, these compounds are considered to be pharmacophores and have been found to be quite stable, including medically important compounds such as xylazine and chlormezanone. [1, 2] Cephem is a fused ring system composed of a 1, 3-thiazine and an azetidine ring. Since the 1970s, cephalosporin's, the most important representative group of cephemas, have been among the most potent and widely used anti-infective drugs [3].Chemically, cephemes can be divided into five different classes: cephalosporin's; cephamycins; oxa-1-cephems; carba-1-cephems; and other. Maximum clinical efficacy can only be achieved by striking a delicate balance between the reactivity

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ofβ -lactam as an acylating agent at the active site of β-lactam-binding proteins and its stability against premature ring opening by water and other nucleophiles en route to the target enzymes. Contributing to the popularity of cephemes in clinical use is their superior chemical stability, particularly to hydrolysis[4].Cephemes, a structural unit found in the most widely used antibiotics,[5,6] have played a fundamental role in medicinal chemistry for almost a century. With the microbe root in medicinal chemistry for almost a century. As the microbes respond to the traditional antibiotics through β-lactamases, the need for novel antibiotics prevails, making the synthesis of new cephemes increasingly important. In addition to their use as antibiotics, cephems are increasingly being used as synthons for other biologically important molecules.[5,7,8] Cephems have been found to act as cholesterol acyltransferase inhibitors, thrombin inhibitors, human cytomegalovirus protease inhibitors, and matrix metalloprotease inhibitors act as inhibitors, cysteine protease, and inducers of apoptosis.[6,9] Biological activity is usually associated with the nature of the groups associated with N-1, C-3, and C-4 of the β-lactam ring.[10] Cephem derivatives containing β-lactam nuclei have a broad spectrum of pharmaceutical activity and are becoming an integral part of the chemotherapeutic arsenal available to today's physicians[5,10].The metabolism of cephemen is analogous to that described forβ -lactam. In terms of their chemical mechanism, cephemene β-lactams are very similar in that they form a covalent bond with peptidoglycan synthetases (PBPs) and cause cell lysis. Susceptible cephemes can be hydrolyzed byβ -lactamases, and indeed some -lactamases are more effective at hydrolyzing cephemes than β -lactam itself. Allergic reactions are not as common in this chemical class as in the β -lactam class [11]. In the present work we have synthesized a new series of cephem and selenacephem derivatives by cycloaddition reactions of ketene with 6H-1, 3-thiazines and 6H-1, 3-selenazines, respectively. The compounds were tested in vivo for acute toxicity, antioxidant, antibacterial, anticancer activity and DNA cleavage.

MATERIALS AND METHODS

Materials and reagents:

All chemicals and solvents used were analytical grade, supplied by Sigma-Aldrich, Fluka, Merck, BDH, HW, USP, GCC, RDH, ALPHA and SCH. p-Anisaldehyde, 4-nitroacetophenone, thiobenzamide, chloroacetyl chloride, sodium borohydride (NaBH4), and MTT stain and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich. Tween-20 (polyoxyethylene (20) sorbitan monolaurate) and dichloromethane were obtained from Fluka. dimethyl sulfoxide, triethylamine, benzonitrile, acetophenone, 4-chlorobenzaldehyde, Na2SO4, NaCl, NaHCO3, NaOH and KOH from Merck product. Selenium powder, chloroform and n-hexane were obtained from BDH. Linoleic acid and carotene were supplied by HW and USP respectively. Hydrochloric acid, acetic acid, and pyridine were also sourced from GCC, RDH, and ALPHA, respectively. Absolute ethanol, ethyl acetate, methanol and benzene were obtained from SCH. Thin layer chromatography (TLC) was performed using silica gel 60F254 (Merck) coated aluminum foil, iodine and ultraviolet (UV) light were used for visualized TLC plates.

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Physical Measurements:

FT-IR spectra as KBr disks were recorded in the 4000-400 cm $^{-1}$ range using a Shimadzu FT-IR model 8400s instrument. The experimental values of ^{1}H and ^{13}C NMR spectra for the studied compounds were recorded in a Brucker spectrophotometer (500 MHz) and using DMSO-d_6 as solvent and TMS as internal standard (Central Laboratory, University of Tehran, Iran) recorded. Mass spectra were measured by the EI technique at 70 eV using an Agilent Technologies 5973C spectrometer. Elemental analysis (C, H, N, S) was performed using the EuroFA Vector EA 3000 elemental analyzer. Melting points were measured on a Bauchi 510 melting point apparatus and are uncorrected.

Synthesis:

The compounds 3-(4-methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**4B$_7$**)and 3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (**4D$_5$**)were prepared and characterization as previously described in literature.[12,13]These compounds gave satisfactory elemental analysis and spectroscopic data and they are not reported.The synthetic procedures for the preparation of compounds (**4B$_7$**and **4D$_5$**)is presented in Scheme 1.

Synthesis of Phenylselenocarboxamide:

The compound Phenylselenocarboxamide was prepared and characterization as previously described in literature. [14]This compound gave satisfactory elemental analysis and spectroscopic data and they are not reported.

Synthesis of 6-(4-substituted phenyl)-4-(4-substituted phenyl)-2-phenyl-6H-1,3-thiazine (or selenazine) (Z4B$_7$, Z4D$_5$, Z4B$_{7'}$ andZ4D$_{5'}$):

All 6H-1, 3-thiazines and 6H-1, 3-selenazines were prepared by the following general procedure according to a literature method [2] with a slight modification.

To a solution of chalcones 4B$_7$and4D$_5$(5 mmol) in ethanol (10 mL) a solution of thiobenzamide (5 mmol, 0.69 gm) or phenylselenocarboxamide (5 mmol, 0.92 gm) in ethanol (10 mL) was added slowly. To this aqueous potassium hydroxide solution (10 mmol, 0.56 gm) was added (prepared from KOH in small amount of distilled water). The reaction mixture was refluxed for 5-7 hrs, the progress of the reaction was monitored by TLC using methanol: chloroform (v/v 1:9) as eluent and ultraviolet (UV) light as appearance, cooled, the resulted compounds were obtained by pouring the reaction mixture onto crushed ice and acidified with conc. HCl. The precipitated solids were filtered, dried and recrystallized from methanol. The chemical structures and some physical properties are listed in Table 1.

Compound 6-(4-chlorophenyl)-2,4-diphenyl-6H-1,3-thiazineZ4D$_5$ was prepared and characterization as previously described in literature.[15,45-60]These compounds gave satisfactory elemental analysis and spectroscopic data.

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General Procedure for Preparation Cephem Compounds ($AZ4B_7$, $AZ4D_5$, $AZ4B_{7'}$ and $AZ4D_{5'}$):

To a solution of 1 mmole of 6H-1,3-thiazine (i.e.$Z4B_7$and $Z4D_5$)or 6H-1,3-selenazine(i.e. $Z4B_{7'}$and$Z4D_{5'}$)in dry dichloromethane(20 mL) was added triethylamine (4 mmol, 0.4 gm). The resulting solution was stirred for 5 min at 0 ^oC under Argon atmosphere then chloroacetylchloride (3 mmol, 0.34 gm) was added drop wise with stirring during 15 min. The reaction mixture was stirred for 6-8 hrs at room temperature then the reaction mixture extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed successively with 1N HCl (20 mL), water (2 × 20 mL), 5% $NaHCO_3$ (20 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated in vacuo. The progress of the reaction was monitored by TLC. The cephem products were recrystallized from hexane.[16-32-45]The R_f values of 7-chloro-4-(4-substituted phenyl)-2-(4-substituted phenyl)-6-phenyl-5-thia (or 5-selena)-1-azabicyclo[4.2.0]oct-2-en-8-one compounds($AZ4B_7$, $AZ4D_5$,$AZ4B_{7'}$and $AZ4D_{5'}$) were determined by using Ethyl acetate: dichloromethane (2:8) as an eluent. The chemical structures and some physical properties are listed in Table 1.

Acute toxicity (LD_{50})

Healthy albino mice of either sex (male and female), age from 7-9 weeks and their body weight ranged between 23-33 g, were used for study acute toxicity of Cephem ($AZ4B_7$) and Selenacephem ($AZ4B_{7'}$) derivatives. The animals were injected intraperitonially with the first dose 500 mg/kg. The result was read death X or life O after 24 hours, and increases or decreases the amount of dose was constant 50 mg/kg and repeat dosing up or down for 4 mice after changing the result death to life and versa. LD_{50} were calculated based on the diagram and equation of DixonLD_{50} = Xf +Kd, where Xf: the last dose, K: the interval between dose levels, d: the tabulated value, Table 2.[17]

	K represented serial tests started with :-				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
	X	XX	XXX	XXXX	
	K represented serial tests started with :-				

Table 1: The tabulated Dixon values

Antibacterial Activity

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The compounds (**AZ4B$_7$**, **AZ4D$_5$**, **AZ4B$_{7'}$** and **AZ4D$_{5'}$**) were screened in vitro for antibacterial properties. The panel of pathogens involved Staphylococcus aurous and Bacillus as a Gram-positive bacterium, Escherichia coli and Pseudomonas aeruginosa as a Gram-negative bacterium, by using agar diffusion method. The antibiotics tetracycline and amoxicillinwere use to calibrate and to comparison with the antibacterial stuff. 0.2 mL of bacterial inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish Mueller Hinton Agar (MHA). The tested compounds and tetracycline drug were dissolved in DMSO with concentrations include (1, 5, 25,125, 250 and 500) mg /mL for each compound. 50 µl from 1-500 mg/mL concentrations of tested compounds and tetracycline were added to every well (7 mm diameter holes cut within the agar gel, 20 mm aside from one another). The plates were incubated for twenty-four h at 36ºC ± 1ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm.[18]Furthermore, values of minimum inhibitory concentration (MIC) of those compounds.[19,33-50] The MIC was recorded because the lowest concentration at which no visible growth was observed.

Antioxidant Activity

The antioxidant activity of the Cephems (AZ4B$_7$andAZ4D$_5$) and Selenacephems (AZ4B$_{7'}$andAZ4D$_{5'}$) were determined according to the β-carotene bleaching method.[20]The β-carotene bleaching method is based on the loss of the yellow color of β-carotene because of its reaction with radicals formed by linoleic acid oxidation in an emulsion and according to previous methods.[21]A solution of β-carotene was prepared by dissolving 0.01 gm of β-carotene in 50 ml of chloroform, 1 ml of this solution was then pipetted into round-bottom rotary flask containing (0.02 ml) of linoleic acid and (0.2 ml) of Tween-20. After removing the chloroform by vacuum evaporation using a rotary evaporator at room temperature, 50ml of distilled water were added to the flask with manual shaking as first stage. The emulsion (3.8 mL) was added to tubes containing 0.2 mL of the prepared compounds and reference (BHT) compound (which prepared by dissolving 0.01 gm of these compounds in 0.2 ml of DMSO) The absorbance was read at 470 nm, the samples were then subjected to thermal autoxidation at 45°C in a water bath for 2 h. Absorbance was measured every 15 min. [20] Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation:

$$\%AA = 1 - [(Ai - At) / (Ai^{*} - At^{*})] \times 100$$

Where, Ai: is the measured absorbance value of sample at zero time. At: is the measured absorbance value of sample after incubation (105) min at 45°C.Ai*: is the measured absorbance value of control atzero time, At*: is the measured absorbance value of control after incubation (105) min at 45°C.

