Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(13):77-90
(http://derpharmachemica.com/archive.html)

Synthesis, Characterization and Pharmacological Evaluation of Some New 1,4-Diazepine Derivatives as Anticancer agents.

Eman A. Fayed[1]* and Hanaa Y. Ahmed[2]

[1]*Organic Chemistry Department, Faculty of Pharmacy, Al-Azhar University (Girls)*
[2]*The Regional Center for Mycology & Biotechnology, Al-Azhar University, Cairo, Egypt*

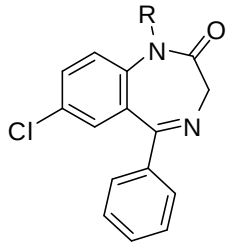
ABSTRACT

The new research work deals with the synthesis of cyclohepta[b]thieno[2,3-e][1,4]diazepine derivatives through three synthetic pathways starting with cycloheptanone. The starting ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (2) and 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b] thiophene-3-carboxamide (5) was synthesized adopting a direct Gewald's method. The starting material 2 was reacted with phenacyl bromide and its chloride derivative to afford 3 a&b which further cyclized through the reaction with different aromatic amines to give 4a-e. Also 3-aryl-7,8,9,10-tetrahydro-1H,6H-cyclohepta[b]thieno[2,3-e][1,4] diazepin-5(4-H)-one (7a-f) was furnished via cyclization of 6 with phenacyl bromides. In addition, the reaction of the same starting material 6 with chloroacetyl chloride afforded 9 through the separated intermediate 8. All of the newly synthesized products were subjected to in vitro anticancer screening against human breast cancer cell line (MCF-7), colon cancer cell line (HCT-116) and against human liver carcinoma (HepG-2). Compounds 7 c, 7 e and 7 f were the most active compounds which exhibited antitumor activity against human hepatocellular carcinoma (HepG-2), human breast adenocarcinoma (MCF-7) and human colon carcinoma (HCT-116) cell lines, with IC50's ranging from 4.4-13 µg/mL in a comparison to slandered vinblastine.

Keywords: Thieno[1,4]diazepine, anticancer activity.

INTRODUCTION

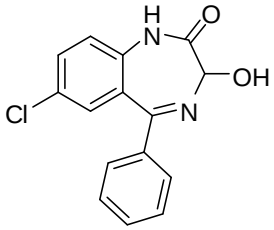
1,4-Diazepine derivatives are the seven membered, nitrogen containing heterocyclic ring systems possessing a wide range of therapeutic applications. These derivatives are predominantly used in the inhibition of signals in the central nervous system which is useful for the synthesis of psychoactive drugs. [1] Certain 1,4-benzodiazepine derivatives such **I (R=CH_3, C_2H_5)** possess sedative properties, [2] and are used as spasmolytic and hypnotic drugs. [3-5] Also **(I, R=H)** was found to have an anticonvulsant, muscle relaxant and tranquilizing activities. [6] Similarly the hydroxy compound **2 (Oxazepam)** has sedative, muscle relaxant, and anticonvulsive effects. [7] Furthermore, the thieno derivative **III** possesses neuroleptic activity. [8]



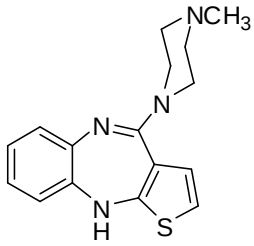
I

R=H,CH_3,C_2H_5

Diazepam (R=CH_3)
N-Desmethyldiazepam (R=H)

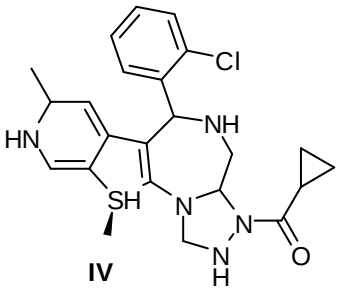


II (Oxazepam)



III

Also, 1,4-diazepines are widely used in the field of peptidomimetics as potential mimetic and molecular scaffolds. [9,10] Analogs of 1,4-diazepine nucleoside with the protected sugar moiety have been made as possible agents against HIV-1 and HIV-2 viruses. [11] 1,4-Diazepines attracted the attention of chemists and druggists for their biological and medicinal activities, such as antimicrobial, [12] anti-HIV, [13] herbicidal, [14] psychotropic [15] and anticancer [16] activities. Furthermore 1,4-diazepines act as antagonists of platelet activation factor (PAF). [17] Such as (*S*)-(+)-6-(2-chlorophenyl)-3-cyclopropane-carbonyl-8,11-dimethyl-1,2,3,3a,4,5,6,8,9,11-decahydropyrido [4',3':4,5][1λ^4]-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]dia-zepine, **(IV)** inhibited thapsigargin-induced production of tumor necrosis factor-*α*. [18]



IV

Moreover, it was found that 1,4-diazepines play a predominant roles in the field of medicinal chemistry because it is the core moiety used for the synthesis of various drug molecules. As the [1,4]diazepine has unique structure that mimics the peptide linkage, the interesting observation completely shifted the interest of medicinal chemist for [1,4]diazepine from CNS acting drugs to anticancer agents. During last few decades, a large number of reports have appeared in the literature highlighting the anticancer activity of [1,4] diazepines. In view of above said importance, thieno[1,4]diazepine derivatives were synthesized and screened for their anticancer activity.

MATERIALS AND METHODS

Chemistry

All melting point were taken on Electrothermal LA9000 series, Digital Melting point Apparatus were uncorrected. IR Spectra were determined using KBr disc technique on Nikolet IR 200 FT IR Spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University, and values are represented in (cm^{-1}). The ^{1}HNMR Spectra were recorded on Gemini 300MHz, and Mercury 400 MHz NMR Spectrometer at the Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defense. DMSO-d_6 was used as solvents; Chemical shifts were measured in δ ppm, relative to TMS as internal standard. Mass Spectrum were recorded at 70 ev on DI-50 unit of Schimadzu GC/ MS- QP5050A Spectrometer at Regional Center for Mycology and Biotechnology (RCMB), At–Al-Azhar University represented as m/z (relative abundance %) (formula). Element Analysis (C,H,N) were carried out at Regional Center for Mycology and Biotechnology, Al-Azhar University, the values were found to be within ±0.4 % of the theoretical ones unless otherwise indicated. Progress of the reaction was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and was visualized using UV lamp.

Ethyl 2-cyano-2-cycloheptylideneacetate (1)
Prepared using reported procedure. [19]

Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (2)
Prepared using reported procedure. [20, 22]

Ethyl 2-(2-oxo-2-phenylethylamino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxy-late and ethyl 2-(2-(4-chlorophenyl)-2-oxoethylamino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]-thiophene-3-carboxylate (3 a & b)
A mixture of compound **2** (2.39 g, 0.01 mol), phenacyl bromide or 4-chloroderivative (1.99 g or 2.33 g, 0.01 mol) and anhydrous K_2CO_3 (0.5 g) in *N*, *N*-dimethylformamide (10 mL) was heated under reflux for 8 h. The reaction mixture was cooled, poured onto ice cold water, filtered, washed with water, then dried and crystallized from ethanol to give compound **3**.

For 3a: Yield: 88%; m.p.: 138-140ºC; IR (KBr) ν cm^{-1}: 3429 (NH); 3061 (Ar-H); 2918, 2853 (aliphatic C-H); 1720-1660 (2C=O); 760, 699 (phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.22-1.27 (t, 3H, CH_3); 1.54 (s, 2H, CH_2 at positions 6); 1.72 (s, 4H, $2CH_2$ at positions 5, 7); 2.57 (s, 2H, $NHCH_2CO$); 2.73 (s, 2H, CH_2 at positions 8); 2.91 (s, 2H, CH_2 at positions 4); 4.13-4.20 (q, 2H, CH_2CH_3); 6.94 (s, 1H, NH exchangeable by D_2O), 7.40-7.95 (m, 5H, Ar-H); MS (m/z %): 357 (2.53) ($M^{\bullet+}$), 239 (100) ($C_{13}H_{19}O_2S^{+}$). Anal. Calcd. for $C_{20}H_{23}NO_3S$; C, 67.20; H, 6.49, N, 3.92; Found: C, 67.51, H, 6.41, N, 3.94.

