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SYNTHESIS, CHARACTERIZATION OF SOME NEW THIAZOLIDIN-4-ONE DERIVATIVES COMPOUNDS AND EVALUATION OF THEIR ANTICANCER ACTIVITY

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ABSTRACT : A new series of derivatives aromatic 4- thiazolidinones were synthesized.ethyl diethylglycinate (1)was obtained by direct reaction of secondary aliphatic amine with chloroethylacetate, then reaction between compound (1) and hydrazine hydrate was carry out in ethanol to form 2-(diethylamino)acetohydrazid (2). After that, Schiff's base formed by addition of four differentaldehydesfinally, cyclocondensationstep was achieved in the presence of thioglycolic acid to form 4-thiazolidinonesheterocyclic ring. The new compounds were determined via the (IR, ^{1}H NMR, ^{13}C NMR, and APT ^{13}C experiments. Two compounds (9 and 7) were evaluated forinhibition oftwocancer cell linesHepG2 and SK-GT2. Compounds, N-(2-(2-bromophenyl)-4-oxothiazolidin-3-yl)-2-(diethylamino)acetamide (9) and N'-((1H-indol-3-yl)methylene)-2-(diethylamino)acetohydrazide(7) depicted potential anticancer behavior.

Key words : Schiff's bases, 4-thiazolidinone, anti-cancer.

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INTRODUCTION

Schiff's bases, a class of organic compound that containing azomethine or imine functionality that noted for their multiple pharmaceutical in the medicinal and pharmaceutical field (Ahmad *et al*, 2013). Many unique biological activities have been reported with a significant result on synthesized Schiff's base such as antitumor activity, antioxidant activity, anti-inflammatory and antibacterial properties (Ahmad *et al*, 2013; Ashraf *et al*, 2011; Baviskar *et al*, 2012). Inaddition,Schiff's bases have many applications in drug design developmentand improvement of drug performances (Choppara *et al*, 2019). For thousands of years' heterocycles have played an important role throughout the world in treating and preventing human diseases. These compound has received amount of attention over the years by medicinal chemists for drug discovery (Cunico *et al*, 2008). Due to interesting structural of the heterocyclic compounds provide additional magic to the researchers and as a result of that much research has been accomplished to discover and develop new methods of synthesis and discover new chemical promising drugs of the future. The introduction of a ring such as thiazolidin-4-one and its derivatives into chemical compounds affects the chemical properties of the organic compounds and often leads to valuable changes in its biological activities (Dikio *et al*, 2017). Thiazolidin-4-one which is an important class of heterocyclic compounds possess a five membered heterocyclic compounds containing two heteroatoms one nitrogen (Huda *et al*, 2021), one sulphur and three carbon atoms including a carbonyl group (Dikio *et al*, 2017; Freshney, 2012). Literature review show versatile pharmacological activities of thiazolidin-4-One derivatives. They have been reported as anti- cancer (Hanson, 2003), anti-viral (Hoan, 2018), anti fung (Huda *et al*, 2021), anti-bacterial agents (Incerti *et al*, 2018) and anti-hyperglycemicactivity (Khalilullah *et al*, 2012). In this study, we report the synthesis and anticancer studies of aromatic thiazolidinone derivatives.

Experimental Materials and Instruments

Chemicals used in this work are supplied from Merck, Sigma-Aldrich, BDH and Fluka companies and are used

without further purification.FTIR spectra were recorded on Perkin Elmer spectrum -65 using KBr discs in the (500-4000) cm^{-1} spectral range. ^{1}HNMR and ^{13}CNMR spectra were recorded on Bruker 400MHz instrument using DMSO-d6 as a solvent and TMS as internal reference. Thin layer chromatography (TLC) was carried out using Fertigfollenprecoated sheets type Polygram Silg, and the plates were developed either by the quenching of UV fluorescence at 254 nm or by treatment with $KMnO_4$ solution and heating. The anticancer activity was performed in Iraqi Center for Cancer and Medical Genetics Research department, Al-Mustansiriyah University.

Experimental

Synthesis of ethyl diethylglycinate (1)

A mixture of diethylamine (27.34mmol)and chloroethylacetate (47.32 mmol) in DMF (30ml) was treated with a catalytic amount of potassium hydroxide (26.73 mmol). The mixture was left stirring at room temperature for 11 hrs. The organic layer was separated and the aqueous layer was extracted with ethylacetate (3 × 30ml). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacue to afford the crude product as a (brown oil 74%). FT-IR- KBr,data in $(cm)^{-1}$: 2979, 2877, 1745, 1625, 1459,1384, 1384,1342, 1276, 1186, 1093, 1033, 991, 868, 781 cm^{-1}. ^{1}H-NMR (400MHz, DMSO, ä in ppm) ä= 4.48 (2H, -O-C*H*$_2$-), 4.26 (2H, N-C*H*$_2$CO-), 2.8(2H, q, N-C*H*$_2$CH$_3$), 1.28-1.08(3H, t, 2C*H*$_3$). ^{13}C-NMR (400MHz, DMSO, ä in ppm) ä= 167.46 (*C*=O), 62.14(N-*C*H$_2$CO), 56.89(O*C*H$_2$), 41.21 (N-*C*H$_2$CH$_3$), 13.74-10.00 (2*C*H$_3$).

Synthesis of 2-(diethyl amino) aceto hydrazid (2)

A mixture of ethyl di ethylglycinate (3.14mmol) and hydrazine hydrate 80%, 5.5ml) in ethanol (30 ml) was left stirring at room temperature for 9 hrs. After cooling solvent was removed and few ice pieces was added. The mixture was poured into saturated brine solution. The organic layer was separated and the aqueous layer was extracted with ethylacetate (3×30 ml). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacue to afford the crude product as (yellow solid 66%). FT-IR- KBr,data in $(cm)^{-1}$: 3444,3302, 3064, 1667,1615, 1528, 1378, 1318, 1147, 1040, 993, 917, 648 cm^{-1}. ^{1}H-NMR (400MHz, DMSO, ä in ppm) ä= 10.06 (1H, s, N*H*), 4.23 (2H,s, -N-C*H*$_2$-CO), 3.88 (2H, q, - N-C*H*$_2$-CH$_3$), 2.39(2H, s, NH*NH*$_2$), 1.05 (3H, t, C*H*$_3$) . ^{13}C-NMR (400MHz, DMSO, ä in ppm) ä= 174.68 (*C*=O), 61.98(N-*C*H$_2$CO), 48.16 (N-*C*H$_2$CH$_3$), 12.04 (2*C*H$_3$).