Anti-Breast Cancer Activity

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A) In vitroMTT cellular viability assay

The Cytotoxicity of samples on MCF-7 cell line were determined by the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazoliumbromide) cell viability assay.[22] Cells at a density of 1 × 10^4 cells/mL (100 µL/well) were seeded in 96-well plates and incubated overnight under 5% CO_2 at 37 °C, followed by exposure to a series of concentrations (6.25, 12.5, 25, 50, 75 and 100 µg/mL) of the tested compounds (**AZ4B$_7$** and **AZ4B$_{7'}$**) and 5-Fluorouracil as reference drug. At the same time, a group only containing culture medium was set as blank control. Each group had three biological repeats. After dosing for 72 h, the cells were washed and then fresh medium (100 µL) supplemented with 28 µL of 2 mg/mL solution of MTT was added to each well. After incubated in the dark for 2 h at 37 °C, removing the MTT solution and the crystals remaining in the wells were solubilised by the addition of 100 µL of DMSO followed by 37 °C incubation for 15 min with shaking.[23] The optical density at 620 (OD620) of each well were measured by plate reader (Synergy H4: Bio-Tek, Winooski, VT, USA). The results are presented as mean ± standard deviation (SD). The survival rate of control cells treated with 0 M the tested compounds was set as 100%. Cell viability was calculated using the following Equation:

Cell viability (%) = [(dosing cell OD – blank OD) / (control cell OD – blank OD)] × 100

B) Acridine Orange/ Ethidium Bromide Staining

Morphological apoptosis of MCF-7 cells treated with different concentrations of the new prepared compounds (**AZ4B$_7$**and**AZ4B$_{7'}$**) and standard (5-Fluorouracil) were assessed using an acridine orange / ethidium bromide (AO/EB) staining kit (Solarbio, Beijing, China, Cat No. CA1140). The density of 1 × 10^4 MCF-7 cells/mL was plated in 6-well plates (1 mL/well) and incubated overnight. The medium was replaced with the tested compounds-containing (6.25, 12.5, 25, 50, 75 and 100 µg/mL) medium and incubated for 48 h under the same conditions mentioned before. Cells were washed with PBS and stained with AO/EB solution (20 µL AO/EB freshly mixed solution of equal volume in 1 mL PBS) for 2–3 min in the dark. After the successive washes, the fluorescent images were taken with an inverted fluorescence microscope (Olympus Corporation, Beijing, China). [24]

Flow Cytometry

This method was conducted according to Zini and Agarwal,[25] to estimate the effect of the two selected compounds (AZ4B$_7$ and AZ4B$_{7'}$) on breast cancer cell line, as % DNA fragmentations index (% DFI) were detected by the Acridine orange by using flow cytometry assay. MCF-7 breast cancercellline (2x 10^5 cell/ ml) were cultivated in RPMI media containing 20% FBS + insulin at 10 ml per petri dish. Upon formation of a monolayer of cells, 100 µL of concentration (100 µg / mL) for each selected compound were added. After 24 h of incubation, cells were harvested by addition of trypsin, centrifuged for 5 min at 1000xg, and finally washed with PBS. Cells were stained according to the protocol and were analyzed. The sample was incubated and analyzed by calibur flow cytometer. The cell Quest software and MOdFit software were used to

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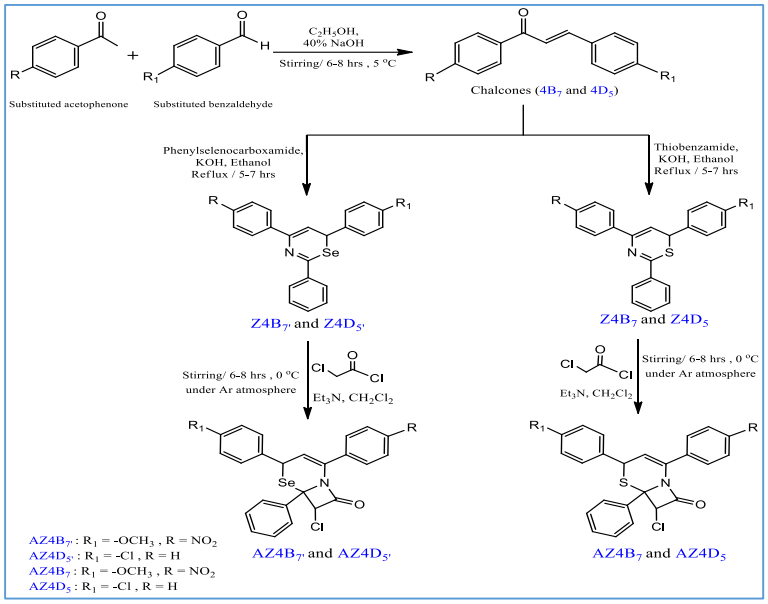
determine (% DFI). In this study the negative control (DMSO) was also maintained against the positive control (5-Fluorouracil). The determinations were performed in duplicates.



Scheme1: Synthesis of Cephems (AZ4B$_7$andAZ4D$_5$) and Selenacephems (AZ4B$_{7'}$andAZ4D$_{5'}$)

RESULTS AND DISCUSSION

The Cephem (AZ4B$_7$andAZ4D$_5$) and Selenacephems(AZ4B$_{7'}$andAZ4D$_{5'}$)compounds were prepared via [2+2] cycloaddition reaction of ketenewith 6H-1,3-thiazines and 6H-1,3-selenazines,respectively.6H-1,3-Thiazine (Z4B$_7$andZ4D$_5$) and1,3-selenazine(Z4B$_{7'}$andZ4D$_{5'}$) derivatives were produced by reactions of thiobenzamideor primary selenoamide with α, β-unsaturated ketones (Michael acceptors)in the presence of KOH to afford 6-(4-substituted phenyl)-4-(4-substituted phenyl)-2-phenyl-6H-1,3-thiazine (or selenazine)by [3+3] cycloaddition, the suggested mechanism for preparing a6H-1,3-thiazines and 6H-1,3-selenazines and their derivatives are shown in scheme 2.Cephem and Selenacephem are stable in air and they are soluble in most non-polar solvents, Also, the existence of interactive unsaturated ketone group in β-lactam ringis accountable for their biological activities. The elemental analysis results C, H, N, S of the studied compounds are in agreement with the theoretical values.The physical properties, percent yield and Rf values are cited in Table 1.

Symbol of com.	**R_f**	Structural formula, Compound name	Reaction time	Colour	Molecular formula	Molecular	Melting point	Yield %	Elemental analysis CHN Practical (Theoretical)

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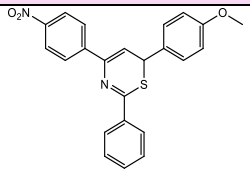
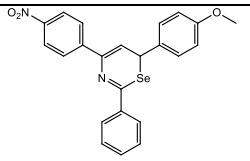
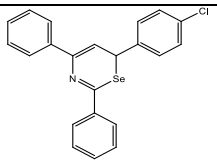
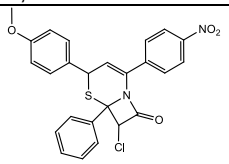
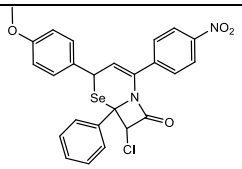
			(h)			weight gm/mol	(°C)		C%	H%	N%	S%
Z4B$_7$	**0.59**	 6-(4-methoxyphenyl)-4-(4-nitrophenyl)-2-phenyl-6H-1,3-thiazine	7	Dark Orange Powder	$C_{23}H_{18}N_2O_3S$	402.47	242-244	71	68.58 (68.64)	4.54 (4.51)	7.01 (6.96)	7.91 (7.97)
Z4B$_{7'}$	**0.55**	 6-(4-methoxyphenyl)-4-(4-nitrophenyl)-2-phenyl-6H-1,3-selenazine	7	Light brown powder	$C_{23}H_{18}N_2O_3Se$	449.36	229-231	63	61.53 (61.48)	4.10 (4.04)	6.29 (6.23)	…..
Z4D$_{5'}$	**0.57**	 6-(4-chlorophenyl)-2,4-diphenyl-6H-1,3-selenazine	7	Maroon powder	$C_{22}H_{16}ClNSe$	408.78	147-149	41	64.58 (64.64)	3.98 (3.95)	3.47 (3.43)	…..
AZ4B$_7$	**0.54**	 7-chloro-4-(4-methoxyphenyl)-2-(4-nitrophenyl)-6-phenyl-5-thia-1-azabicyclo[4.2.0]oct-2-en-8-one	8	Dark orange oil	$C_{25}H_{19}ClN_2O_4S$	478.95	Oil	43	62.64 (62.69)	4.09 (4.00)	5.79 (5.85)	6.72 (6.69)
AZ4B$_{7'}$	**0.65**		8	Brown oil	$C_{25}H_{19}ClN_2O_4Se$	525.84	Oil	47	57.17 (57.10)	3.68 (3.64)	5.27 (5.33)	…..

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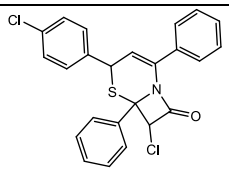
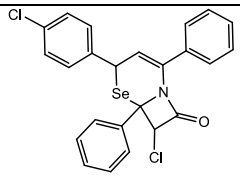
		7-chloro-4-(4-methoxyphenyl)-2-(4-nitrophenyl)-6-phenyl-5-selena-1-azabicyclo[4.2.0]oct-2-en-8-one										
AZ4D$_5$	**0.51**	 7-chloro-4-(4-chlorophenyl)-2,6-diphenyl-5-thia-1-azabicyclo[4.2.0]oct-2-en-8-one	7	Brown oil	$C_{24}H_{17}Cl_2NOS$	438.37	Oil	72	65.83 (65.76)	3.96 (3.91)	3.17 (3.20)	7.37 (7.31)
AZ4D$_{5'}$	**0.68**	 7-chloro-4-(4-chlorophenyl)-2,6-diphenyl-5-selena-1-azabicyclo[4.2.0]oct-2-en-8-one	8	Brown oil	$C_{24}H_{17}Cl_2NOSe$	485.26	Oil	39	59.34 (59.40)	3.55 (3.53)	2.82 (2.89)	…..