For 3 b: Yield: 90%; m.p.: 118-120ºC; IR (KBr) ν cm^{-1}: 3399 (NH); 3087 (Ar-H); 2915, 2851 (aliphatic C-H); 1710-1659 (2C=O); 830, 768 (*p*- substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.22-1.30 (t, 3H, CH_3); 1.53 (s, 2H, CH_2 at positions 6); 1.81 (s, 4H, $2CH_2$ at positions 5, 7); 2.57 (s, 2H, $NHCH_2CO$); 2.72 (s, 2H, CH_2 at positions 8); 2.88 (s, 2H, CH_2 at positions 4); 4.12-4.17 (q, 2H, CH_2CH_3); 6.97 (s, 1H, NH exchangeable by D_2O), 7.29-8.44 (m, 4H, Ar-H); MS (m/z %): 393 (3.09) ($M+2^{\bullet+}$), 391 (25.23) ($M^{\bullet+}$), 141 (100) ($C_7H_{11}NS^{\bullet+}$ &/or $C_7H_6ClO^{+}$). Anal. Calcd for $C_{20}H_{22}ClNO_3S$; C, 61.29; H, 5.66, N, 3.57; Found: C, 61.31, H, 5.67, N, 3.53.

3,4-Diaryl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-one (4a-j)
General procedure:
A mixture of the appropriate ketoester 3 a&b (0.01mol) and the respective amine (0.01mol) in glacial acetic acid (10 mL) was heated under reflux for 10 h. After cooling, the reaction mixture was concentrated under reduced pressure and the product was triturated with diethyl ether. The separated solid was filtered and crystallized from the suitable solvent.

3,4-Diphenyl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-one (4a)
Compound **4a** was prepared as described from **3a** and aniline. Crystallized from ethanol; Yield: 85 %; m.p.: 168-170ºC; IR (KBr) ν cm^{-1}: 3283 (NH); 3053 (Ar-H); 2917, 2860 (aliphatic C-H); 1661 (C=O); 750, 695 (phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 2H, CH_2 at position 8); 2.03 (s, 4H, $2CH_2$ at positions 7,9); 2.78 (s, 2H, CH_2 at positions 10); 2.88 (s, 2H, CH_2 at positions 6); 6.31 (s, 1H, CH at positions 2); 6.98-7.95 (m, 10 H, Ar-H); 9.82 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 386 (6.55) ($M^{\bullet+}$), 77 (100) ($C_6H_5^{+}$). Anal. Calcd for $C_{24}H_{22}N_2OS$; C, 74.58; H, 5.74, N, 7.25; Found: C, 74.76, H, 5.81, N, 7.38.

4-(4-Chlorophenyl)-3-phenyl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diaz-epin-5(4*H*)-one (4b)
Compound **4b** was prepared as described from **3a** and 4-chloroaniline. Crystallized from ethanol; Yield: 73 %; m.p.: 215-217ºC; IR (KBr) ν cm^{-1}: 3413 (NH); 3057 (Ar-H); 2920, 2851(aliphatic C-H); 1662 (C=O); 822, 700, 690 (*p*-substituted phenyl & phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.82 (s, 2H, CH_2 at positions 8); 2.03 (s, 4H, $2CH_2$ at positions 7, 9); 2.73 (s, 2H, CH_2 at positions 10); 2.89 (s, 2H, CH_2 at positions 6); 7.30-7.95 (m, 10H, 9 Ar-H and 1H at position 2-), 9.98 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 422 (0.98) ($M+2^{\bullet+}$), 420 (1.01) ($M^{\bullet+}$), 57 (100) ($C_2H_5^{+}$, CHN_2O^{+} &/or $C_2H_3NO^{\bullet+}$). Anal. Calcd for $C_{24}H_{21}ClN_2OS$; C, 68.48; H, 5.03, N, 6.65; Found: C, 68.69, H, 5.08, N, 6.67.

4-(5-Oxo--3-phenyl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-4(5*H*)yl) benzonitrile (4 c)

Compound **4c** was prepared as described from **3a** and 4-aminobenzonitrile. Crystallized from benzene; Yield: 85 %; m.p.: 220-222ºC; IR (KBr) ν cm^{-1}: 3269 (NH); 3050 (Ar-H); 2921, 2860 (aliphatic C-H); 2219 (CN); 1668 (C=O); 835, 755, 702 (*p*-substituted phenyl & phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 2H, CH_2 at positions 8); 2.08 (s, 4H, $2CH_2$ at positions 7, 9); 2.72 (s, 2H, CH_2 at positions 10); 2.89 (s, 2H, CH_2 at positions 6); 7.60-7.91 (m, 10H, 9Ar-H and 1H at position 2-); 10.31 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 411 (11.70) ($M^{\bullet+}$), 202 (100) ($C_{11}H_{12}N_3O^{+}$ &/or $C_{11}H_8NOS^{\bullet}$). Anal. Calcd for $C_{25}H_{21}N_3OS$; C, 72.97; H, 5.14, N, 10.21; Found: C, 73.24, H, 5.17, N, 10.31.

4-(4-Hydroxyphenyl)-3-phenyl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diaz-epin-5(4*H*)-one (4d)

Compound **4d** was prepared as described from **3a** and 4-hydroxyaniline. Crystallized from ethanol; Yield: 80 %; m.p.: 175-177ºC; IR (KBr) ν cm^{-1}: 3267 (br OH&NH); 3050 (Ar-H); 2919, 2860 (aliphatic C-H); 1655 (C=O); 826, 699 (*p*-substituted phenyl & phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.83 (s, 2H, CH_2 at positions 8); 1.97 (s, 4H, $2CH_2$ at positions 7,9); 2.17 (s, 2H, CH_2 at positions 10); 2.23 (s, 2H, CH_2 at positions 6); 6.65-6.67 (d, *J*= 6Hz, 2H, Ar-H); 6.80-6.83 (d, *J*= 9Hz, 2H, Ar-H); 7.01-7.04 (d, *J*= 9Hz, 2H, Ar-H); 7.30-7.33 (m, 4H, 3Ar-H and 1H at position 2-); 7.55-7.58 (d, *J*= 9Hz, 2H, Ar-H), 9.05 (s, 1H, NH exchangeable by D_2O); 9.57 (s, 1H, OH exchangeable by D_2O); MS (m/z %): 402 (1.20) ($M^{\bullet+}$), 239 (100) ($C_{15}H_{13}NS^{\bullet+}$). Anal. Calcd for $C_{24}H_{22}N_2O_2S$; C, 71.62; H, 5.51, N, 6.96; Found: C, 71.80, H, 5.62, N, 7.11.

4-(4-Methoxyphenyl)-3-phenyl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diaz-epin-5(4*H*)-one (4e)

Compound **4e** was prepared as described from **3a** and 4-methoxyaniline. Crystallized from glacial acetic acid; Yield: 81 %; m.p.: 200-201ºC; IR (KBr) ν cm^{-1}: 3261 (NH); 3075 (Ar-H); 2928, 2839 (aliphatic C-H); 1652 (C=O); 830,770, 703 (*p*-substituted phenyl & phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 4H, $2CH_2$ at positions 7,9); 1.99 (s, 2H, CH_2 at positions 8); 2.36 (s, 2H, CH_2 at positions 10); 2.62 (s, 2H, CH_2 at positions 6); 3.70 (s, 3H, OCH_3); 6.83-7.47 (m, 10H, 9Ar-H and 1H at position 2-); 9.69 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 416 (0.15) ($M^{\bullet+}$), 93 (100) ($C_6H_7N^{\bullet+}$). Anal. Calcd for $C_{25}H_{24}N_2O_2S$; C, 72.09; H, 5.81, N, 6.73; Found: C, 72.18, H, 5.94, N, 6.89.

3-(4-Chlorophenyl)-4-phenyl-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diaz-epin-5(4*H*)-one (4f)

Compound **4f** was prepared as described from **3b** and aniline. Crystallized from ethanol; Yield: 75 %; m.p.: > 300ºC; IR (KBr) ν cm^{-1}: 3438 (NH); 3050 (Ar-H); 2985, 2860 (aliphatic C-H); 1637 (C=O); 850, 700 (*p*-substituted phenyl & phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 4H, $2CH_2$ at positions 7,9); 2.03 (s, 4H, $2CH_2$ at positions 8,10); 2.72 (s, 2H, CH_2 at positions 6); 7.01-7.54 ((m, 10H, 9Ar-H and 1H at position 2-); 9.85 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 422 (1.08) ($M+2^{\bullet+}$), 420 (1.35) ($M^{\bullet+}$), 73 (100) ($C_2H_3NS^{\bullet+}$). Anal. Calcd for $C_{24}H_{21}ClN_2OS$; C, 68.48; H, 5.03, N, 6.65; Found: C, 68.72, H, 5.07, N, 6.80.