Synthesis of1H-indole-3-carbaldehyde(3)

$POCl_3$(20.80mmol) was slowly added to a solution of pre-cooled indole) (17.07 mmol) in dry DMF (15 ml) at –5°C. After completion of addition, the temperature was a raise to 40°C and the reaction mixture was stirred at 40°C for 2 h, before quenched with crushed ice followed by NaOH (1-2 ml, 1M). The resulting was heated rapidly to the boiling point and then allowed to cool to room temperature, before placed in refrigerator over-nights. The precipitate was filtered off and washed three time with 100 ml water to furnish the title compound as (red solid, 88%). FT-IR- KBr,data in $(cm)^{-1}$: 3411, 3201, 3171, 3105, 3045, 2931, 2817, 1697, 1634,1613,1577, 1519, 1495, 1448, 1393, 1333, 1243, 1126, 1084, 1006, 787 cm^{-1}. ^{1}H-NMR (400MHz, DMSO, ä in ppm) ä = 12.21 (1H, s, N*H*), 9.93 (1H,s, COH), 8.28-7.21 (5H, indole and ArH). ^{13}C-NMR (400MHz, DMSO, ä in ppm) ä= 184.85 (*C*=O), 138.28-112.30 (Ar-C and C-indole).

General procedure that adopted for the synthesisof four Schiff's base (4-7)

The solution of 2-(diethylamino)acetohydrazid**2** (2.06 mmol) and the suitable aldehyde (2.06 mmol) in ethanol (15 ml) was heated under reflux for 11 h in the presence of AcOH (10 drops). The resulting mixture wasallowed to cool to room temperature and the precipitated solid thus obtained was filtered, washed with ethyl acetate then with ice-cold water and recrystallized from hotethanol.

Synthesis of 2-(diethylamino)-N-(4-nitrobenzylidene) acetohydrazide (4)

Yellow crystals (87%)FT-IR- KBr, data in $(cm)^{-11}$: 3303, 3177, 3093, 2973, 2835, 1751, 1682, 1643, 1604, 1522, 1477, 1390, 1345, 1333, 1288, 1222, 1135, 1093, 1078, 1030 ,952, 877, 817, 736 cm^{-1}. ^{1}H-NMR (400MHz, DMSO, ä in ppm) ä= 11.65 (1H, s, CO-N*H*), 8.51-7.73 (1H, s, N=C*H*) and (4H, d, Ar-*H*), 2.25-2.00(2H, q, N-C*H*$_2$CH$_3$), 1.24-1.07 (3H,t, NCH$_2$C*H*$_3$). ^{13}C-NMR (400MHz, DMSO, ä in ppm) ä= 172.24 (C=O), 146.19 (N=*C*H), 143.35-120.66 (Ar-*C*), 20.25 (*C*H$_3$).

Synthesis of N-(2-bromobenzylidene)-2-(diethylamino)acetohydrazide (5)

Yellow solid (67%). FT-IR- KBr,data in $(cm)^{-1}$: 3205, 2995, 2948, 1655, 1628, 1610, 1589, 1556, 1464, 1431, 1314, 1269, 1024, 952, 749, 639 cm^{-1}.^{1}H-NMR (400MHz, DMSO, ä in ppm) ä= 9.1 (1H, s, CO-N*H*), 8.17 (1H, s, N=C*H*), 7.80-7.49 (4H, d-t, *Ar-H*), 3.90 (2H, s, N-C*H*$_2$CO), 2.09 (2H,q, NC*H*$_2$CH$_3$), 1.13(3H,t, NCH$_2$C*H*$_3$).

Synthesis of N-(4-chlorobenzylidene)-2-(diethylamino)acetohydrazide (6)

Brightyellow crystals (75%).FT-IR- KBr,data in $(cm)^{-1}$: 3185, 1646, 1625, 1592, 1565, 1487, 1401, 1293, 1090 ,

1012, 958, 824, 701 cm $^{-1}$. ^{1}H-NMR (400MHz, DMSO, ä in ppm) ä= 11.73 (1H, s, CO-N*H*), 8.73 (1H, s, N=C*H*), 7.9 (2H, d, Ar-*H*), 7.61 (2H, d, Ar-*H*), 4.40(2H, s, N-CH_2CO), 2.09 (2H, q, NCH_2CH$_3$), 1.06(3H, t, NCH$_2$CH_3).

Synthesis of N-(1H-indol-3-yl)methylene)-2-diethylamino) acetohydrazidea (7)

Brown solid. (72 %). FT-IR- KBr,data in (cm)$^{-1}$: 3193, 3109 , 3061, 2930, 1672, 1622, 1242, 1120, 955, 806 ,707, 603 cm $^{-1}$. ^{1}H-NMR (400MHz, DMSO, ä in ppm) ä= 11.78 (1H, s, N*H*, indole), 8.92 (1H, s, CO-N*H*), 8.07 (1H, s, N=C*H*),8.07-7.23 (5H, m, Ar-*H*), 3.94(2H, s, N-CH_2CO), 2.19 (2H, q, NCH_2CH$_3$), 1.07 (3H, t, NCH$_2$CH_3).

General Procedure for the Synthesis of thiazolidin-4-one derivatives (8–11)

A mixture of appropriate Schiff's bases (4–7) (2.1mmol), thioglycolic acid (3mmol) and catalytic amount of zinc chloride anhydrous in 20 mL of toluene or THF was refluxed for 13-15 h. The resulting solution was quenched with cold distilled water and neutralized with an aqueous solution of NaOH 10%. The resulting of precipitate was filtered off, washed with distilled water and dried in an oven.