Table 1: The symbol, Synthetic formula, compounds name, analytical and physical data of the 6H-1, 3-thiazines and 6H-1,3-selenazines and their derivatives

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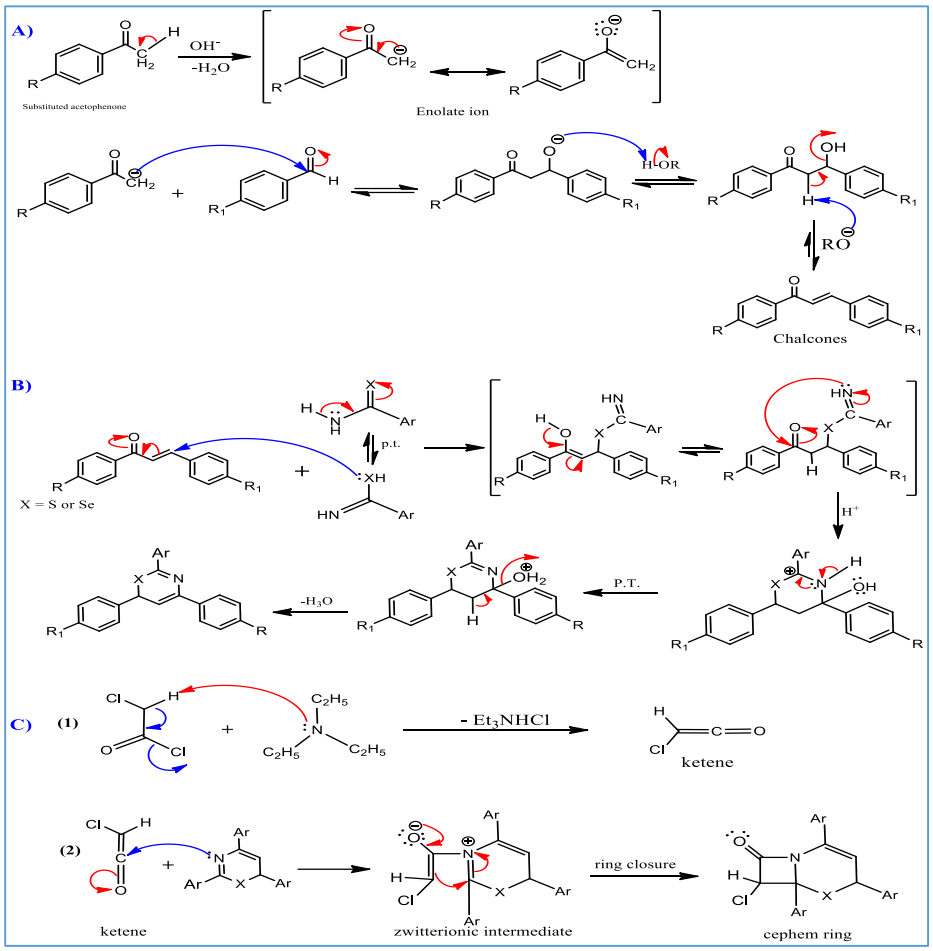

Scheme 2: The suggested mechanism for preparing compounds

Spectroscopic analysis

Spectral studies including the observed spectroscopic results for the title compounds are discussed. All the synthesized compounds gave a spectroscopic analysis consistent with the empirical structures. A complete set of spectral data of studied compounds is given in Supplementary data.

Infrared spectra (FT-IR):

The infrared spectra show the position and the intensities of the peaks which corresponds to various groups present in each compound. On comparing the IR spectral data of the 6H-1,3-Thiazines and 6H-1,3-selenazineswith the IR spectra of cephemes and Selenacephems, respectively the following can be pointed out (Table 3):All the infrared spectra of the 6H-1,3-thia- or selenazine and their derivatives were characterized by a strong to medium band at1253–1280cm^{-1}which corresponds to the v(C-N) stretching vibration.[26]The infrared spectra of the 1,3-Thiazines and 6H-1,3-selenazines were characterized by a strong band at 1589–1598cm^{-1}which corresponds to the azomethine

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ν(C=N) stretching vibration.[2]Also, the IR spectra of the prepared compounds(Z4B$_7$, Z4B$_{7'}$, AZ4B$_7$ and AZ4B$_{7'}$) show featured bands at the range 1508-1512cm^{-1} and in 1334-1350cm^{-1}, which assigned to asymmetrical and symmetrical stretching vibration respectively of (NO_2) group.[27]The spectrum was distinguished by the appearance of distinct absorption bands for ν(C-S-C) at the range 2357-2364cm^{-1}which assigned to stretching vibration for the 6H-1,3-Thiazines and cephemes (Z4B$_7$,AZ4B$_7$ and AZ4D$_5$). [2]Furthermore, the medium to weak bands which appeared in the range 524-592 cm^{-1} are attributed to the ν(C-Se) stretching vibration for the 6H-1,3-selenazines and Selenacephems (Z4B$_{7'}$,Z4D$_{5'}$AZ4B$_{7'}$ and AZ4D$_{5'}$).[28]The structure of cephems or selenacephems(i.e. compounds AZ4B$_7$,AZ4B$_{7'}$,AZ4D$_5$and AZ4D$_{5'}$) were established by IR spectroscopy which showed the disappearance of C=N bands in the region 1589–1598 cm^{-1} combined with the appearance of absorption bands at 1708-1734cm^{-1}and at 1537-1597 cm^{-1}due to ν(C=O) and ν(C-N), respectively for β-lactam ring.[16, 29]In the region 694-821 cm^{-1} strong to weak peaks appeared related to stretching ofν(C-Cl)for thecompoundsZ4D$_{5'}$, AZ4B$_7$, AZ4B$_{7'}$, AZ4D$_5$and AZ4D$_{5'}$.

Com.	ν(C-H)$_{Str.}$ Benzene	ν(C-H)$_{Str.}$ Thiazine	ν(C-S-C)$_{Str.}$ Thiazine	ν(C=N) Thiazine	ν(C=C)$_{Aro.}$	ν(NO_2) Asym Sym	ν(C-N) Thiazine	ν(C-H)$_{Aro.}$ Bending	ν(C-Se) Selenazine	ν(Others)
Z4B$_7$	3059 w	2839 w	2364 m	1598 s	1444 m	1510 s 1338 m	1257 s 1174 m	825 m 777 m 690 m	…..	2958 w ν$_{Str.}$(C-H)$_{ali.}$ 1215 m ν(C-O) 659 w ν(C-S)$_{ring}$
Z4B$_{7'}$	3063 w	2838 w	…..	1593 s	1458 m	1508 s 1334 m	1253 m 1172 s	825 m 775 w 690 w	532 m	2963 w ν$_{Str.}$(C-H)$_{ali.}$ 1215 m ν(C-O)
Z4D$_5$	3059 m	2854 w	…..	1589 s	1489 s 1446 m	…..	1257 m 1157 m	829 s 759 m	540 m	2924 m ν$_{Str.}$(C-H)$_{ali.}$ 694 s ν(C-Cl)
Com.	**ν(C-H)$_{Str.}$ Benzene**	**ν(C-S-C)$_{Str.}$ Thiazine**	**ν(C=O) β-Lactam**	**ν(C=C) Thiazine**	**ν(C-N) β-Lactam**	**ν(C=C)$_{Aro.}$**	**ν(NO_2) Asym Sym**	**ν(C-N) Thiazine**	**ν(C-Se) Selenazine**	**ν(Others)**
AZ4B$_7$	3110 w	2357 w	1712 m	1643 s	1597 m	1512 m 1465 m	1512 m 1350 m	1253 m 1172 s	…..	2985 s ν$_{Str.}$(C-H)$_{ali.}$ 2951, 2665 w ν$_{Str.}$(C-H)$_{ali., ring}$ 1219 m ν(C-O) 779 w ν(C-Cl) 698 m ν (C-S)$_{Str.}$
AZ4B$_{7'}$	3036 w	…..	1708 m	1651 m	1597 s	1458 m	1508 s 1342 m	1253 s 1172 s	524 w	2955 m ν$_{Str.}$(C-H)$_{ali.}$ 2839 m ν$_{Str.}$(C-H)$_{ali., ring}$ 1026 s ν(C-O) 821 s ν(C-Cl)
AZ4D$_5$	3061 w	2362 w	1726 m	1595 m	1537 m	1485 m 1456 m	…..	1280 m 1170 w	…..	2931 m ν$_{Str.}$(C-H)$_{ali.}$ 2870, 2744 w ν$_{Str.}$(C-H)$_{ali., ring}$ 752 w ν(C-Cl) 698 m ν (C-S)$_{Str.}$
AZ4D$_{5'}$	3062 w	…..	1734 m	1595 m	1575 m	1533 w 1473 m	…..	1261 w 1174 m	592 m	2974 m ν$_{Str.}$(C-H)$_{ali.}$ 2742 m ν$_{Str.}$(C-H)$_{ali., ring}$ 758, 696 m ν(C-Cl)

Table 3: Important IR spectral data cm^{-1} of the studied compounds (s: strong, m: medium, w: weak, br: broad)

^{1}HNMR and ^{13}CNMRSpectra: The ^{1}HNMR and ^{13}CNMR spectral data of synthesized compounds have been listed in Table 4. The structures of all new compounds were confirmed and the formation of four-membered ring by ^{1}HNMR spectra. The ^{1}HNMR spectra of the cephem and Selenacephems compounds show a single signal at the range δ4.12-4.28ppm, which attributed to the (CH-Cl) protons. The cephem and selenacephem derivatives are characterized by showing doublet signal at δ 5.56-6.65ppm and at δ 5.74-6.30 ppm, which can be assigned to the (CH-S) and (CH-Se) protons, respectively.[1,16]The ^{1}HNMR spectra of the cephems and selenacephems (AZ4B$_7$,

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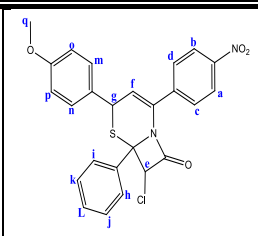
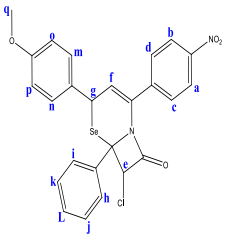
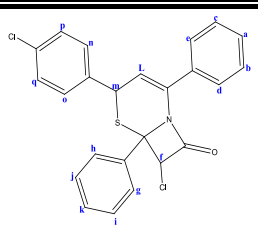
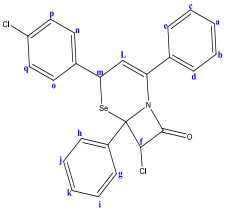
AZ4B$_{7'}$, AZ4D$_5$ and AZ4D$_{5'}$) are characterized by showing doublet signal at δ 6.09-7.08ppm, which attributed to the (CH=C)protons of 1,3-Thia- orselenazine ring.[16]The compounds (AZ4B$_7$ andAZ4B$_{7'}$) shows a single signal at δ (3.04 – 3.79) ppm due to the methoxy protons.[30]In addition, multiple signals that appear at δ 6.88-8.31ppm can be attribute to aromatic rings of all the studied compounds.[29,16]Therefore, the ^{1}HNMR result supports the formation of cephemor selenacephemring.

The ^{13}C-NMR spectra of all studied compounds show signal at the range δ (152.17 - 139.24) ppm and signal at δ (112.77 - 110.36)ppm which attribute to the 4-C and 5-C of 1,3-Thia- or selenazine ring, respectively.[2,16]The ^{13}C-NMR spectra of the prepared compounds (Z4B$_7$, Z4B$_{7'}$, Z4D$_5$ and Z4D$_{5'}$) show signal at the range δ 163.43 - 165.52 ppm is due to the imine functional group (C=N) in 6H-1,3-thia- or selenazine ring.[2]Also, the spectra of all prepared compounds exhibited signal at δ (41.06-44.39)ppm and signal at δ (34.32-43.27)□ppm which can be assigned to the C-S and C-Se in 6H-1,3-Thiazine and 6H-1,3-selenazine ring, respectively.[1,2]The ^{13}C-NMR spectra of cephems and selenacephems show signal at the range δ (170.91 - 172.03)ppm and signal at δ (168.49 - 170.68)ppm which attribute to cyclic carbonyl carbon (C=O), respectively.[16, 31]The cephem or selenacephem derivatives are characterized by showing two signals at (70.46-73.40)ppm and δ (71.92-74.01)ppm and which can be assigned to the C-Cl and C-N in β-lactam ring, respectively.[16, 10]Furthermore, the signal of the methoxy group observed at the range δ (50.57-56.21)□ppm for compounds (Z4B$_7$,Z4B$_{7'}$,AZ4B$_7$ and AZ4B$_{7'}$).Additionally, the signals of aromatic carbons of these synthesized compounds represented at δ (113.59-159.28)□ppm.[30,60-88]The^{13}C-NMR spectral data of the cephemsandselenacephemsare in accord with suggested structures.