3,4-Bis(4-chlorophenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5-(4*H*)-one (4g)

Compound **4g** was prepared as described from **3b** and 4-chloroaniline. Crystallized from ethanol; Yield: 90 %; m.p.: 180-182 ºC; IR (KBr) ν cm^{-1}: 3295 (NH); 3050 (Ar-H); 2919, 2853 (aliphatic C-H); 1662 (C=O); 826, 744 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 2H, CH_2 at positions 8); 2.03 (s, 4H, $2CH_2$ at positions 7,9); 2.56 (s, 2H, CH_2 at positions 10); 2.72 (s, 2H, CH_2 at positions 6); 7.30-7.60 ((m, 9H, 8Ar-H and 1H at position 2-); 9.99 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 456 (1.96) ($M+2^{\bullet+}$), 454 (4.15) ($M^{\bullet+}$), 44 (100) ($CS^{\bullet+}$). Anal. Calcd for $C_{24}H_{20}C_{l2}N_2OS$; C, 63.30; H, 4.43, N, 6.15; Found: C, 63.53, H, 4.41, N, 6.28.

4-(3-(4-Chlorophenyl--5-oxo-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diaz-epin-4(5*H*)-yl) benzonitrile (4h)

Compound **4h** was prepared as described from **3b** and 4-aminobenzonitrile. Crystallized from benzene; Yield: 85 %; m.p.: 208-210ºC; IR (KBr) ν cm^{-1}: 3227 (NH); 3086 (Ar-H); 2922, 2855 (aliphatic C-H); 2226 (CN); 1696 (C=O); 794, 680 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 4H, $2CH_2$ at positions 7,9); 2.08 (s, 2H, CH_2 at positions 8); 2.78 (s, 2H, CH_2 at positions 10); 2.94 (s, 2H, CH_2 at positions 6); 7.60-7.96 ((m, 9H, 8Ar-H and 1H at position 2-); 10.35 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 445 (1.79) ($M^{\bullet+}$), 136 (100) ($C_8H_{10}NO^{+}$ &/ or $C_8H_8S^{\bullet+}$). Anal. Calcd for $C_{25}H_{20}ClN_3OS$; C, 67.33; H, 4.52, N, 9.42; Found: C, 67.51, H, 4.60, N, 9.53.

3-(4-Chlorophenyl)-4(4-hydroxyphenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*] [1,4]diazepin-5(4*H*)-one (4i)

Compound **4i** was prepared as described from **3b** and 4-hydroxyaniline. Crystallized from ethanol; Yield: 74 %; m.p.: 283-285ºC; IR (KBr) ν cm^{-1}: 3402 (br OH&NH); 3050 (Ar-H); 2920, 2870 (aliphatic C-H); 1649 (C=O); 821, 738 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ ppm: 1.90 (s, 2H, CH_2 at positions 7); 1.99 (s, 2H, CH_2 at positions 9); 2.03 (s, 4H, $2CH_2$ at positions 8,10); 2.23 (s, 2H, CH_2 at positions 6); 6.64-7.33 ((m, 9H, 8Ar-H and 1H at position 2-); 9.10 (s, 1H, NH exchangeable by D_2O); 9.60 (s, 1H, OH exchangeable by D_2O); MS (m/z %): 438 (1.88) ($M+2^{\bullet+}$), 436 (5.27) ($M^{\bullet+}$), 86 (100) ($C_3H_6N_2O^{\bullet+}$). Anal. Calcd for $C_{24}H_{21}ClN_2O_2S$; C, 65.97; H, 4.84, N, 6.41; Found: C, 66.14, H, 4.89, N 6.48.

3-(4-Chlorophenyl)-4(4-methoxyphenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*] [1,4]diazepin-5(4*H*)-one (4 j)

Compound **4 j** was prepared as described from **3b** and 4-methoxyaniline. Crystallized from ethanol; Yield: 86 %; m.p.: >300ºC; IR (KBr) ν cm^{-1}: 3428 (NH); 3054 (Ar-H); 2921, 2849 (aliphatic C-H); 1652 (C=O); 821, 740 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.90 (s, 4H, $2CH_2$ at positions 7,9); 1.99 (s, 4H, $2CH_2$ at positions 8,10); 2.57 (s, 2H, CH_2 at positions 6); 3.70 (s, 3H, OCH_3); 6.83-6.86 ((d, *J*=9Hz, 5H, 4Ar-H and 1H at position 2-); 7.44-7.47 (d, *J*=9Hz, 4H, Ar-H) 9.75 (s, 1H, NH exchangeable by D_2O); MS (m/z %): 452 (0.79) ($M+2^{\bullet+}$), 450 (0.91) ($M^{\bullet+}$), 108 (100) ($C_6H_4S^{\bullet+}$). Anal. Calcd for $C_{25}H_{23}ClN_2O_2S$; C, 66.58; H, 5.14, N, 6.21; Found: C, 66.69, H, 5.18, N, 6.27.

2-Cyano-2-cycloheptylideneacetamide (5)

Prepared using reported procedure. [19, 23]

2-Amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (6)

Prepared using reported procedure. [19]

3-Aryl-,7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7)

General procedure:

A mixture of compound **6** (0.01mol) and the respective phenacyl bromide (0.01mol) in *N*,*N*-dimethylformamide (10 mL) and anhydrous K_2CO_3 (0.5 g) was heated under reflux for 8 h. The reaction mixture was poured onto ice cold water (≈20 mL); the formed solid, was filtered, dried and crystallized from the suitable solvent.

3-Phenyl-,7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7a)

Compound **7a** was prepared as described from **6** and phenacylbromide. Crystallized from ethanol; Yield: 77%; m.p.: 250-251˚C; IR: 3399 (br. 2NH); 3066 (Ar-H); 2919, 2854 (aliphatic C-H); 1658 (C=O); 776, 661 (phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.61 (s, 4H, $2CH_2$ at position 7,9); 1.85 (s, 2H, CH_2 at position 8); 2.72 (s, 2H, CH_2 at position 10); 2.88 (s, 2H, CH_2 at position 6); 7.22-8.11 (m, 6H, 5Ar-H and 1H at position 2-); 12.48 (s, 2H, 2 NH exchangeable by D_2O); MS m/z: 310 (2.02) ($M^{\bullet+}$), 135 (100) ($C_8H_7S^+$). Anal. Calcd. for $C_{18}H_{18}N_2OS$, C, 69.65, H, 5.84; N, 9.02; Found: C, 69.63; H, 5.80, N, 9.07.

3-(4-Methylphenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7b)

Compound **7b** was prepared as described from **6** and 4-methyl phenacylbromide. Crystallized from ethanol; Yield: 53%; m.p.: 146-147 ^{o}C; IR: 3425, 3241 (2NH); 3039 (Ar-H); 2918, 2857 (aliphatic C-H); 1660 (C=O); 823 (*p* -substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.62 (s, 4H, $2CH_2$ at position 7,9); 1.85 (s, 2H, CH_2 at position 8); 2.36 (s, 3H, CH_3); 2.72 (s, 2H, CH_2 at position 10); 2.88 (s, 2H, CH_2 at position 6); 7.06-8.00 (m, 5H, 4Ar-H and 1H at position 2-); 12.51 (s, 2H, 2 NH exchangeable by D_2O); MS m/z: 324 (6.50) ($M^{\bullet+}$), 119 (100) ($C_8H_7O^+$). Anal. Calcd for $C_{19}H_{20}N_2OS$; C, 70.34; H, 6.21; N, 8.63; Found: C, 70.30; H, 6.28; N, 8.63.