(diethylamino)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)acetamide (8)

Light brown solid (47 %, in toluene), FT-IR-KBr,data in (cm)$^{-1}$: 3245, 3076, 2979, 2925, 2864 ,1722,1689, 1598, 1523, 1390, 1345 ,1266, 1224 ,1173, 1106, 1058, 1013 ,997, 928, 898, 849, 810 ,735 cm $^{-1}$.^{1}HNMR (400MHz, DMSO, ä in ppm) ä=10.16 (1H, s, CO-N*H*), 8.41-7.69 (4H, m, Ar*H*)6.64 (1H, s, N-C*H*thiazolidinering), 5.94 (2H, s, N-*C*H_2), 4.04-3.73 (2H, m, CH_2S thiazolidine ring), 1.77-2.0 (2H, q, NCH_2-CH$_3$) 1.07 (3H, t, NCH$_2$-CH_3). APT^{13}C-NMR(400MHz, DMSO, ä in ppm) ä= 167.75-170.00 (2*C*=O), 130.35-123.60 (Ar-*C*), 60.30 (*N*-*C*H), 29.59 (*C*H_2S), 20.04 (*C*H$_3$).

N-(2-(2-bromophenyl)-4-oxothiazolidin-3-yl)-2-(diethylamino)acetamide (9)

White precipitate (66%, in THF), FT-IR-KBr, data in (cm)$^{-1}$: 3162, 2923, 2674, 2564, 1718 ,1465, 1396, 1293, 1176, 1022, 901, 747 cm^{-1}. ^{1}HNMR (400MHz, DMSO, ä in ppm) ä = 10.45(1H, s, CO-N*H*), 7.21–8.21 (4H d-dd, Ar*H*)6.68 (1H, s, N-C*H* thiazolidine ring), 5.37 (2H, s, N-*C*H_2), 4.06-3.89 (2H 2d, CH_2S thiazolidine ring), 2.04 (2H, q, NCH_2-CH$_3$) 1.28 (3H, t, NCH$_2$-CH_3).

N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-2-(diethylamino)acetamide(10)

White precipitate (52%, in THF), FT-IR- KBr, data in (cm)$^{-1}$: 2938, 2659, 2564, 1714, 1491, 1407,1289 ,1176, 1176, 1088, 1015, 901, 832, 751, 656 cm^{-1}. ^{1}HNMR (400MHz, DMSO, ä in ppm) ä=10.19 (1H, s, CO-N*H*), 8.74-7.48 (4H d-dd, Ar*H*)6.79 (1H, s, N-C*H*thiazolidine ring), 5.79 (2H, s, N-*C*H_2), 4.00-3.85 (2H, 2d, *C*H_2S thiazolidine ring), 2.21 (2H, q, NCH_2-CH$_3$) 1.24 (3H, t, NCH$_2$-CH_3). ^{13}C-NMR (400MHz, DMSO, ä in ppm) ä= 167.35 (*C*=O), 160.36 (*C*=O), 149.07- 138.3 (Ar-*C*), 59.56 (*N*-*C*H), 29.69 (*C*H_2S), 19.97 (*C*H$_3$).

N-(2-(1H-indol-3-yl)-4-oxothiazolidin-3-yl)-2-(diethylamino)acetamide (11)

Pink precipitate (75%, in THF), FT-IR- KBr,data in (cm)$^{-1}$:3193, 3109, 3061, 2930, 1672, 1622, 1242, 1120, 955, 86,707, 603 cm^{-1}. ^{1}HNMR (400MHz, DMSO, ä in ppm) ä=10. 98 (1H, s, CO-N*H*), 10.53 (1H, s, indole-NH), 8.34-7.18 (Ar-H)6.17 (, 1H, s, N-C*H*thiazolidine ring), 4.69 (2H, s, N-*C*H_2), 3.87-3.69 (2H, m, *C*H_2S thiazolidine ring), 2.28 (2H, q, NCH_2-CH$_3$) 1.07 (3H, t, NCH$_2$-CH_3).