Symbol of com.	Structural formula	Chemical shift □ (ppm)
ZA4B$_7$		8.08 (dd, 2H, *J* = 10 Hz, H-a, H-b) 7.95 (d, 2H, *J* = 5 Hz, H-c, H-d) 7.83 (d, 2H, *J* = 15 Hz, H-h, H-i) 7.56 (t, 2H, J_1 = 25 Hz, J_2 = 35 Hz, H-j, H-k) 7.27 (t, 1H, J_1 = 10 Hz, J_2 = 5 Hz, H-L) 6.93 (d, 2H, *J* = 10 Hz, H-m, H-n) 6.88 (d, 2H, *J* = 5 Hz, H-o, H-p) 6.73 (d, 1H, *J* = 10 Hz, H-f) 6.65 (d, 1H, *J* = 10 Hz, H-g) 4.15 (s, 1H, H-e) 3.79 (s,3H, CH$_3$-q)
ZA4B$_{7'}$		7.87 (dd, 2H, *J* = 10 Hz, H-a, H-b) 7.79 (dd, 2H, *J* = 10 Hz, H-c, H-d) 7.68 (d, 2H, *J* = 15 Hz, H-h, H-i) 7.50 (t, 2H, J_1 = J_2 = 25 Hz, H-j, H-k) 7.22 (t, 1H, J_1 = 10 Hz, J_2 = 5 Hz, H-L) 6.92 (dd, 2H, *J* = 10 Hz, H-m, H-n) 6.61 (d, 2H, *J* = 5 Hz, H-o, H-p) 6.09 (d, 1H, *J* = 15 Hz, H-f) 5.74 (d, 1H, *J* = 15 Hz, H-g) 4.28 (s, 1H, H-e) 3.04 (s,3H, CH$_3$-q)
Symbol of com.	**Structural formula**	**Chemical shift □ (ppm)**

Symbol of com.	Structural formula	Chemical shift □ (ppm)
AZ4D$_5$		7.91 (d, 2H, *J* = 5 Hz, H-d, H-e) 7.68 (t, 1H, J_1 = J_2 = 5 Hz, H-a) 7.66 (d, 2H, *J* = 5 Hz, H-g, H-h) 7.64 (t, 2H, J_1 = J_2 = 5 Hz, H-b, H-c) 7.59 (t, 2H, J_1 = J_2 = 20 Hz, H-i, H-j) 7.43 (t, 1H, J_1 = 15 Hz, J_2 = 20 Hz, H-k) 7.33 (d, 2H, *J* = 5 Hz, H-p, H-q) 7.24 (d, 2H, *J* = 10 Hz, H-n, H-o) 6.84 (d, 1H, *J* = 10 Hz, H-L) 5.56 (d, 1H, *J* = 10 Hz, H-m) 4.24 (s, 1H, H-f)
AZ4D$_{5'}$		8.31 (d, 2H, *J* = 10 Hz, H-d, H-e) 8.13 (d, 2H, *J* = 5 Hz, H-g, H-h) 7.91 (t, 1H, J_1 = J_2 = 5 Hz, H-a) 7.68 (t, 2H, J_1 = J_2 = 5 Hz, H-b, H-c) 7.65 (t, 2H, J_1 = J_2 = 5 Hz, H-i, H-j) 7.58 (dd, 2H, *J* = 10 Hz, H-p, H-q) 7.47 (t, 1H, J_1 = 5 Hz, J_2 = 10 Hz, H-k) 7.27 (dd, 2H, *J* = 5 Hz, H-n, H-o) 7.08 (d, 1H, *J* = 10 Hz, H-L) 6.30 (d, 1H, *J* = 20 Hz, H-m) 4.12 (s, 1H, H-f)
Symbol of com.	**Structural formula**	**Chemical shift □ (ppm)**

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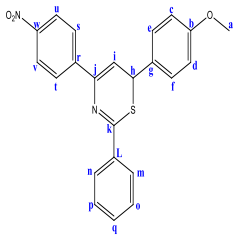
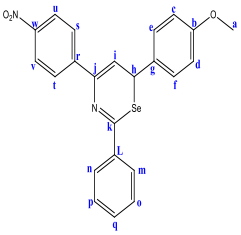
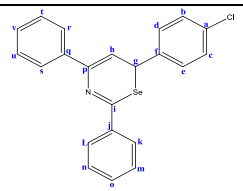
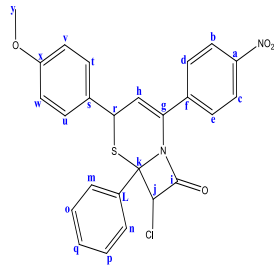
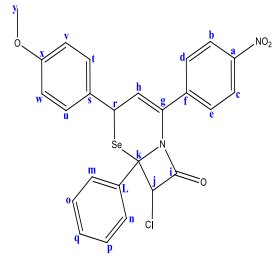
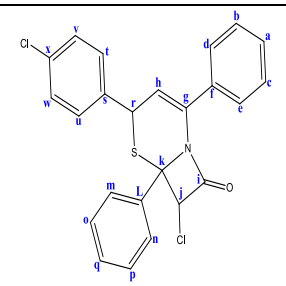
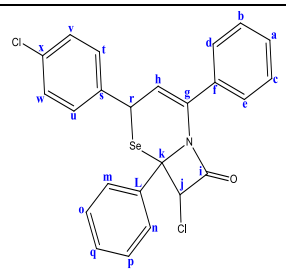
Z4B$_7$		163.90 (1C, C - k) 157.00 (1C, C - b) 152.17 (1C, C - j) 146.91 (1C, C - w) 137.92 (1C, C - r) 133.91 (1C, C - L) 131.89 (1C, C - g) 130.78 (1C, C - q) 129.07 (2C, C – o, C – p) 128.44 (2C, C – e, C – f) 128.13 (2C, C – s, C – t) 127.99 (2C, C – m, C – n) 123.30 (2C, C – u, C – v) 113.59 (2C, C – c, C – d) 110.36 (1C, C - i) 52.57 (1C, CH_3 - a) 43.98 (1C, C - h)
Z4B$_{7'}$		165.52 (1C, C - k) 159.28 (1C, C - b) 150.72 (1C, C - j) 142.90 (1C, C - w) 138.02 (1C, C - r) 135.46 (1C, C - L) 133.24 (1C, C - g) 129.64 (1C, C - q) 126.96 (2C, C – o, C – p) 126.70 (2C, C – e, C – f) 126.43 (2C, C – s, C – t) 126.23 (2C, C – m, C – n) 121.34 (2C, C – u, C – v) 113.84 (2C, C – c, C – d) 112.47 (1C, C - i) 56.05 (1C, CH_3 - a) 36.24 (1C, C - h)
Z4D$_5$		163.43 (1C, C - i) 142.37 (1C, C - f) 139.24 (1C, C - p) 137.58 (1C, C - j) 133.31 (1C, C - q) 132.43 (1C, C - a) 130.47 (1C, C - o) 129.89 (1C, C - v) 129.46 (2C, C – m, C – n) 129.10 (2C, C – b, C – c) 128.42 (2C, C – t, C – u) 127.96 (2C, C – d, C – e) 127.46 (2C, C – r, C – s) 125.77 (2C, C – k, C – L) 112.40 (1C, C - h) 34.32 (1C, C - g)
ZA4B$_7$		170.91 (1C, C - i) 158.91 (1C, C - x) 149.53 (1C, C - a) 142.11 (1C, C - g) 141.47 (1C, C - f) 136.73 (1C, C - L) 135.87 (1C, C - s) 131.67 (2C, C – t, C – u) 130.74 (1C, C – q) 128.90 (2C, C – o, C – p) 128.00 (2C, C – d, C – e) 125.05 (2C, C – m, C – n) 124.46 (2C, C – b, C – c) 115.57 (2C, C – v, C – w) 112.37 (1C, C - h) 73.51 (1C, C - k) 72.25 (1C, C - j) 50.57 (1C, CH_3 - y) 41.06 (1C, C - r)
ZA4B$_{7'}$		168.49 (1C, C - i) 157.67 (1C, C - x) 153.50 (1C, C - a) 142.87 (1C, C - g) 142.73 (1C, C - f) 133.96 (1C, C - L) 133.79 (1C, C - s) 130.29 (2C, C – t, C – u) 128.19 (1C, C – q) 127.87 (2C, C – o, C – p) 127.35 (2C, C – d, C – e) 125.24 (2C, C – m, C – n) 123.43 (2C, C – b, C – c) 115.98 (2C, C – v, C – w) 112.57 (1C, C - h) 74.01 (1C, C - k) 73.40 (1C, C - j) 56.21 (1C, CH_3 - y) 37.07 (1C, C - r)
AZ4D$_5$		172.03 (1C, C - i) 142.94 (1C, C - g) 139.96 (1C, C - s) 136.99 (1C, C - f) 135.17 (1C, C - L) 134.95 (1C, C - x) 130.08 (1C, C - a) 128.90 (2C, C – v, C – w) 128.61 (2C, C – t, C – u) 128.42 (2C, C – b, C – c) 128.19 (1C, C – q) 127.64 (2C, C – o, C – p) 125.94 (2C, C – m, C – n) 125.66 (2C, C – d, C – e) 112.77 (1C, C - h) 73.47 (1C, C - k) 70.46 (1C, C - j) 44.39 (1C, C - r)
AZ4D$_{5'}$		170.68 (1C, C - i) 142.70 (1C, C - g) 139.14 (1C, C - s) 135.96 (1C, C - f) 134.23 (1C, C - L) 133.81 (1C, C - x) 129.69 (1C, C - a) 129.26 (2C, C – v, C – w) 128.87 (2C, C – t, C – u) 128.58 (2C, C – b, C – c) 128.35 (1C, C – q) 127.91 (2C, C – o, C – p) 126.53 (2C, C – m, C – n) 125.85 (2C, C – d, C – e) 110.44 (1C, C - h) 71.92 (1C, C - k) 71.34 (1C, C - j) 43.27 (1C, C - r)

Table 4:^{1}H and ^{13}C NMR spectral data of studied compounds

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EI-mass: Mass spectrometry as a powerful structural characterization technique in coordination chemistry has been successfully used to confirm the molecular ion peaks of the cephems (AZ4B$_7$andAZ4D$_5$) and Selenacephems (AZ4B$_{7'}$andAZ4D$_{5'}$).The electron impact spectrum of the synthesized compounds is differentiating by high to low relative intensity molecular ion peaks.[1,33] The mass spectrum of all studied compounds detects the molecular ion peaks $[M]^+$ are in excellent acceptance with the suggested structures. The potential suggested ion fragments with the appearance of the result of fragmentation of these synthesized compounds are shown in schemes 3 and 4, furthermore the peaks intensity gives an idea about the stability of fragments primarily with the base peaks.