3-(4-Benzylphenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7c)

Compound **7b** was prepared as described from **6** and 4-phenyl phenacylbromide. Crystallized from benzene; Yield: 81%; m.p.: 215-216 ^{o}C; IR: 3424, 3180 (2 NH); 3045 (Ar-H); 2917, 2870 (aliphatic C-H); 1661 (C=O); 841, 749, 690 (*p* -substituted phenyl & phenyl); MS m/z: 386 (3.33) ($M^{\bullet+}$), 234 (100) ($C_{16}H_{12}NO^{\bullet+}$). Anal. Calcd for $C_{24}H_{22}N_2OS$; C, 74.58; H, 5.74; N, 7.25; Found: C, 74.60; H, 5.77; N, 7.27.

3-(4-Chlorophenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7d)
Compound **7d** was prepared as described from **6** and 4-chloro phenacylbromide. Crystallized from glacial acetic acid; Yield: 79%; m.p.: 180-182 ^{o}C; IR: 3420, 3150 (2NH); 3069 (Ar-H); 2918, 2855 (aliphatic C-H); 1658 (C=O); 831 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.60 (s, 4H, 2CH_2 at position 7,9); 1.85 (s, 2H, CH_2 at position 8); 2.72 (s, 2H, CH_2 at position 10); 2.88 (s, 2H, CH_2 at position 6); 7.46-8.13 (m, 5H, 4Ar-H and 1H at position 2-); 12.51 (s, 2H, 2 NH exchangeable by D_2O); MS m/z: 346 (2.31) (M+2┐$^{\bullet+}$), 344 (3.79) (M$^{\bullet+}$), 139 (100) (C_8H_8Cl ┐$^{+}$), 75 (27.69%) (C_3H_4Cl ┐$^{+}$). Anal. Calcd for $C_{18}H_{17}ClN_2OS$; C, 62.69; H, 4.97; N, 8.12; Found: C, 62.72; H, 4. 99; N, 8.11.

3-(4-Hydroxyphenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7e)
Compound **7e** was prepared as described from **6** and 4-hydroxy phenacylbromide. Crystallized from glacial acetic acid; Yield: 76%; m.p.: 198-200 ^{o}C; IR: 3408, 3159 (NH & OH); 3050 (Ar-H); 2919, 2860 (aliphatic C-H); 1657 (C=O); 831 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.59 (s, 4H, 2CH_2 at position 7,9); 1.83 (s, 2H, CH_2 at position 8); 2.72 (s, 2H, CH_2 at position 10); 2.88 (s, 2H, CH_2 at position 6); 6.63-8.03 (m, 5H, 4Ar-H and 1H at position 2-); 11.68 (s, 1H, OH exchangeable by D_2O); 12.47 (s, 2H, 2 NH exchangeable by D_2O); MS m/z: 326 (21.50) (M$^{\bullet+}$), 121 (100) (C_8H_9O ┐$^{+}$). Anal. Calcd for $C_{18}H_{18}N_2O_2S$; C, 66.23; H, 5.56; N, 8.58; Found: C, 66.23; H, 5.60; N, 8.53.

3-(4-Methoxyphenyl)-7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones (7f)
Compound **7f** was prepared as described from **6** and 4-methoxy phenacylbromide. Crystallized from ethanol; Yield: 50%; m.p.: 229-230 ^{o}C; IR: 3428, 3180 (2NH); 3058 (Ar-H); 2917, 2854 (aliphatic C-H); 1658 (C=O); 854 (*p*-substituted phenyl); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.60 (s, 4H, 2CH_2 at position 7,9); 1.84 (s, 2H, CH_2 at position 8); 2.72 (s, 2H, CH_2 at position 10); 2.88 (s, 2H, CH_2 at position 6); 3.87 (s, 3H, OCH_3); 6.99-8.13 (m, 5H, 4Ar-H and 1H at position 2-); 12.45 (s, 2H, 2 NH exchangeable by D_2O); MS m/z: 340 (0.92) (M$^{\bullet+}$), 234 (100) ($C_{16}H_{12}NO$┐$^{\bullet+}$). Anal. Calcd for $C_{19}H_{20}N_2O_2S$; C, 67.03; H, 5.92; N, 8.23; Found: C, 67.08; H, 5.94; N, 8.27.

2-(2-Chloroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[b]thiophene-3-carboxamide (8) Chloroacetyl chloride (1.13g, 0.8mL, 0.01mol) was added to a stirred solution of **6** (0.01mol) in dry toluene (50 mL). The reaction mixture was stirred at room temperature for one h. The solid product was then filtered and crystallized from ethanol; Yield: 90%; m.p.: 218-220 ^{o}C; IR: 3389 (NH); 3247, 3180 (NH_2); 2920, 2852 (aliphatic C-H); 1637 (C=O); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.56 (s, 4H, 2CH_2 at position 7,9); 1.79 (s, 2H, CH_2 at position 8); 2.67-2.75 (m, 4H, 2CH_2 at position 6,10); 4.24, 4.41 (2s, 2H, -$COCH_2Cl$); 7.42 (s, 2H, NH_2 exchangeable by D_2O); 11.10 (s, 1H, NH exchangeable by D_2O); MS m/z: 288 (M+2┐$^{\bullet+}$) (20.33), 286 (58.51) (M$^{\bullet+}$), 269 (100) ($C_{11}H_{10}ClN_2O_2S$┐$^{+}$ &/or $C_{12}H_{12}ClNO_2S$┐$^{\bullet+}$); 271 (38.97) (B+2┐$^{\bullet+}$). Anal. Calcd for $C_{12}H_{15}Cl\ N_2O_2S$; C, 50.26; H, 5.27; N, 9.77; Found: C, 50.28; H, 5.28; N, 9.80.

7,8,9,10-Tetrahydro-1*H*, 6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-2,5(3*H*,4*H*)-dione (9)
Method 1
A mixture of **6** (0.01mol) in dry toluene (50mL) was stirred at room temperature while chloroacetyl chloride (1.13g, 0.8mL, 0.01mol) was added dropwise. The reaction mixture was then heated under reflux for 5 h. The solvent was then evaporated under reduced pressure and the oily residue was extracted with ether until solidification. The solid was then crystallized from ethanol.

Method 2:
A mixture of **8** (0.01mol) in dry toluene (50mL) was heated under reflux for 5 h. The solvent was then evaporated under reduced pressure and the oily residue was extracted with ether until solidification. The solid was then crystallized from ethanol. Yield: 85% for method 1 and 93 % for method 2; m.p.: 152-152 ^{o}C; IR: 3421 (br. 2NH); 2922, 2860 (aliphatic C-H); 1662 (2 C=O); ^{1}HNMR: 1.75 (s, 4H, 2CH_2); ^{1}HNMR (DMSO-d_6) δ/ppm: 1.58 (s, 4H, 2CH_2 at position 7,9); 1.80 (s, 2H, CH_2 at position 8); 2.63-2.71 (m, 4H, 2CH_2 at position 6,10); 4.25, 443 (s, 2H,-$COCH_2$-NH); 11.80 (s, 2H, 2NH exchangeable by D_2O); MS m/z: 250 (7.50) (M$^{\bullet+}$), 193 (100) ($C_{10}H_{11}NOS$ ┐$^{\bullet+}$). Anal. Calcd for $C_{12}H_{14}N_2O_2S$; C, 57.58; H, 5.64; N, 11.19; Found: C, 57.53; H, 5.66; N, 11.18.