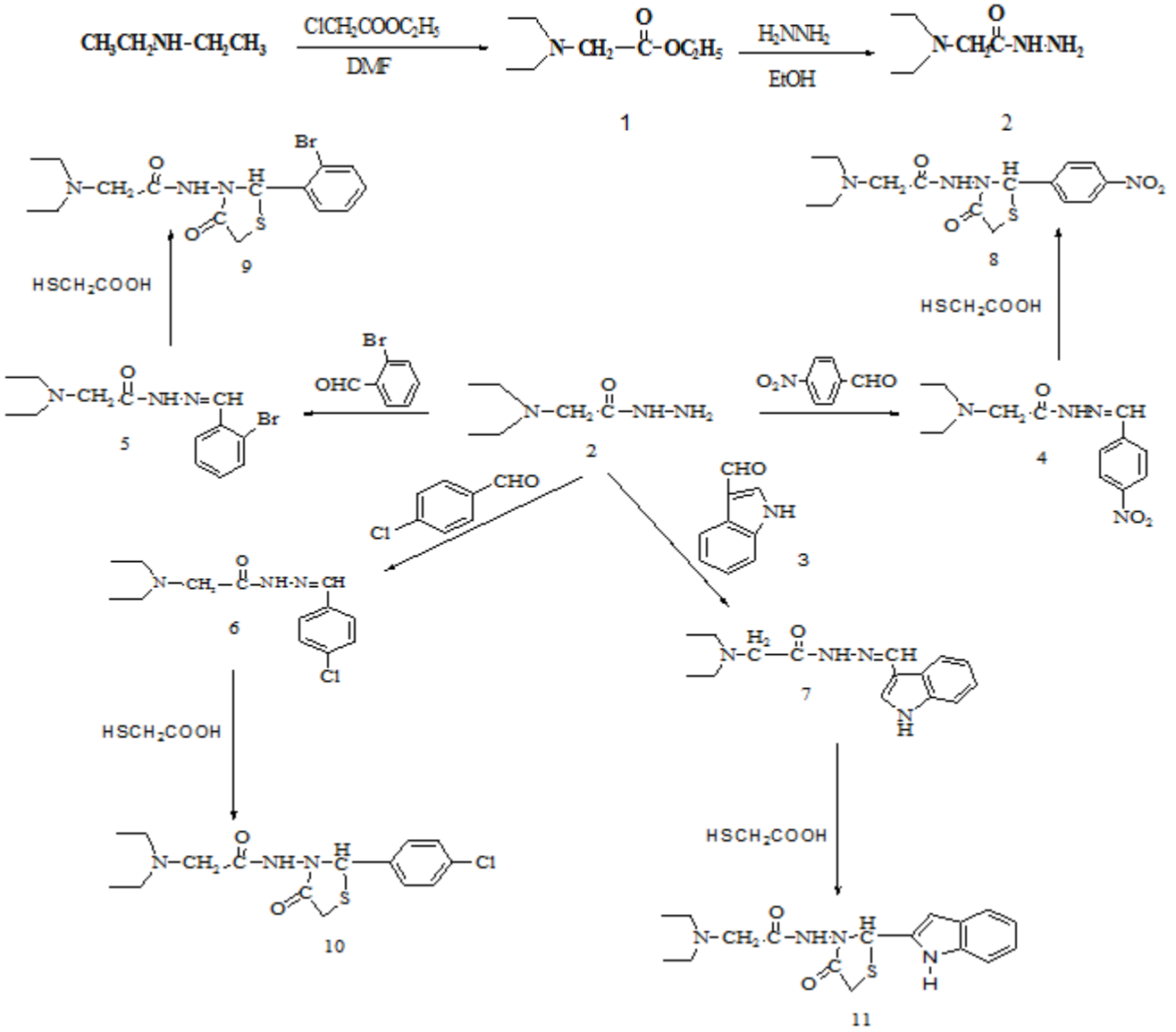
RESULTS AND DISCUSSION

The synthesized compounds (1-11) are shown in Scheme 1. The synthesized compounds were subject to TLC; spectral studies like ^{1}H NMR, ^{13}C NMR, APT, ^{13}C NMR and FTIR and results of some synthesized compound are discussed below.

The IR spectrum of compound **(1)** showed strong absorption corresponding to the carbonyl group of ester at 1745cm^{-1} (Fig. 1).

Analysis of the ^{1}H NMR spectrum of crude materials compound **(1)** obtained after work showed two new signals characteristic at ä 4.48 and 4.26 ppm corresponding to protons of O-C***H***$_2$ and N-C***H***$_2$CO. Furthermore, appearance series of signals at ä 167.46, 62.14, 56.89, 41.21 and 13.74 ppm in ^{13}C NMR spectrum corresponding to the resonances of the carbon of C=O of ester, N-***C***H$_2$C=O, O-***C***H$_2$, N***C***H$_2$CH$_3$ and 2***C***H$_3$ groups confirmed our assumption.The critical intermediate 2-(diethylamino)acetohydrazid (2) is prepared by procedure as describe in literature (Küçükgüzel *et al*, 2006) ethyl diethylglycinate (1) was dissolved in 20 ml of ethanol. Excess of hydrazine hydrazine hydrate 80% was added and the reaction mixture was left stirring at room temperature for 9 hrs. The TLC analysis showed the presence of a new material with complete consumption of starting material. FT-IR, ^{1}HNMR and ^{13}CNMR allowed the identification of the desired new compound.

The7 FT-IR 7 spectrum (Fig. 2) displayed two stretching bands at 3444 and 3302 cm^{-1} belongtothe NH$_2$



Scheme 1 : Proposal synthetic of compounds 1-11.

and NH[14], in addition absorption band at 1667 and 1615.9 cm^{-1}due to C=O and NH bending.

A new feature in the ^{1}H NMR spectrum was a signal at ä 10.06 and ä 2.39 ppm corresponding to the protons of NH and NH_2 (Mir *et al*, 2018) of hydrazide group respectively along with resonances at ä 4.23 and ä 3.88 ppm corresponding to the protons of N-C***H***$_2$CO and N-C***H***$_2$CH$_3$ groups. Furthermore, the structure of compound was also confirmed by ^{13}C-NMR spectrum. Analysis of ^{13}C NMR spectrum allowed to identified the carbonyl group of amide at ä 174.68 ppm and three carbonssignals at ä 61.98, 48.16 and 12.04 ppm, which were attributed to the carbons of the N-CH_2CO, N***C***H_2CH$_3$ and NCH$_2$***C***H$_3$ groups, respectively. The 1H-indole-3-carbaldehyde **(3)** was synthesized using procedure described by Choppara *et al* (2019). TLC analysis suggested that new compound had been formed with completely consume of starting material.FT-IR spectrum (Fig. 3) of the new compound showed absorption at 3411, and 1697 cm^{-1} that referred to NH of indole and C=O of aldehyde, respectively. Absorption starching at 2817 cm^{-1} for aldehyde group confirmed, we had the desired compound.

The ^{1}H NMR spectrum (Fig. 4) of the crude product obtained after work up showed peaks corresponding to the proton of aldehyde group and NH of indole at ä 9.93 and ä 12.21 ppm respectively with additional peaks in aromatic region belong to the protons of rings.