The mass spectrum of the compound AZ4B$_7$showsseveral fragmentation peaks at m/z 363, m/z 329, m/z270, m/z 224, m/z 184 and m/z 86, these peaks can be assigned to $[C_{19}H_{22}ClNO_2S]^{\bullet+}$, $[C_{19}H_{23}NO_2S]^{\bullet+}$, $C_{16}H_{16}NO_3^+$, $[C_{16}H_{16}O]^{\bullet+}$, $C_9H_{11}ClNO^+$ and $C_4H_8NO^+$ions, respectively. On other hand the mass spectrum of compound AZ4D$_{5'}$characterized by the appearance of six fragmentation peaks at m/z 450, m/z 410, m/z 327, m/z 178, m/z 149 and m/z 86, which can be attributed to $[C_{24}H_{18}ClNOSe]^{\bullet+}$, $C_{22}H_{18}ClNSe^+$, $C_{14}H_{15}ClNOSe^+$, $[C_5H_9NOSe]^{\bullet+}$, $C_3H_4NOSe^+$and $C_4H_8NO^+$ions respectively. The base peaks at m/z 86 can be assigned to $C_4H_8NO^+$ ion for most cephemcompounds.Successive degradation of the target compound and appearance of different peaks duet various fragments are good evidence for the molecular structure of the investigated compounds, Table 5.

Symbol of com.	m/z	Relative intensity %	Fragment
AZ4B7	479	1	$[m]^+$, $[C_{25}H_{19}ClN_2O_4S]^{\bullet+}$
	363	3.6	$[m-116]^+$, $[C_{19}H_{22}ClNO_2S]^{\bullet+}$
	329	1.2	$[m-150]^+$, $[C_{19}H_{23}NO_2S]^{\bullet+}$
	270	1.1	$[m-209]^+$, $C_{16}H_{16}NO_3^+$
	224	1.6	$[m-255]^+$, $[C_{16}H_{16}O]^{\bullet+}$
	184	1.5	$[m-295]^+$, $C_9H_{11}ClNO^+$
	86	100	$[m-393]^+$, $C_4H_8NO^+$
AZ4D5	439	1.7	$[m]^+$, $[C_{24}H_{17}Cl_2NOS]^{\bullet+}$
	426	3	$[m-13]^+$, $[C_{24}H_21Cl_2NS]^{\bullet+}$
	294	7.8	$[m-145]^+$, $C_{18}H_{16}NOS^+$
	180	71	$[m-259]^+$, $C_{10}H_{14}NS^+$
	138	31.2	$[m-301]^+$, $[C_8H_{10}S]^{\bullet+}$
	86	100	$[m-353]^+$, $C_4H_8NO^+$
AZ4B7'	526	8	$[m]^+$, $[C_{25}H_{19}ClN_2O_4Se]^{\bullet+}$
	494	15	$[m-32]^+$, $C_{24}H_{16}ClN_2O_3Se^+$
	436	5.5	$[m-90]^+$, $C_{23}H_{21}N_2O_2Se^+$
	390	2	$[m-136]^+$, $[C_{23}H_{21}NSe]^{\bullet+}$
	263	100	$[m-263]^+$, $C_9H_{11}ClNOSe^+$
	204	9	$[m-322]^+$, $[C_7H_{11}NOSe]^{\bullet+}$
AZ4D5'	488	1.4	$[m]^+$, $[C_{24}H_{17}Cl_2NOSe]^{\bullet+}$
	450	1	$[m-38]^+$, $[C_{24}H_{18}ClNOSe]^{\bullet+}$
	410	2	$[m-78]^+$, $C_{22}H_{18}ClNSe^+$
	327	7.5	$[m-161]^+$, $C_{14}H_{15}ClNOSe^+$
	178	4.5	$[m-310]^+$, $[C_5H_9NOSe]^{\bullet+}$
	149	45	$[m-339]^+$, $C_3H_4NOSe^+$
	86	100	$[m-402]^+$, $C_4H_8NO^+$

Table 5: Mass spectroscopic of prepared compounds

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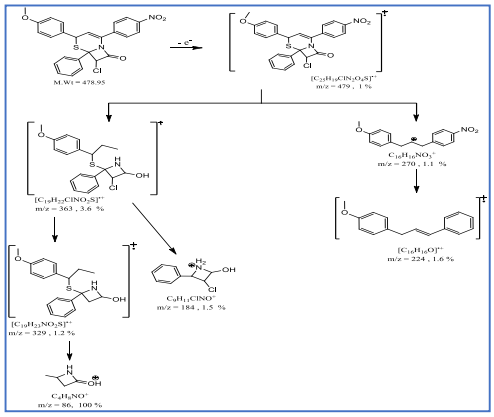
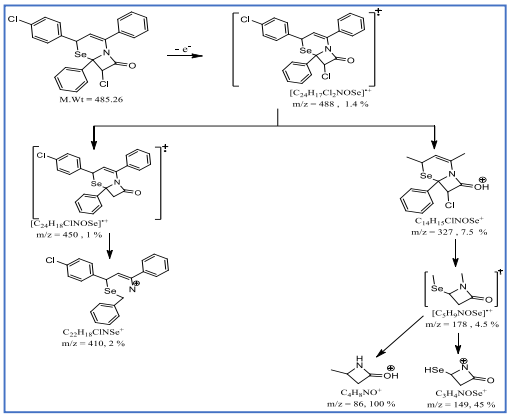


Scheme 3: The fragmentation pattern proposed for compound



Scheme 4: The fragmentation pattern proposed for

Biological activity

Median lethal dose (LD_{50})

The lethal dose (LD_{50}) of the studied compounds ($AZ4B_7$ and $AZ4B_{7'}$) in-vivo was determined in mice via intraperitonially injecting dosages ranging from 500-750 mg/kg with equal spacing (concentrations) between doses. Our data revealed that LD_{50} values were 718.6 and 758.45 mg/kg for the compounds $AZ4B_7$and$AZ4B_{7'}$, respectively. The results may give an indicated about the moderately toxicity effect of the studied compounds and clinical change that observed in the mice after giving different doses. The toxic signs observed in injected mice may be manifested in some behaviours such as tremors, straight tail, salivation, urination, lacrimation, defecation, shortness of breath, excitation, muscle fasciculation's, capillary bulge, convulsions and also the tortuous reflex in some treatments, and finally Death at high toxic doses, Table 6.[34,35,88-100]

Test characterization	**Results**	
	$AZ4B_7$	**$AZ4B_{7'}$**
Doses range	500-700 = 200 mg/kg	500-750=250 mg/kg
First dose	500 mg/kg	500 mg/kg
Last dose	700 mg/kg	750 mg/kg
Up and down dose	50 mg/kg	50 mg/kg
Median lethal dose (LD_{50}) mg/kg	718.6 mg/kg	758.45 mg/kg
Effective dose (LD_{50} /10) mg/kg	71.86 mg/kg	75.84 mg/kg
No. of mice	8 (XXOOOOXX)	8 (XXOXOOXO)
Onset of toxic signs	5-16 minutes	5-24 minutes
Toxic signs	Rolling convulsions, excitation, salivation, choreoathetosis, tremors, death	Salivation, dyspnoea, convulsions, excitation, tremors, muscle fasciculation, death

Table 6: Toxicity results (LD_{50}) of and toxic signs on mice

Antibacterial activity

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The sensitivity of four human pathogenic microbes (two of Gram-positive bacteria: Staphylococcus aureus, Bacillus and two of Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa) to the new synthetic heterocyclic compounds ($AZ4B_7$, $AZ4D_5$, $AZ4B_{7'}$ and$AZ4D_{5'}$) was tested and compared to that of commercially available antibacterial antibiotics tetracycline and amoxicillin. Our study confirmed that the cephem and selenacephem compounds had antibacterial activity (increases as the compound concentration increases) against the studied bacteria, also minimum inhibitory concentration MIC which can define as the lowest concentration of the compound in medium which out visible growth of the test organisms in concentration ranging from 1-500 mg/mL, as shown in Table 7.

All the scientific studies reported that the antibiotics had the ability to introduce the main basis for the therapy of microbe's infections. On the other hand, the bacteria had a highly genetic variability which enables them to rapidly evade the effect of antibiotics via developing antibiotic resistance. Furthermore, the development in recent years of the ability of pathogenic bacteria and parasites to resist multi-drugs has resulted in major clinical problems in the treatment of infectious diseases.[36] The toxicity of some antimicrobial drugs on host tissues and other problems have raised the need for attention in the search for new antimicrobial substances. Moreover, Escherichia coli is one of the most dangerous microbes that cause many common diseases in humans, frequently associated with urinary tract infections, a common problem in stressed people and office owners who share communal toilets and followed by the risk of pseudomonas aeruginosa infection, which is often associated with infant diseases. Also, the main human bacterial agent causing a variety of variety of potentially serious infections and clinical manifestations is Staphylococcus aureus if allowed to enter the bloodstream or internal tissues [37].

In the present work, the antibacterial activity of the new synthetic compounds may be attributed to the fact that these two groups of bacteria differ by its cell wall component and its thickness. The ability of these new compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall, thereby inhibiting the microbial growth [37].Among the new synthetic heterocyclic compounds, $AZ4B_{7'}$ was found to be more effective than positive control (tetracycline and amoxicillin) against Gram-negative bacteria (E. coli) with an inhibition zone (IZ) of 27 mm at the concentration of **5**mg/mL. This result may come from the fact that the membrane of Gram-negative bacteria is surrounded by an outer membrane containing lip polysaccharides, which makes the compound able to combine with the lipophilic layer in order to enhance the permeability of the membrane to Gram-negative bacteria. In conclusion, the antibacterial activity of any compound may be related to the cell wall structure of bacteria due to the importance of this wall for bacterial survival. Thus, the ability of antibiotics to kill or inhibit the growth of bacteria is may be through inhibition of a step in peptidoglycan synthesis by gram positive bacteria.[38,39]

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In the case of antibacterial activity against Gram-positive bacteria (Staphylococcus aureus and Bacillus), all compounds were found to have activity ranged between high and moderate. Our results indicated that the compound AZ4B$_{7'}$possessed the highest antibacterial activity against Gm+Ve (Staphylococcus aureus) with an IZ of (30, 36, 26, and 29 mm) at concentrations of (5, 25, 125 and 250 mg/mL). Also, AZ4B$_{7'}$ compound showed more potent compared to the positive control (IZ= 4-25) mm at the same concentration. From the other hand, our data pointed out that compound AZ4B$_7$ showed a good antibacterial activity against Gm+Ve (Bacillus) with an IZ ranging from (12-32)mm as compared to tetracycline (IZ= 11-30mm) at the concentrations (5-250)mg/mL.