Biological Activities

Cell Lines

Human colon (HCT-116), breast (MCF-7) and hepatocellular (Hep-G2) carcinoma cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown in RPMI-1640 medium, supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Evaluation of the antitumor activity by MTT assay:

Viability of control and treated cells were evaluated using the MTT assay in triplicate. MTT assay is a laboratory test and a standard colorimetric assay (an assay which measures changes in color) for measuring cellular growth, Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) was reduced to purple formazan in the mitochondria of living cells. A solubilization solution (dimethyl sulfoxide) was added to dissolving the insoluble purple formazan product into a colored solution. Briefly, three tumor cell lines were seeded in 96-well plates containing 100µL of the growth medium at a density of $1x10^4$ cells/well. Cells were permitted to adhere for 24h till confluence, washed with PBS, and then treated with different concentration of compounds in fresh maintenance medium from 50 to 1.56 µg and incubated at 37ºC for 24h. A control of untreated cells was made in the absence of test compound. Untreated cells used as negative control. Serial two-fold dilutions of the tested compounds were added into a 96-well tissue culture plate using multichannel pipette (eppendorff, Germany). After treatment (24h), the culture supernatant was replaced by fresh medium. Then, the cells in each well were incubated at 37˚C with 100µl of MTT solution (5mg/ml) for 4h. After the end of incubation the MTT solution was removed, then 100µl of DMSO was added to each well. The absorbance was detected at 570nm using a microplate reader (SunRise TECAN, Inc, USA).The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments. [24]

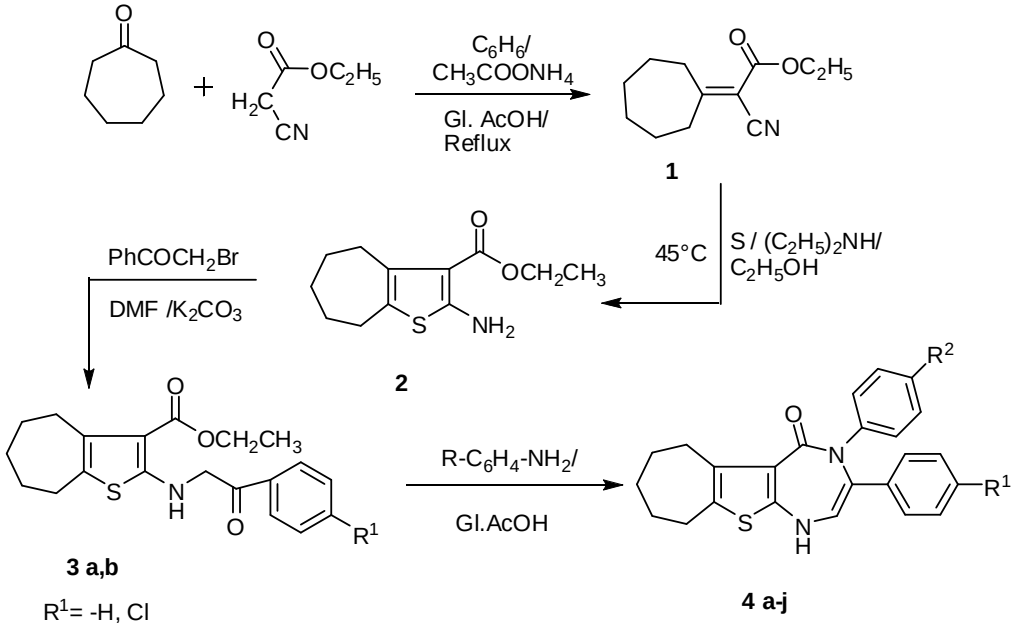
Data analysis

The percentage cell viability was calculated using the Microsoft Excel®. Percentage cell viability was calculated as follows: according to the following calculation: the percentage of cell viability = [1−(ODt/ODc)] × 100%, where ODt is the mean optical density of wells treated with the tested compound and ODc is the mean optical density of untreated cells. The test compounds were compared using the IC50 value, i.e., the concentration of an individual compound leading to 50% cell death that was estimated from graphical plots of surviving cells vs compound concentrations.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the target compounds is depicted in Schemes 1&2. **Scheme 1** starting with the reaction of cycloheptanone with ethyl cyanoacetate followed by cyclization with sulfur applying Gewald's reaction conditions to afford ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate **(2)**. Reaction of **2** with phenacyl bromide and its chloride derivative yielded **3a&b**. The presence of signals of aromatic protons in the **^{1}HNMR** spectrum in addition to out of plane bending absorption bands at 760 & 699 cm^{-1} pointed to the presence of phenyl groups and the formation of compound **3a,** in addition to out of plane bending absorption bands at 830 &768 cm^{-1} for *p*-substituted phenyl of compound **3b**. Target compounds **4 a-j** were obtained via reaction of **3 a&b** with different anilines in a glacial acetic acid. Confirmations of structures **4a-j** were achieved by spectral data and element analyses. The absence of ester group in **IR** and **^{1}HNMR** and the appearance of -C=O amidic at range 1661-1637 cm^{-1} in **IR** spectrum indicate the formation of these compounds. In addition to the presence of functional groups such as cyano group in compound **4 c&h** at 2219 and 2226 cm^{-1} respectively. Also the presence of absorption bands at 3267 and 3402 cm^{-1} in **IR** spectrum indicate the presence of –OH group in addition to -NH for **4 d&i**. The **^{1}HNMR** for the same compounds shows two singlet signals at δ 9.05 and 9.57 ppm for -NH and -OH which exchangeable by D_2O for compound **4d**. Another singlet signals appeared at δ 9.10 and 9.60 ppm indicate the presence of -NH and -OH which exchanged by D_2O for compound **4i**. Furthermore **^{1}HNMR** for compounds **4e&j** indicate the presence of a methoxy group (-OCH_3) as a singlet signal at δ 3.70 ppm. Mass spectrum also proves the structures of these compounds. The structure of these compounds were confirmed by the mass spectra as presence of M+2 in compounds containing chloride (-Cl) in addition to the presence of molecular ion peaks such as **4b,f,g,i&j**. (Scheme 1)



Scheme 1:

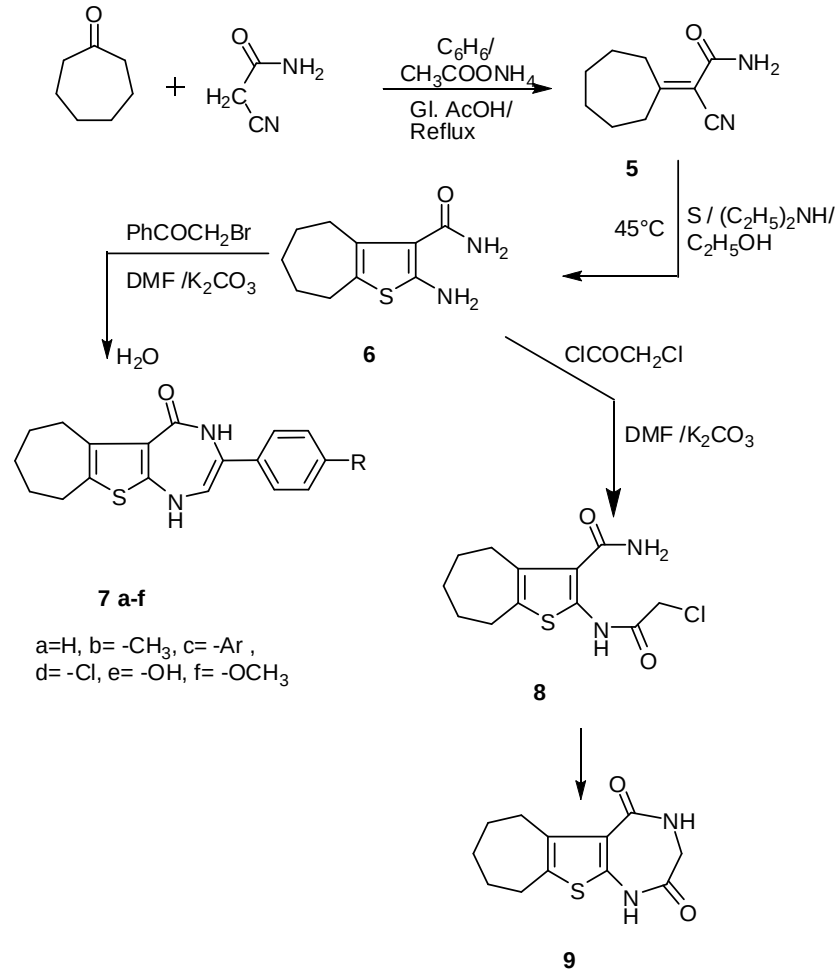
Compd. 4	R^1	R^2	Compd. 4	R^1	R^2
a	-H	-H	**f**	-Cl	-H
b	-H	-Cl	**g**	-Cl	-Cl
c	-H	-CN	**h**	-Cl	-CN
d	-H	-OH	**i**	-Cl	-OH
e	-H	$-OCH_3$	**j**	-Cl	$-OCH_3$

Scheme 2, starting with the reaction of cycloheptanone in dry benzene, with cyanoacetamide, ammonium acetate and glacial acetic acid afforded 2-cyano-2-cycloheptylideneacetamide (5). Cyclization of 5 with sulfur applying Gewald's reaction conditions yielded 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (6).