A signal in the ^{13}CNMR spectrum (Fig. 5) at ä 184.85 ppm corresponding to the carbonyl carbon of aldehyde group was observed along with eight carbon signals at ä

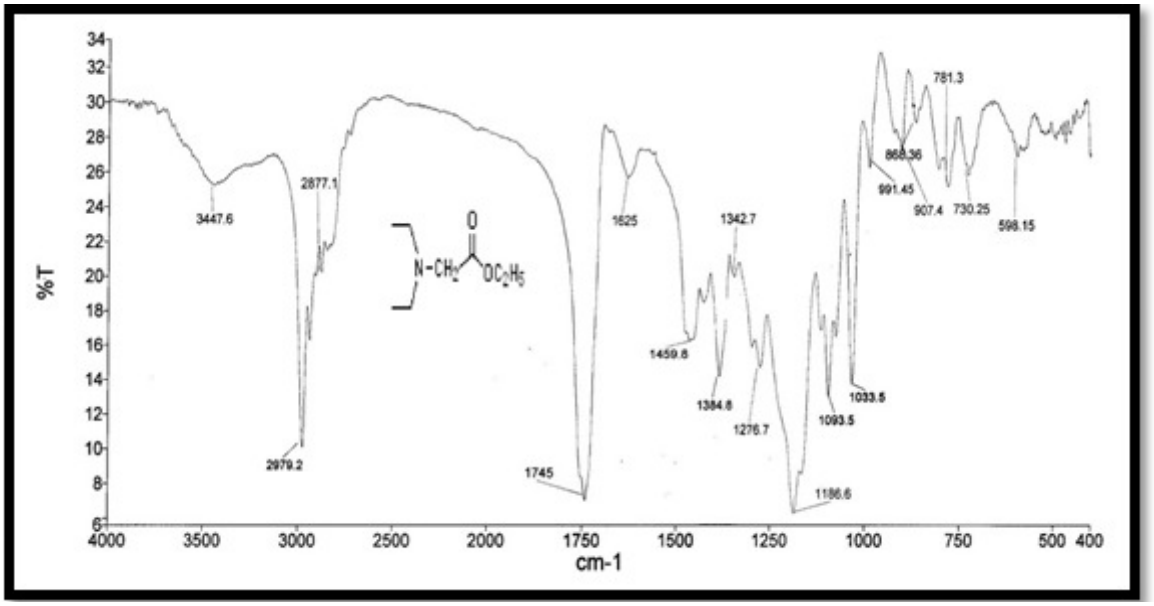


Fig. 1 : IR spectrum of ethyl diethylglycinate (1).

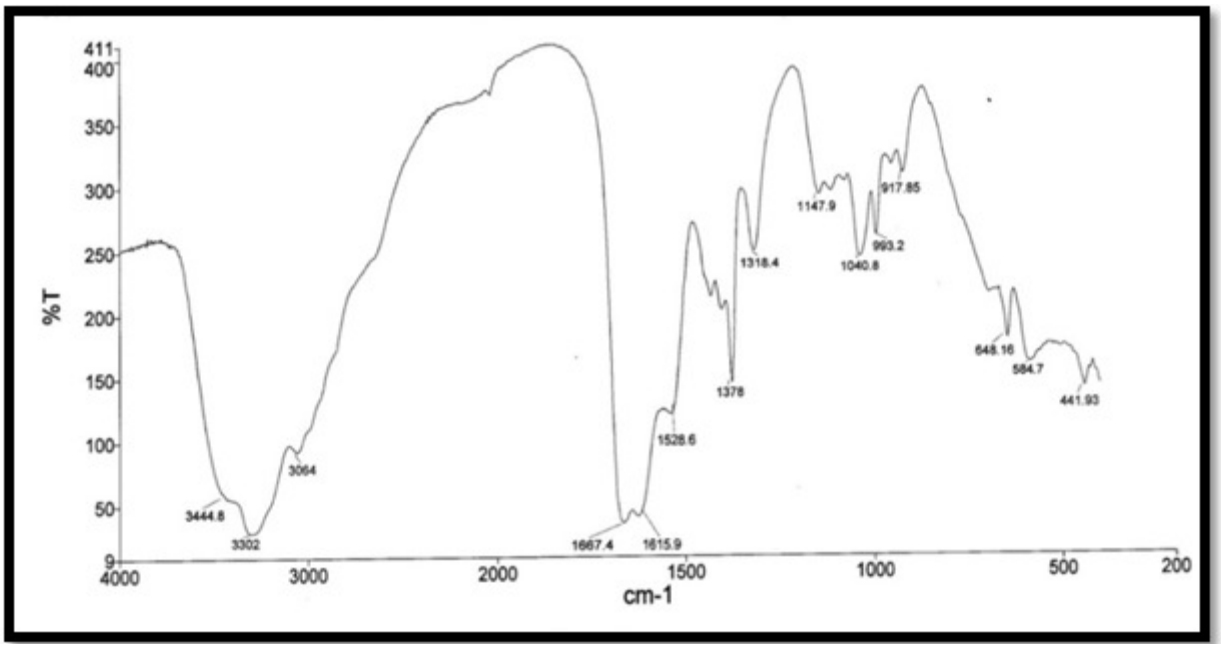


Fig. 2 : IR spectrum of 2-(diethylamino)acetohydrazid (2).

138.28 - 112.30 ppm, which were attributed to the carbons of the rings.

Synthesis of Schiff's base compounds (4-7) was carry out from procedure as describe in literature [17]were solution of (2- diethylamino)acetohydrazid **(2)** was reacted with suitable aldehyde in ethanol under reflux for 11 h. in the presence of acetic acid. The structure of synthesized compounds has been supported by different spectral data (IR, ^{1}HNMR, ^{13}CNMR and APT^{13}CNMR). For example, the FT-IR spectrum of compound (4) (Fig. 6) showed a strong absorption corresponding to the carbonyl group of amide at 1682 cm^{-1} along with of stretching band at 1643 cm^{-1} corresponding to C=N group. The structure also was confirmed by analysis of the ^{1}H NMR and ^{13}CNMR spectrum.

^{1}H NMR spectrum (Fig. 7) showed doubling up of all peaks signal such as CO-NH proton at ä 11.65 and ä 11.49 ppm along with two signals in aliphatic region at ä 2.25-1.07 ppm tentatively assigned to the methylene and methyl groups. All these a new feature indicated that there is a mixture of two isomers of the desired compound.