The antimicrobial activity of these new synthetic heterocyclic compoundsis may attribute to the basis of their structures, mainly possessing electron withdrawing groups like chlorine. Also, the presence of halogen atom in the molecule increases the lipophilicity of the molecule and facilitates hydrophobic interactions of the molecule with specific binding sites on either receptor or enzymes. Furthermore, the Cl^-ion in the compounds AZ4B$_7$, AZ4B7', AZ4D5 and AZ4D5' can enhance the antibacterial activity due to the killing microbes or inhibiting their multiplication by blocking their active site. Moreover, the presence of heteroatom's resulted in an increase in the antimicrobial activity [40,41] .Finally, all cephem drugs are selective inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria.[42] The biological activity of cephem skeleton is believed to be associated with the chemical reactivity of the ring and on the substituent's especially at nitrogen of β-lactam ring.[43]

The MIC of tested compounds in this study against the test organisms ranged between (5-25) mg/mL,Table 7. Antimicrobial agents with low activity against an organism had a high MIC while a highly active antimicrobial agent gave a low MIC.The most resistant microorganisms were Escherichia coli and Pseudomonas aeruginosa, whereas the most sensitive microorganisms were Staphylococcus aureus and Bacillus. The lowest MIC value of (5) mg/mL was recorded on S. Aureus and on Bacillus with compoundsAZ4B$_7$, AZ4B$_{7'}$ and AZ4D$_5$. The compounds AZ4B$_7$and AZ4B$_{7'}$were more active as compared with positive control (amoxicillin) and had the lowest MIC value of (5) mg/mL was obtained on Pseudomonas aeruginosa. However, the highest MIC value of 25 mg/mL was recorded on E. coli and on Pseudomonas aeruginosa with compounds (AZ4D$_5$ and AZ4D$_{5'}$), whereas the highest MIC value of (25) mg/mL was obtained on Staphylococcus aureus and on Bacillus with compound AZ4D$_{5'}$. The results of the present study suggest that the cephem and selenacephem compounds possess remarkable toxic activity against bacteria and may assume pharmacological importance.[6, 44]

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Compounds	Diameter of inhibition zone (mm) Bacillus							Compounds	Diameter of inhibition zone (mm) Staphylococcus aureus						
	Concentration (mg/mL)								Concentration (mg/mL)						
	1	5	25	125	250	500	MIC		1	5	25	125	250	500	MIC
AZ4B$_7$	NI	12	22	31	32	36	5	**AZ4B$_7$**	NI	23	33	26	28	29	5
AZ4B$_{7'}$	NI	10	28	30	32	33	5	**AZ4B$_{7'}$**	NI	30	36	26	29	29	5
AZ4D$_5$	NI	15	17	17	19	20	5	**AZ4D$_5$**	NI	15	25	20	20	20	5
AZ4D$_{5'}$	NI	NI	12	25	25	29	25	**AZ4D$_{5'}$**	NI	NI	20	16	17	22	25
Amoxicillin*	5	8	33	38	44	52	1	**Amoxicillin***	10	23	40	49	51	58	1
Tetracycline*	5	11	14	22	30	50	1	Tetracycline*	NI	4	10	14	25	48	5
Compounds	**Diameter of inhibition zone (mm) Escherichia coli**							**Compounds**	**Diameter of inhibition zone (mm) Pseudomonas aeruginosa**						
	Concentration (mg/mL)								**Concentration (mg/mL)**						
	1	**5**	**25**	**125**	**250**	**500**	**MIC**		**1**	**5**	**25**	**125**	**250**	**500**	**MIC**
AZ4B$_7$	NI	23	28	28	29	30	5	**AZ4B$_7$**	NI	22	29	24	25	26	5
AZ4B$_{7'}$	NI	27	30	25	28	29	5	**AZ4B$_{7'}$**	NI	26	32	20	20	22	5
AZ4D$_5$	NI	14	11	12	12	12	5	**AZ4D$_5$**	NI	NI	10	12	12	14	25
AZ4D$_{5'}$	NI	NI	12	13	14	15	25	**AZ4D$_{5'}$**	NI	NI	11	12	12	12	25
Amoxicillin*	NI	23	39	46	51	57	5	**Amoxicillin***	NI	NI	NI	NI	NI	17	500
Tetracycline*	NI	8	11	15	21	44	5	Tetracycline*	NI	6	8	17	30	52	5

***Standard, NI = No Inhibition**

Table 7: Sensitivity of human pathogenic selected microbes to the new synthetic heterocyclic compounds

Antioxidant Activity

Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals are being generated during bioorganic redox process and normal

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cellular metabolism, play a significant role in oxidative stress related to the development and pathogenesis of life-limiting various diseases such as cancer, diabetes mellitus, arteriosclerosis, rheumatoid arthritis, and others.[21] It is scientifically known that exposure of a normal cells to free radical lead to damage structures via interfering with functions of enzymes and critical macromolecules within cell such as lipids, proteins and nucleic acids. Conversely, antioxidants are man-made or natural substances which possess the ability to prevent or delay some types of cell damage caused by free radical-induced oxidative stress. In the past decade, the scientists of medical chemists, food chemists, and biologists have focused their attention largely on the research and testing of a variety of new and effective natural or synthetic antioxidants as a preventive strategy against human diseases in order to reduce and/or inhibit oxidative damage related to free radical reactions.[21]

In the present study, antioxidant activity of the new synthetic compounds was quantified by the β-carotene bleaching method. In this method, linoleic acid undergoes an oxidation reaction to form unstable hydro peroxides which easily attack and oxidize the β-carotene molecules rich in double bonds, causing the beta-carotene molecule to lose its colour and double bond rapidly. In this method, linoleic acids undergoes oxidation reaction to unstable hydroperoxides which easily attack and oxidation of the double bonding rich β-carotene molecules making it a rapid decolorization and lose their double bonds. Hence, presence of antioxidant compound can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.[21]Accordingly, the absorbance values were decreased rapidly in the samples devoid of antioxidants, while in the presence of one of the antioxidants it was observed that they retained their colour and therefore their absorbance was high for a longer period.[45]

The results in Table 8 and Figure 1-2were indicated an increase in the antioxidant activity of the synthetic compounds and standard in the order of$AZ4B_7$<$AZ4D_5$< BHT with corresponding percentages values of (53, 55.4 and 82.3) %, respectively. On the other hand, the lowest activity was observed for compounds $AZ4D_{5'}$ and $AZ4B_{7'}$ with corresponding inhibition ratio (24.2 and 18.1)%, respectively. A possible explanation for the higher antioxidant activity of these compounds ($AZ4D_5$ and $AZ4B_7$) might be due to the following reasons; first, since compound $AZ4B_7$ have an additional methoxy group which increase the antioxidant activity, this activity may be correlated with the introduction of electron donor substituent which stabilizes the generated radical during oxidation.[46] Second, compounds $AZ4D_5$ and $AZ4B_7$have (–S-C-N–) moieties in 6H-1,3-thiazine ring which can act as a scavenger for radicals to prevent oxidative cellular damage and thus enhance antioxidant properties.[4]Third, compounds ($AZ4D_5$ and $AZ4B_7$) have a β-lactam ring which can act as a scavenger for radicals to prevent oxidative cellular damage and thus enhance antioxidant properties.[47,90-102]

The finding that compound $AZ4D_5$possessed a strong protective effect is interesting and points to the potential use of this new compound as an agent to overcome oxidative stress

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that associated with cellular metabolism and disease conditions.[47] The mechanism by which $AZ4D_5$ protects the body's cells from oxidative damage may require further study and investigation. Interestingly, the relative antioxidant effect of some β-lactamantibiotics such as ampicillin on oxygen-reactive species (ROS) has been reported and a possible therapeutic role for β-lactamagents in protecting host tissues from oxidative damage has been proposed. Actually, keto lactam ring or thiazolidine ring is responsible to initiate the free radical scavenging activity due to its N-H and C=O moieties.[47, 48,80-101]

Notably, scientific studies have confirmed that compounds in general, including those that have antioxidant properties, may be subjected to metabolism in vivo through specialized enzymatic systems in the body, which often convert lipophilic chemical compounds into polar products that are easily secreted. Moreover, because the metabolism of any compound can result in an increase or a decrease in its toxicity.[21] Therefore, we expect that $AZ4D_5$ and other new synthetic compounds to enter different metabolic pathways in the body that may differently modify from their structure and/or toxicity and this require further researches. Again, the possible exact mechanism via which compound $AZ4D_5$ and the new other synthetic compounds protects against oxidative damage will be the matter of future studies and must be confirmed in a more controlled experimental design.[21]

Comp. symbol	Aj	At	Aj*	At*	AA%
BHT	0.582±0.01	0.544±0.011	0.456±0.031	0.241±0.016	82.3
$AZ4B_7$	0.500±0.019	0.399±0.024	0.456±0.031	0.241±0.016	53
$AZ4B_{7'}$	0.535±0.027	0.359±0.008	0.456±0.031	0.241±0.016	18.1
$AZ4D_5$	0.577±0.023	0.481±0.012	0.456±0.031	0.241±0.016	55.4
$AZ4D_{5'}$	0.560±0.007	0.397±0.017	0.456±0.031	0.241±0.016	24.2

Table 8: Antioxidant Activity of Prepared Compounds, the values is the mean ± SD

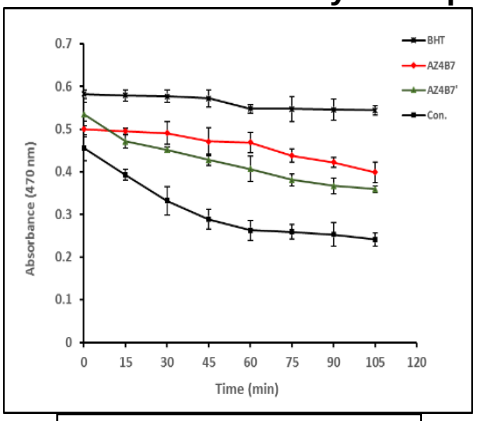

Fig.1: Antioxidant Activity of Compounds $AZ4B_7$ and $AZ4B_{7'}$

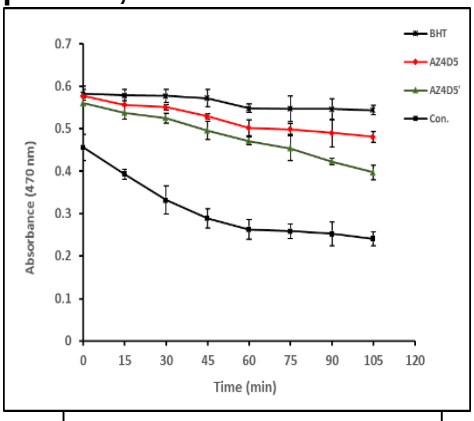

Fig.2: Antioxidant Activity of Compounds $AZ4D_5$ and $AZ4D_{5'}$

Cell Cytotoxicity (anticancer) study

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The process of carcinogenesis initiates from a set of mutations induced by carcinogens, that affect regulation of proliferation and involves series of molecular events which trigger progressive changes from pre-invasive histological transformation to an invasive neoplastic process.[49] On the other hand, Chemo preventive intervention involves a pharmacological approach that utilizes natural, synthetic or biologic chemical agents with an objective to reverse, suppress or prevent carcinogenic progression. Also, the efficacy of a Chemo preventive agent depends on its ability to inhibit the development of invasive cancer, either by blocking the transformative, hyper proliferative and inflammatory processes that initiate carcinogenesis or by arresting or reversing the progression of premalignant cells to malignant by suppressing angiogenesis and metastasis. Furthermore, the appropriate use of Chemo preventive agent depends on the understanding of its mechanism of action at all levels i.e. at molecular, cellular, tissue and organs levels, as well as in the animal as a whole.[50]