The main start 6 reacted in two pathways,

firstly with phenacylbromide derivatives in *N*,*N*-dimethylformamide (*N*,*N*-DMF) to yield 3-aryl-,7,8,9,10-tetrahydro-1*H*,6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-5(4*H*)-ones **(7)**. Both elemental analysis and spectral data are in agreement with the proposed structure. **IR** indicate the presence of absorption band at 3066 cm^{-1} which indicate the presence of aromatic protons in addition to the presence of out of plain bending at 776, 661 cm^{-1} for **7a** . **^{1}HNMR** for compound **7b** indicate the presence of a singlet signal at δ 2.36 ppm for $-CH_3$ group absorption. Also the presence of a molecular ion peak at m/z: 386 for **7 c**, M+2 and $M^{+\cdot}$ at m/z: 346 and 344 in a percentage 3:1 indicate the presence of **-Cl** in the compound **7 d**. Moreover for compound **7 e** there was an absorption bands in **IR** at 3408, 3159 cm^{-1} for -NH & -OH. **^{1}HNMR** for the same compound represented a singlet signal at δ 11.68 and 12.47 ppm which indicate the presence of -OH& -NH which exchanged by D_2O. **^{1}HNMR** for **7f** showed a singlet signal at δ 3.87 ppm for $-OCH_3$ group. Furthermore the reaction of 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide **(6)** with chloroacetylchloride afforded 2-(2-Chloroacetamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[b]thiophene-3-carboxamide **(8).** The structure of **8** was confirmed by spectral and elemental analyses. The **IR** spectrum indicates the presence of -NH&-NH_2 absorption at 3389, 3247& 3180 cm^{-1}. Also the **^{1}HNMR** for the same compound represents two singlet signals at δ 4.24 & 4.41 ppm for $-COCH_2Cl$ group in addition to another signals at δ 7.42 ppm for $-NH_2$ and 11.10 for -NH which exchangeable by D_2O. Finally cyclization of **8** in dry toluene afforded **9**. The structure of 7,8,9,10-tetrahydro-1*H*, 6*H*-cyclohepta[*b*]thieno[2,3-*e*][1,4]diazepin-2,5(3*H*,4*H*)-dione **(9)** was improved by spectral in addition to elemental analyses. The **IR** spectra showed an absorption band at 3421 cm^{-1} responsible for 2-NH absorption in addition to 2 -C=O at 1662 cm^{-1}. **^{1}HNMR** showed a singlet signal at δ 11.80 ppm for 2 -NH and the disappearance of a signal at 7.42 ppm confirmed that the disappearance of $-NH_2$ absorption and this improve that the compound was closed at this step. The mass spectral data also confirmed the cyclization that the appearance of molecular ion peak at m/z: 250 (7.50 %).

Scheme 2:



Biological activity:

All of the newly synthesized compounds were screened for their anticancer activity against three cell lines **MCF-7, HepG-2** and **HCT 116** cells. Most of the tested compound showed the anticancer activity against **MCF-7** cells with **IC50** ranging from **4.4** to **49.5** µg **(Table 4)**. Compound **7e** elicited the highest antitumor activity against the **MCF-7** cell lines with **IC50 4.4 µg** compared with the standard Vinblastine followed by compound **7 c& f** which has **IC50 5.9 & 6 µg** respectively. For **HepG-2** cell line, it was found that the most active compounds were **7 c, e & f**. Also these compounds showed the highest activity against **HCT-116** cell line. Studying the structure-activity relationships of 1,4-diazepine derivatives (**4 a-j** and **7a-f**) revealed that, compounds **7** showed higher activity than **4** in all tested cell lines. Comparing anticancer activities of compounds **4a-j** where the diazepine ring is substituted with substituted phenyl ring at 3 & 4 positions**,** compounds **4f-j** imparts higher activity with **IC 50** ranging from 25-50 µg against **MCF-7** and **HCT-116** cell lines, while compounds **4 b, e-i** showed anticancer activity against **HepG-2** cell line with **IC 50** ranging from 33-50 µg. On the other hand compounds **7 a-f** which have 1,4-diazepine ring substituted at position 3- with substituted phenyl showed the highest activity against three cell lines. Compounds **7 c, e &f** were the most active compounds in which the phenyl group at position 3- substituted with 4-phenyl, -OH and -OCH_3 groups. Therefore; the electron withdrawing groups in this position is favored for anticancer activity. These three groups possess **-I** effect in addition to **+ M** effect. Introduction of non-substituted phenyl or substituted phenyl with electron donating group such as –CH_3 decrease the anticancer activity. On the other hand when the substitution was 4-Cl phenyl which has –I effect only the activity was decreased. Among compounds with heterocyclic ring **7a-f**, it was obvious that, compound **7e** bearing 4-hydroxy phenyl ring has slight better activity than **7c&f** having phenyl or 4-methoxy phenyl ring against **MCF-7** with IC 50 **4.4 µg**. (Table 4 and figure 4)

Table 1: % Viability of MCF-7 cells

compounds	Viability %						
	0.00 µg	1.56 µg	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg
4a	100	100	100	98.76	90.50	78.24	65.12
4b	100	100	100	100	90.71	75.68	60.41
4c	100	100	100	100	95.85	89.43	75.21
4d	100	100	100	96.70	89.41	73.56	69.44
4e	100	100	100	98.21	75.98	63.43	52.19
4f	100	100	97.75	80.29	73.67	50.42	28.57
4g	100	100	100	95.44	79.58	60.97	42.85
4h	100	100	100	90.18	76.34	69.45	50.31
4i	100	100	95.22	89.31	78.62	67.81	46.98
4j	100	100	100	100	97.33	70.13	49.56
7a	100	100	100	98.76	83.49	75.58	60.94
7b	100	100	100	95.49	78.89	64.89	50.34
7c	100	89.19	66.39	47.98	39.56	22.34	15.66
7d	100	100	100	100	85.48	66.97	54.27
7e	100	78.49	50.96	48.54	39.89	27.98	13.24
7f	100	90.96	65.66	49.29	37.98	25.46	19.58
9	100	100	100	97.58	80.23	73.58	69.43
Vinblastine	**100.00**	**67.24**	**56.54**	**42.35**	**29.26**	**15.18**	**7.82**