It is important to write that, the ^{1}H NMR of the product showed very weak signal of N-CH_2 between ä (4.0-5.0) ppm. In ^{13}CNMR spectrum (Fig. 8), showed two signals for amide carbon at ä 172.24 and 165.98 ppm along with Spectra data (IR and ^{1}HNMR) of all

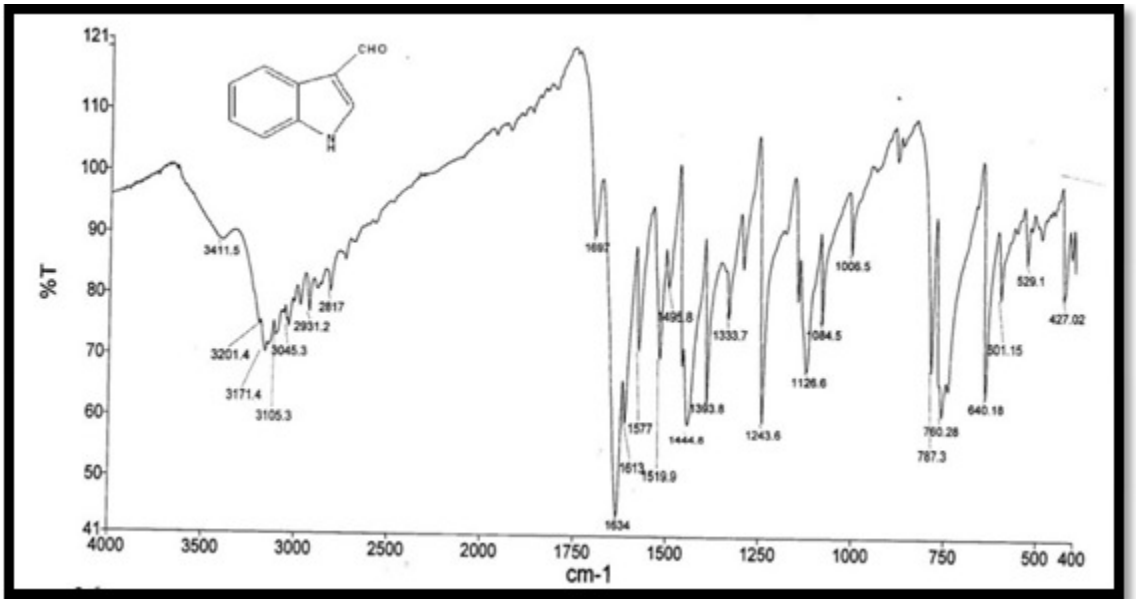


Fig. 3 : FT-IR spectrum of compound 1H-indole-3-carbaldehyde (3).

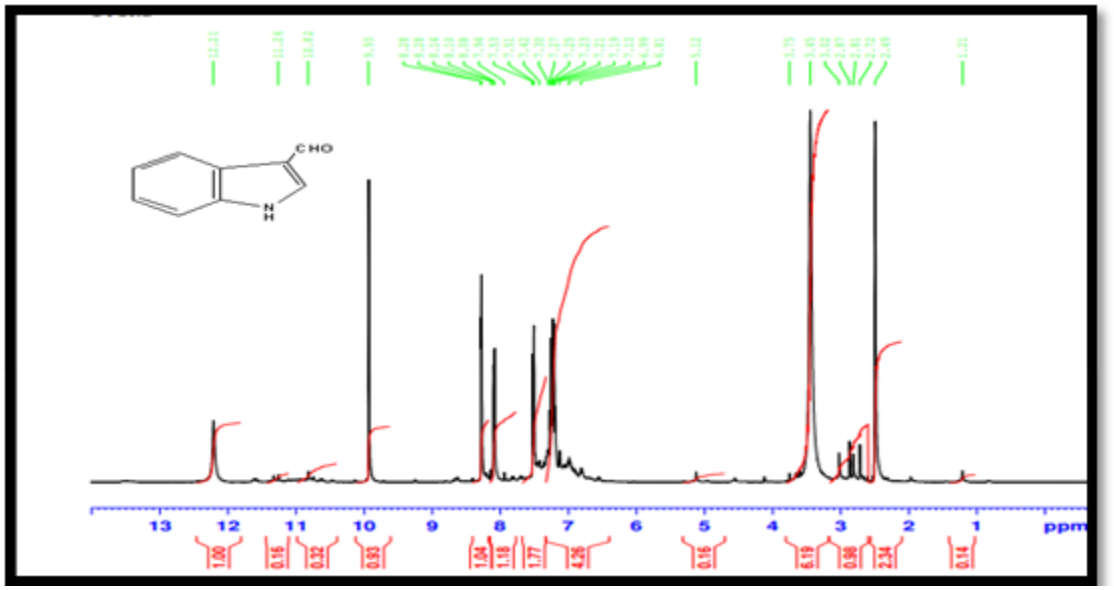


Fig. 4 : [1]HNMR spectrum of compound 1H-indole-3-carbaldehyde (3).

synthesized compounds has been described in experimental section.

Synthesis of thiazolidin ring(8–11)

Preparationthe desired 4-thiazolidinone ring was carried out according to the literature procedure of Ahmad *et al* (2013). After 15 hours refluxing in oil bath the TLC revealed the total consumption of starting material along with the formation of a new spot.The FT-IR spectrum of (**8**) (Fig. 9) allowed to identification of all the functional group. The presence of two peaks at 1722 and 1689cm^{-1}are corresponding to the carbonyl group of thiazolidinone ring and amide with a broad peak at 3245cm^{-1}, which assign to the NH group confirm the proposal compound.

The ^{1}H NMR spectrum (Fig. 10) of this compound showed signal characteristic proton of CO-NH in aromatic region at ä 10.16 ppm (Raza *et al*, 2013). Signals at ä 6.64 ppm corresponding to the resonance of N-CHAr of thiazolidine ring (Reddy *et al*, 2013) along with a clean signals of protons of CH_2S at ä 4.04-3.73 ppm indicating the formation of thiazolidine ring (Reddy *et al*, 2013).

APT^{13}CNMR resultswere further used to characterize the compound **(8)**A signal in the APT^{13}C NMR spectrum (Fig. 11) at (negative side below of the spectra)at ä 60.30 ppmcorresponding to the N-$\underline{C}$H of thiazolidinone ring and a signal at ä 29.59 ppm for $\underline{C}H_2$-S of thiazolidinone ring that appeared at (positive side above of the spectrum) and disappearance a signal of N=CH group confirmed that we achieved cyclization step.