Hence, an interest in the pharmacological effects of bioactive compounds, both of prepared or isolated from natural products, on cancer treatments and prevention has increased dramatically over the past twenty years. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells.[51]

For this, one of the first goals of researchers and scientists is to discover and develop a new anti-cancer drug that has good efficacy and does not cause any of the side effects of current chemotherapy drugs. Therefore, the need for a time-saving, low-cost, high-throughput drug efficacy testing system has led to the emergence of an in vitro Model cytotoxicity testing on human cancer cell lines.[50]

In this work, the cytotoxic effects of the synthesized compounds against breast cancer cell line (MCF-7) were evaluated using 5-fluorouracil (5-FU) as a reference cytotoxic drug. The IC_{50} and cell viability percent of MCF-7 cancerous at different concentrations ranging from 6.25-100 µg/mL are given in Table 9. The results showed that compounds $AZ4B_7$ and $AZ4B_{7'}$were comparable to that of 5-FU (positive control), while compound $AZ4B_{7'}$(IC_{50} = 44.78 µg/mL) is more cytotoxic agent than 5-FU (IC_{50} = 97.47 µg/mL), Table 9. It is evident that, the tested compounds showed anticancer activity in all concentrations and the effects of these compounds were dose dependent, i.e. by increasing the concentration in the culture media; the percentage of cells viability is decreased (this means that the percentage of dead cells has increased). IC_{50} values ranged from 44.78 to 97.47 µg/mL. Also, we can note that the cytotoxic activity of compound $AZ4B_{7'}$ was higher in cancerous cells when compared with the compound $AZ4B_7$ especially at a concentration 100 µg/mL.

β-lactam compounds revealed their pharmaceutical significance as anticancer agents. Numerous antitumor β-lactams that are currently used to treat cancer, such as anthracyclines, bleomycin, mitomycin C, dactinomycin, and mithramycin. The major mechanism of action for these antitumor β-lactams is inhibition of cell wall synthesis, DNA intercalation or inhibition of DNA synthesis.[6]The presence of β-lactam ring in the

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molecular structure of compounds AZ4B$_{7'}$ and AZ4B$_7$ is related to anticancer activity by inhibiting the transpeptidase enzyme, which catalyzes the cross-linking of the peptidoglycan strands in the cell wall phase of the cancer cell wall biosynthesis. The β-lactam ring can bind to the active site of the transpeptidase enzyme since its structure resembles that of the substrate, which is the terminal D-ala-D-ala dipeptide of the pentapeptide of each monomer unit. Note that D-ala-D-ala dipeptide of the substrate can exist in multiple conformations formed by rotation around the C–C single bonds but a β-lactam molecule has a limited variety of conformation because of the rigidity of the four-membered lactam ring. Of the many conformations possible for the terminal dipeptide the one that binds to the enzyme resembles the structure of the β-lactam ring, and thus, the two can compete for binding to the active site of the enzyme. The –C (O)–N bond of the β-lactam mimics the –C(O)–N of the peptide bond of the terminal dipeptide. Therefore, inhibition the formation of the cancer cell wall, which leads to cells death.[6] In addition, found that a class of β-lactams, the N-thiolated β-lactams, induce tumor cell apoptosis by introducing DNA damage in a potent, and more importantly, a tumor cell-specific manner with little or no effect on normal cells.[52,53] Cainelliet al., describe that 4-alkylidene-bβ lactams inhibit matrix metalloproteinases-2, and -9 (MMP), essential for the tumor-induced neovascularisation. [6]Baniketal., also show that β-lactams with polyaromatic substituent's induce tumor cell death in a variety of breast cancer cell lines.[6] As well, the presence of (-Se-C-N-) moieties in the selenacephem compounds is related to anticancer activity by the interaction with the active site of protein through hydrogen bonding bringing about the hindrance development of cells,[44, 5] however, several novel classes of β-lactams have been shown to possess anticancer properties as well.[6]

On the other hand, the present results clearly indicated that the compound AZ4B$_{7'}$ had an ability to induced apoptosis of MCF-7 Cells, as illustrated in Figure7. Acridine orange (AO) is a vital dye and will stain the nuclei of both live and dead cell to green while ethidium bromide (EB) will stain only cells that have lost membrane integrity to red. Thus, live cells will appear uniformly green while early apoptotic cells will have condensed or fragmented nuclei with bright green color. Late apoptotic cells will show condensed and fragmented orange chromatin. The results showed that increased the compound AZ4B$_{7'}$ concentration resulted in gradual increases in orange and red staining accompanied by reductions in green staining of nuclei, indicating cell damage and apoptosis (Figure7). Therefore, high concentration (100 µg/mL) of AZ4B$_{7'}$ could cause serious membrane damage in around 92% of cells. Moreover, these results indicate that apoptotic rate gradually increase with the AZ4B$_{7'}$ concentration and treatment time. It is verified that at around 100 µg/mLAZ4B$_{7'}$ can induce half of the cells to undergo apoptosis at 48 h, which is consistent with the IC$_{50}$ results.

	Cell Viability %	

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Compounds	Concentration (µg/mL)						IC_{50} µg/mL
	6.25	12.5	25	50	75	100	
$AZ4B_7$	99.35±0.11	98.63±1.15	98.05±0.61	92.11±0.46	90.93±0.25	85.82±1.23	…..
$AZ4B_{7'}$	98.04±1.65	98.58±1.36	93.39±0.74	38.58±0.08	17.17±0.08	7.87±0.03	44.78
5-Fluorouracil	83.13±0.86	80.69±1.07	72.76±0.86	66.57±1.06	58.93±0.61	49.29±0.06	97.47

Table 9: The IC_{50}Values and the Percent of Cell Viability of the Tested

Compounds in Breast Cancer Cell LineMCF-7, the values are the mean ± SD

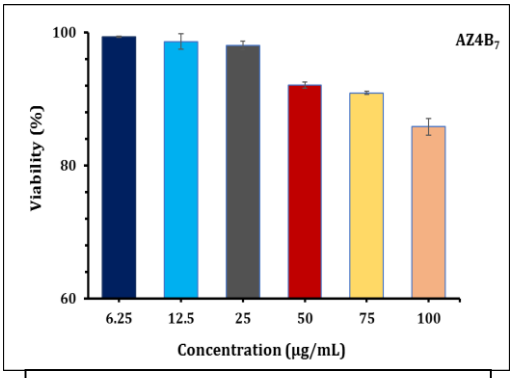


Figure 3: Anticancer Activity of Compound**$AZ4B_7$**at (6.25-100) µg/mL

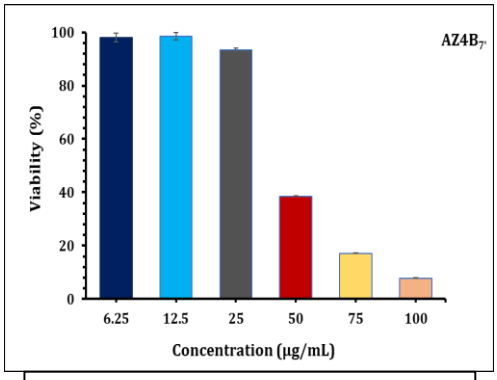


Figure 4: Anticancer Activity of Compound**$AZ4B_{7'}$**at (6.25-100) µg/mL

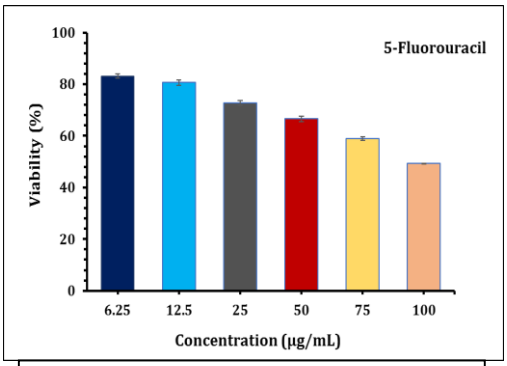
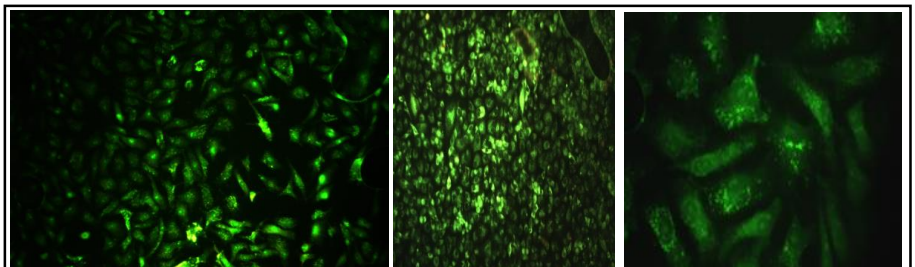


Figure 5: Anticancer Activity of drug **5-Fluorouracil** at (6.25-100) µg/mL



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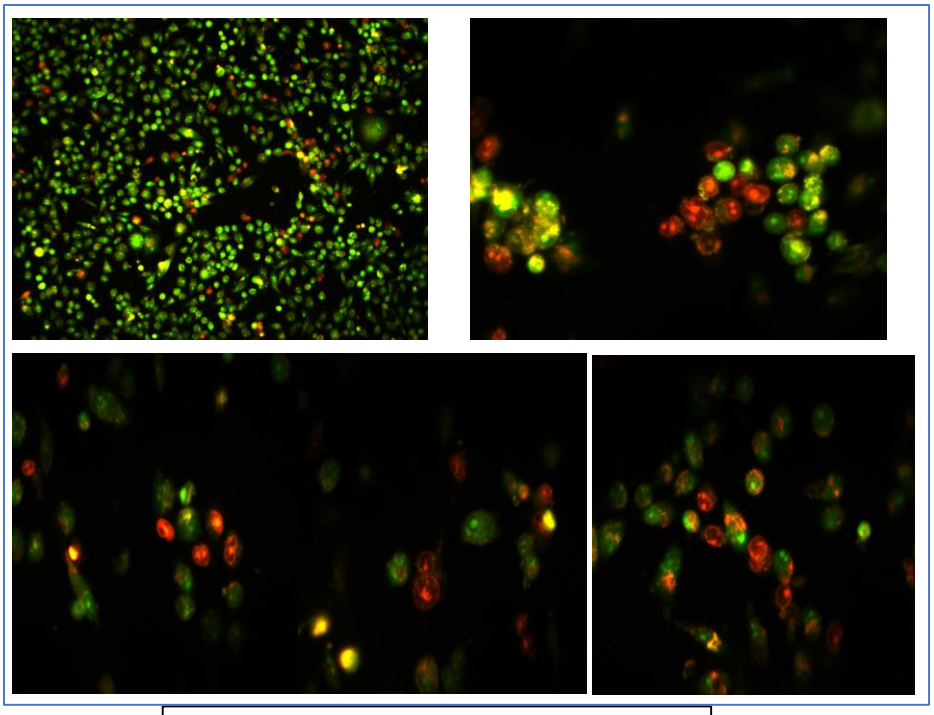

Figure 7: Anticancer Activity of Compound**AZ4B$_7$**at

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DNA Cleavage Study (Genotoxicity assay)

In the present work, we used the flow cytometry technique and acridine orange staining to estimate the genotoxicity of the selected compounds AZ4B$_7$ and AZ4B$_{7'}$, on MCF-7 breast cancer cell line. The dyeacridine orange (AO) interacts with DNA and RNA by intercalation and electrostatic attraction, respectively. This dye is cell permeable and interacts with double-strand DNA by intercalation and fluoresces green, while electrostatic single-strand RNA and DNA fluoresces red. Acridine orange staining has been shown to be useful for measuring apoptosis (a process of programmed cell death that occurs in multi cellular organisms).[54]We tested the ability of the studied compounds to causes DNA fragmentation. The MCF-7-treated cells showed DNA fragmentation, which is the signature feature of apoptosis reported by previous studies.[55, 77-95] The results after 24h incubation at concentration 100µg/mL are shown in Figure8-11.We found that all the tested compounds have DNA fragmentation index DFI% to a good extent, the results listed in Table 10 which establish (DFI%) of the chosen compounds in comparison with theanti-tumor drug 5-Fluorouracil(positive control).The results showed the DFI % is dose-dependent manner and the viable cells (lower left,Figure8-11) are drastically reduced with increasing overlap of these compounds on MCF-7 compared to the negative control at concentration 100 µg/mL.