Figure 1 : % Viability of MCF-7 cells

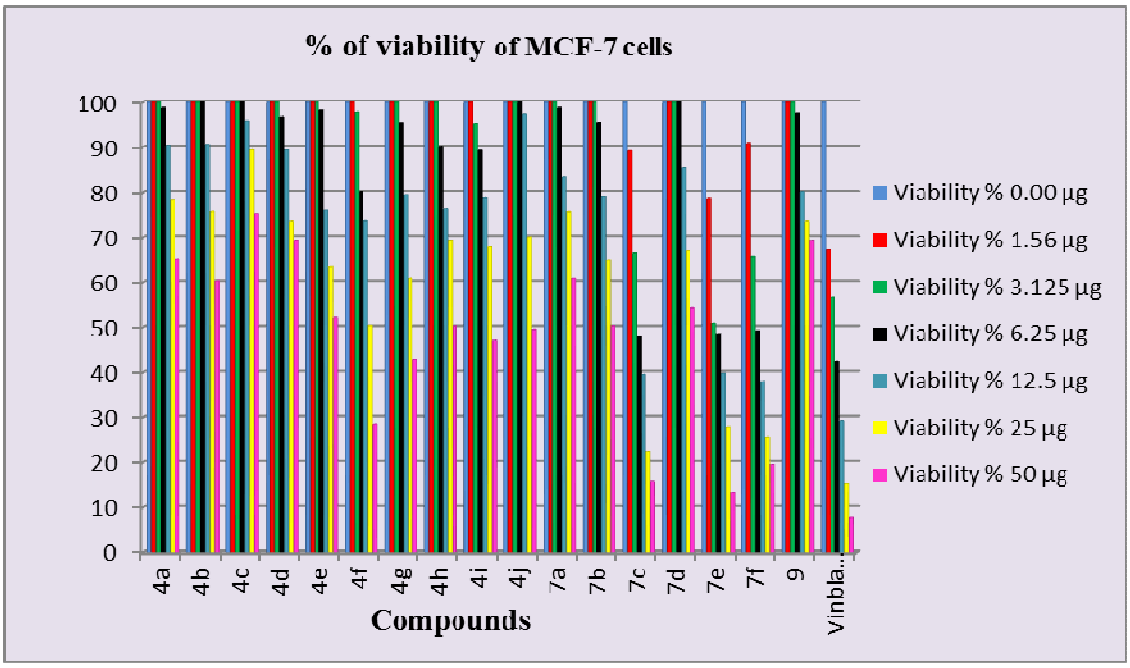


Table 2 % Viability of HepG-2 cells

compounds	Viability %						
	0.00 µg	**1.56 µg**	**3.125 µg**	**6.25 µg**	**12.5 µg**	**25 µg**	**50 µg**
4a	100	100	100	100	85.94	73.51	60.26
4b	100	100	95.31	80.29	67.94	59.26	45.67
4c	100	100	100	94.56	82.15	75.94	60.23
4d	100	100	100	98.43	85.61	78.33	67.51
4e	100	100	94.68	79.93	68.42	56.75	43.94
4f	100	100	95.98	79.45	64.96	55.76	38.21
4g	100	100	100	95.63	77.42	59.74	45.16
4h	100	100	100	98.14	85.62	70.89	50.12
4i	100	100	100	90.96	78.67	63.94	40.86
4j	100	100	100	100	85.59	69.45	54.33
7a	100	100	100	100	95.43	86.71	70.95
7b	100	100	100	87.74	71.14	67.89	50.66
7c	100	94.84	80.79	65.45	44.78	31.69	23.49
7d	100	100	100	100	88.42	75.91	60.32
7e	100	95.74	83.59	60.36	49.53	35.69	26.95
7f	100	95.76	75.48	59.69	48.54	40.46	30.14
9	100	100	100	100	98.54	86.11	70.47
Vinblastine	**100.00**	**76.82**	**69.23**	**53.85**	**43.59**	**27.35**	**15.38**

Figure 2 : % Viability of HepG-2 cells

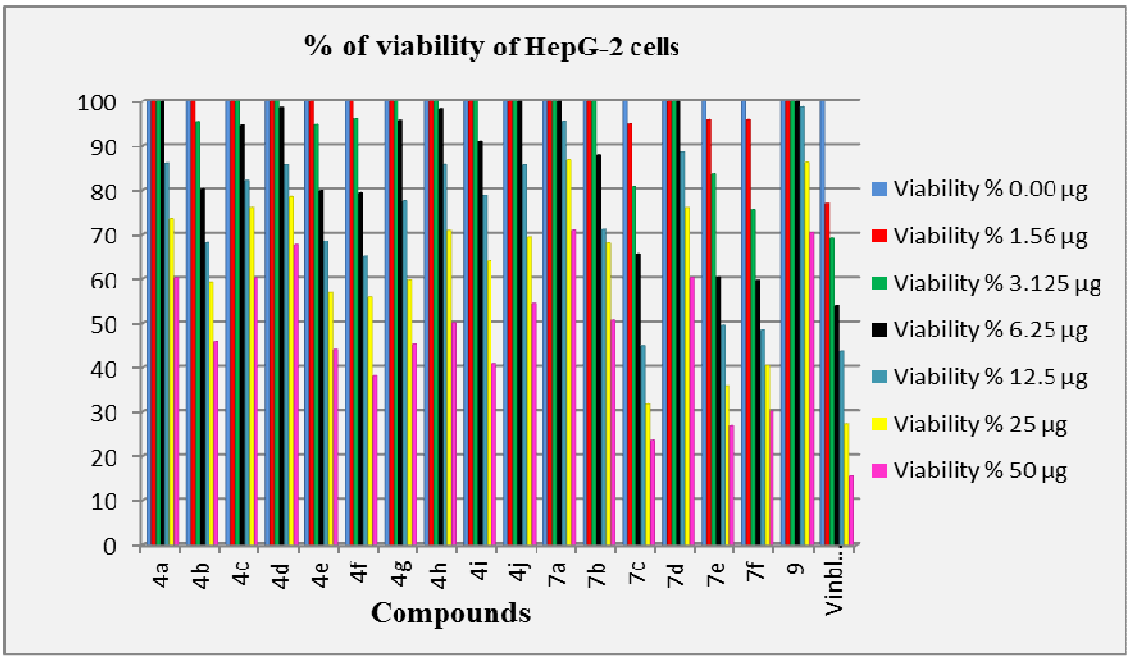


Table 3 % Viability of HCT cells

compounds	Viability %						
	0.00 µg	**1.56 µg**	**3.125 µg**	**6.25 µg**	**12.5 µg**	**25 µg**	**50 µg**
4a	100	100	100	100	98.68	85.43	64.95
4b	100	100	95.49	88.68	75.32	60.07	50.11
4c	100	100	100	100	95.49	89.73	78.65
4d	100	100	100	100	98.78	86.64	76.50
4e	100	100	100	87.95	73.53	66.67	50.04
4f	100	100	98.56	85.42	69.87	50.16	35.16
4g	100	100	96.22	89.72	70.92	55.63	40.12
4h	100	100	90.65	75.12	62.87	50.22	42.91
4i	100	100	95.21	77.87	65.49	50.78	44.64
4j	100	100	100	96.18	86.46	60.31	45.48
7a	100	100	100	100	95.67	80.56	76.47
7b	100	100	100	95.43	80.96	69.78	55.69
7c	100	98.44	85.76	60.94	49.44	35.96	23.54
7d	100	100	100	98.49	89.96	74.67	65.96
7e	100	98.35	87.65	69.97	50.45	39.56	27.94
7f	100	80.81	69.43	54.78	47.95	32.86	20.94
9	100	100	100	100	100	93.48	75.96
Vinblastine	**100.00**	**58.11**	**47.30**	**39.86**	**18.92**	**15.54**	**12.16**

Figure 3 : % Viability of HCT cells

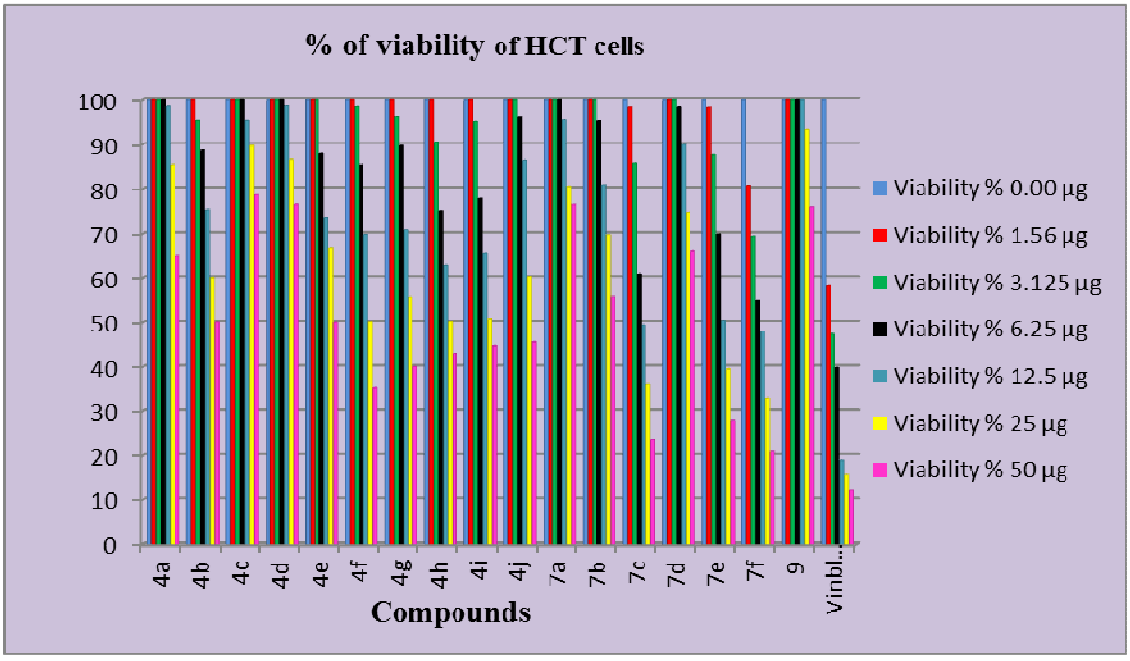
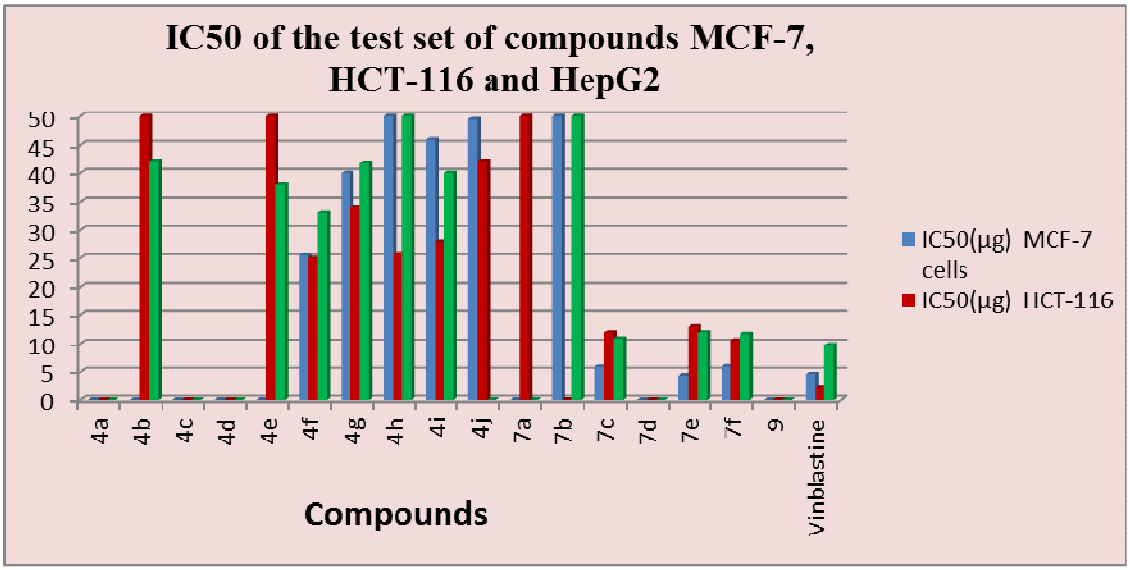