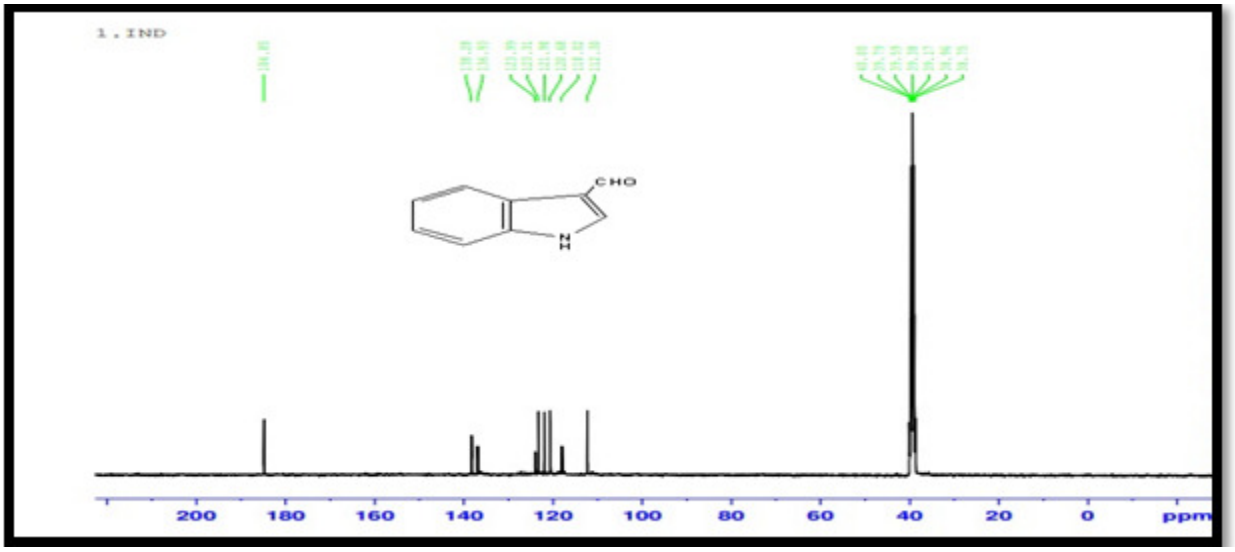


Fig. 5 : ^{13}C NMR spectrum of compound 1H-indole-3-carbaldehyde (3).

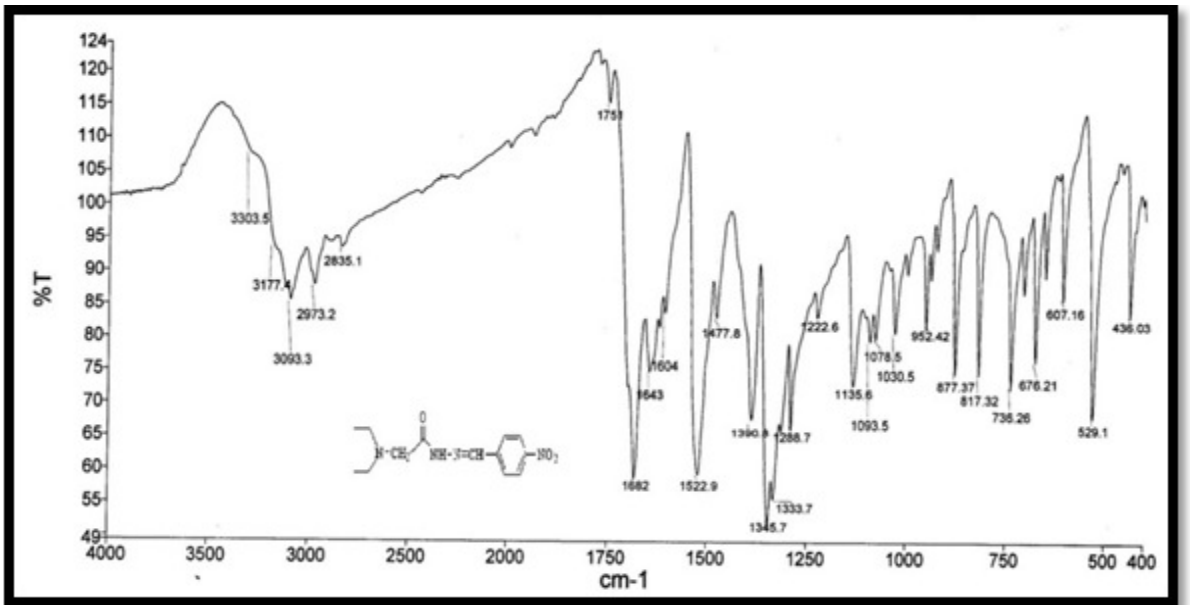


Fig. 6 : FT-IR spectrum of compound 2-(diethylamino)-N-(4-nitrobenzylidene) acetohydrazide (4).