The results showed that Compound AZ4B$_{7'}$ having a higher percentage with 60.2%compared with5-Fluorouracil (46.95%) and the lowest DFI % was the compound AZ4B$_7$ which recorded 43.9 % at the same concentration.

Previous studies demonstrate the biological activity of β-lactam compounds such as in hibition of" DNA, RNA.[6,52,90-100] Also, Cephems which can bind or cleave DNA are now in great consideration due to their importance in the development of anticancer agents,[5]"additionally, the data regarding with the explanation of in vitro % DFI established, by which the percentage of DFI less than 15% DFI can be represented as an excellent pattern for the high integrity status of DNA,[56,89-102] these results approved the using of these compounds as biomedical and nano medicine applications and gene delivery systems. In conclusion, the obtained results show that the tested compounds exhibit promising potentials as an anticancer compound according to anticancer and DNA fragmentation study.

Sample	Concentration(100 µg / mL)		
	DFI (%) (1)	DFI (%) (2)	DFI (%) Average
AZ4B$_7$	46.2	41.6	43.9
AZ4B$_{7'}$	59.1	61.3	60.2
Positive Control (**5-Fluorouracil**)	45.2	48.7	46.95
Negative Control	1.10	1.40	1.25

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Table 10: DNA Fragmentation Percent (% DFI) of Compounds AZ4B$_7$andAZ4B$_{7'}$ Using MCF-7breast cancercell line

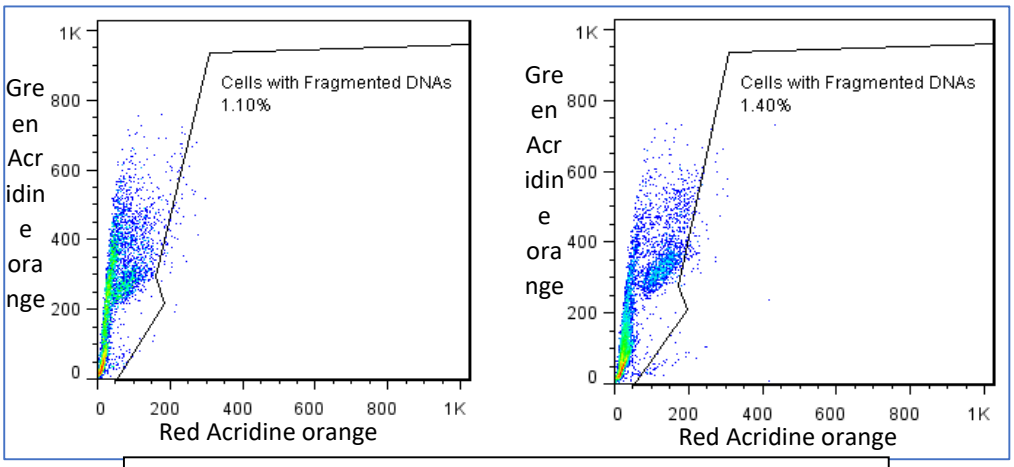

Figure 8: DNA fragmentation of Negative Control

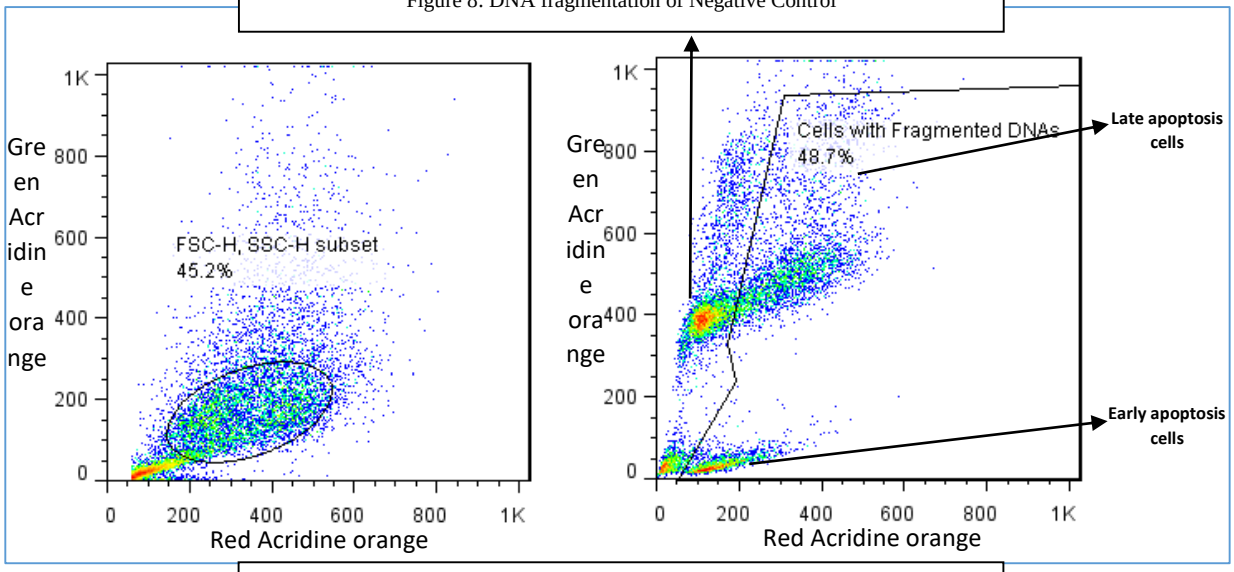

Figure 9: DNA Fragmentation of Positive Control

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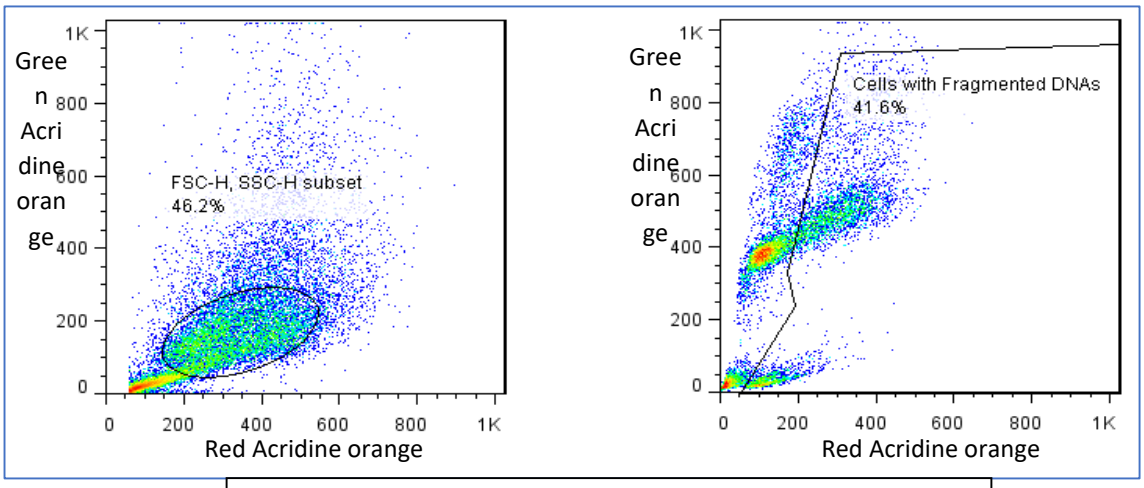

Figure 10: DNA fragmentation of the compound**AZ4B$_{7}$**at (100) µg/mL

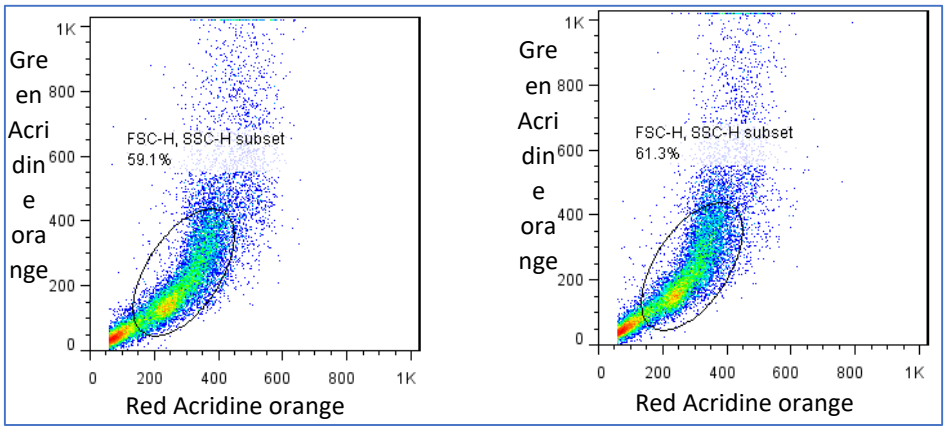

Figure 11: DNA fragmentation of the compound **AZ4B$_{7'}$** at (100) µg/mL

Conclusion

The present study concluded that the Cephemand Selenacephem compounds derived from 6H-1, 3-thia- or selenazines were prepared, characterized and biological evaluated as antibacterial, Cephem or selenacephemring in studied compounds likewise assumed a significant job in the restraint of receptor enzyme. Presence of the 6H-1,3-thia- or selenazines in the biologically active molecules has appeared to assume a vital job in their antioxidant and anticancer agents. The compounds show moderate antibacterial activities against Staphylococcus aureus, Bacillus, Escherichia coli and Pseudomonas aeruginosa. The most elegant result as antibacterial activity was obtained for compounds AZ4B$_{7}$and AZ4B$_{7'}$, while the synthesized compound AZ4D$_{5}$ showed high activity as an antioxidant agent. Compound AZ4B$_{7'}$have greater anticancer activity and the percentage inhibition of cell viability by compound was 38.58 % at concentration 50 µg/mL and the results showed that Compound AZ4B$_{7'}$ having a higher percentage with % DNA fragmentations index (60.2%) compared with 5-Fluorouracil (46.95 %). The present study

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reported moderate in vivo toxic effects by LD_{50} measurement of new compounds ($AZ4B_7$and $AZ4B_{7'}$).

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