Table 4: IC50 of the test set of compounds against human breast cancer cells MCF-7, human hepatocellular carcinoma cell line HepG2 and human colon cancer cells (HCT-116)

compounds	IC_{50}(µg) MCF-7 cells	IC_{50}(µg) HepG-2 cells	IC_{50}(µg) HCT-116
4a	>50	>50	>50
4b	>50	42	50
4c	>50	>50	>50
4d	>50	>50	>50
4e	>50	38	50
4f	**25.5**	33	**25**
4g	40	41.7	34
4h	50	50	**25.8**
4i	46	40	28
4j	49.5	>50	42
7a	>50	>50	50
7b	50	50	>50
7c	**5.9**	**10.9**	**12**
7d	>50	>50	>50
7e	**4.4**	**12**	**13**
7f	**6**	**11.7**	**10.6**
9	>50	>50	>50
Vinblastine	**4.6**	**9.8**	**2.38**

Figure 4: IC50 of the test set of compounds against human breast cancer cells MCF-7, human colon cancer cells (HCT-116), and human hepatocellular carcinoma cell line HepG2



Acknowledgement
The authors would like to thank Dr. Bothaina Abd El-Fattah Mousa Prof. of Organic Chemistry- Faculty of Pharmacy-Cairo University for her real help.

REFERENCES

[1] Dikeos DG, Theleritis CG, Soldatos CR. Benzodiazepines: effects on sleep. In: Pandi-Perumal SR, Verster JC, Lader M, Langer SZ. Boca Raton, FL: CRC Press, Taylor & Francis Group, 220–2 (**2008**).
[2] Yoshio, B. and Masako, N.; Japan. 76 30,074 (Cl. C07D243/24), 30 Aug 1976, Appl. 68/6,884, 06 Feb 1968; *through Chem. Abstr*., 86, 106676r (**1977**).
[3] Hisao, Y.; Shigeho, I.; Tadashi, O.; Toshiyuki, H.; Kikoo, I.; Isamu, M.; Michihiro, Y.; Kazuo, M. and Tsuyoshi, K.; Japan. 71 02, 983 (Cl. C 07*d*, A 61*k*), 25 Jan 1971 Appl. 26 Apr 1968; *through Chem. Abstr*., 78, 141892g (**1973**).

[4] Ludwig, S.; Ger. Offen. 2,043,614 (Cl. C 07*d*), 25 Apr 1971 Austrian Appl. 19 Sep 1969-23 Jun 1970; *through Chem. Abstr*., 78, 141899q (**1973**).
[5] Kanji, M.; Hiroyuki, T. and Yutaka, K.; Ger. Offen. 2,042,758 (Cl. C 07*d*), 11 Mar 1971 Japan. Appl. 01 Sep 1969; *through Chem. Abstr*., 78, 141900h (**1973**).
[6] Rodney, I. F. and Armin, W.; Fr. Demande 2,252,103 (Cl. A61K31/55), 20 Jun 1975 US Appl.419,563, 28 Nov 1973; *through Chem. Abstr*., 86, 106672m (**1977**).
[7] Umakant, D. S.; Ger. Offen. 2,622,937 (Cl. C07D405/12), 02 Dec 1976, Brit. Appl.75/22,057, 22 May 1975; *through Chem. Abstr*., 86, 106675q (**1977**).
[8] Ramon, M. P.; Carlos, C. S.; Joaquin, D. R. Z.; Nelida, U. C.; Cristobal, M. R. and Antonio, Vila-Coro B.; Ger. Offen. 2,233,457 (Cl. C 07*d*), 22 Feb 1973, Span. Appl. 393, 101, 09 Jul 1971; *through Chem. Abstr*., 78, 124645u (**1973**).
[9] Weitz IS, Pellegrini M, Mierke DF, Chorev M. Synthesis of a Trisubstituted 1,4-Diazepin-3-one-Based Dipeptidomimetic as a Novel Molecular Scaffold. *J Org Chem*., 62, 2527–34 (**1997**).
[10] Weitz IS, Pellegrini M, Royo M, Mierke DF, Chorev M. *Lett Pept Sci*., 5, 83–6 (**1998**).
[11] Zia-ul-haq M, Hameed S, Duddeck H, Ahmed R. *Turk J Chem*., 26:807–13 (**2002**).
[12] Parmar NJ, Barad HA, Pansuriya BR, Teraiya SB, Gupta VK, Kant R. *Bioorg Med Chem Lett*., 22, 3816–21(**2012**).
[13] Fader LD, Bethell R, Bonneau P, Bös M, Bousquet Y, Cordingley MG, et al. *Bioorg Med Chem Lett*., 21, 398–404 (**2011**).
[14] Vicentini CB, Guarneri M, Scatturin A, Giori P, Heilman W. *Il Farmaco*., 51, 609–12 (**1996**).
[15] Childress SJ, Gluckman MI. *J Pharm Sci*., 53, 577–90 (**1964**).
[16] Sandra CM, Eduardo CC, Simon HO, Teresa RA, Antonio NC, Lijanova IV, *et al*. *Med Chem*.,12, 611–8 (**2012**).
[17] Casals-Stenzel J. *Lipids*., 26, 1157–61 (**1991**).
[18] Yamada, M.; Tanimoto, A.; Ichinowatari, G.; Yaginuma, H. and Ohuchi, K.; *Eur. J. of Pharmacol*., 374, 341 (**1999**).
[19] Arya, V. P.: *Indian J. Chem*., 10, 1141(**1972**).
[20] Gewald, k.; Z. Chem. 1962, 2, 305; through chem. Abstr. 58, 6770 (**1963**).
[21] Gewald, K.; Schinke, E.; Bőttcher, H.; *Chem Ver*., 99, 94 (**1966**).
[22] Chusheng, H.; Zhe, Z.; Shuhua, L.; Yulin, L.; *J. Chem. Res* (S)., 148 (**1999**).
[23] Fougaud, A.; Person et, H. and Robert, A; *J. Bull. chem. Soc. France*, 8, 1873 (**1964**).
[24] Wilson, A.P. Cytotoxicity and viability assays in animal cell culture: A practical approach, 3rd ed. (ed. Masters, J.R.W.) *Oxford University Press:* Vol.1. (**2000**).