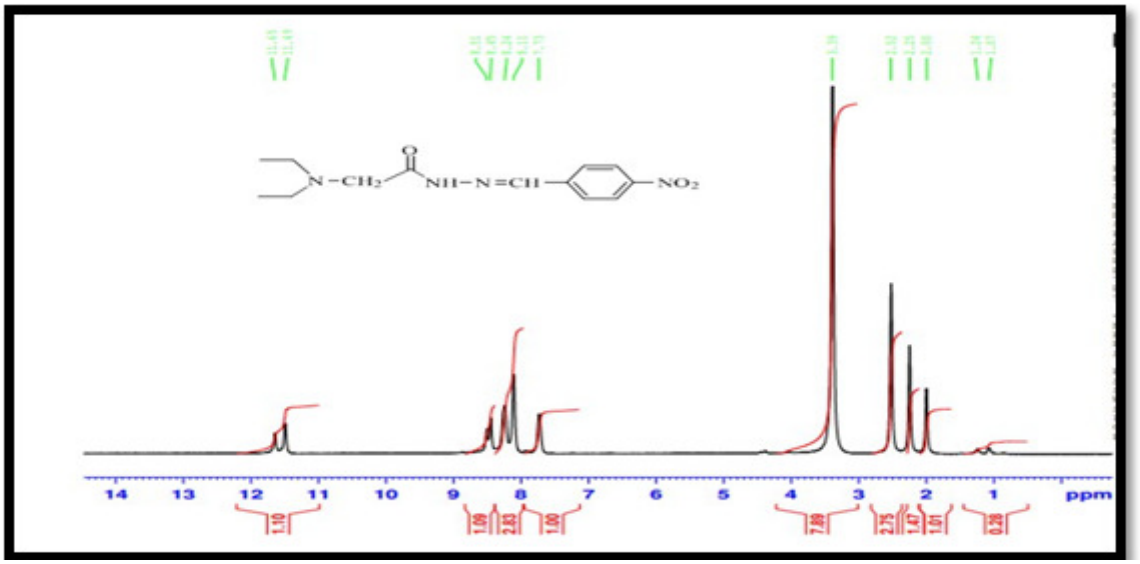


Fig. 7 : ^{1}HNMR spectrum of compound 2-(diethylamino)-N-(4-nitrobenzylidene) acetohydrazide (4).

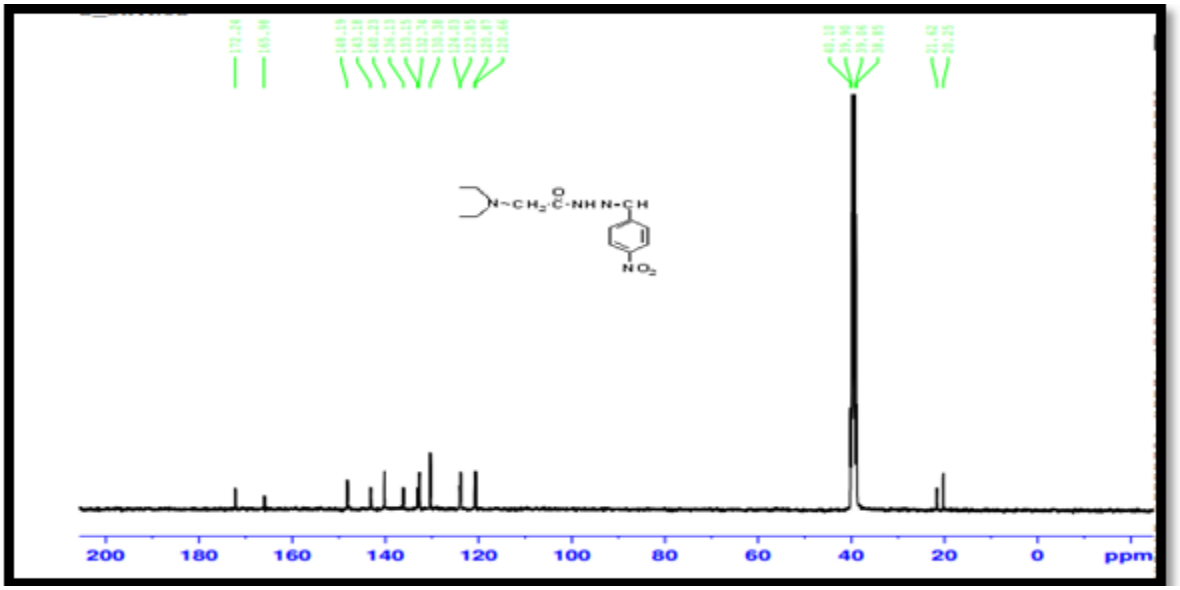


Fig. 8 : ^{13}C NMR spectrum of compound2-(diethylamino)-N-(4-nitrobenzylidene)acetohydrazide (4).

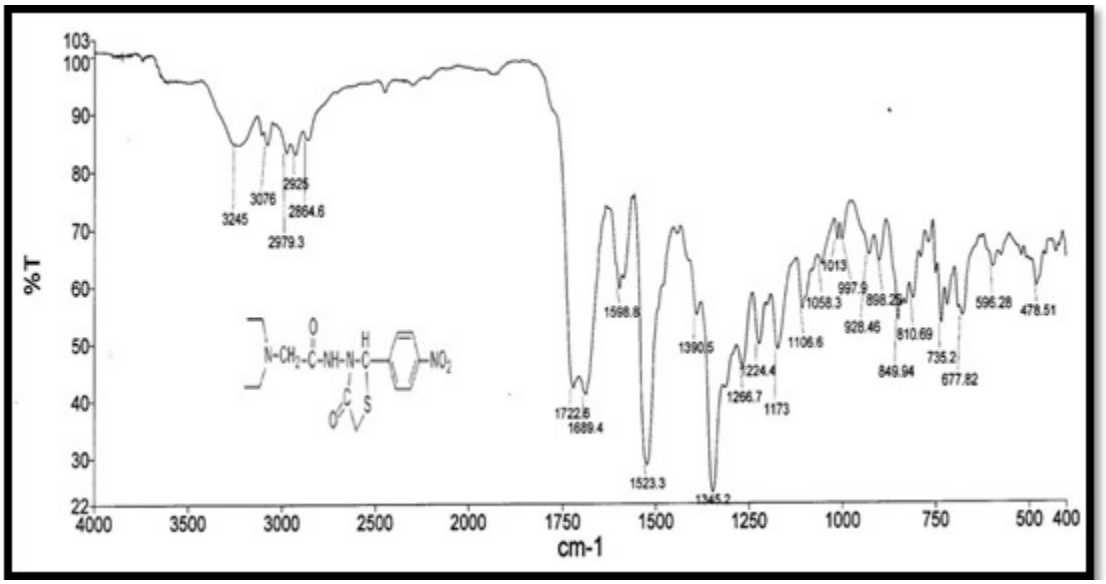


Fig. 9 : FT-IR spectrum of compound2- (diethylamino)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)acetamide (8).

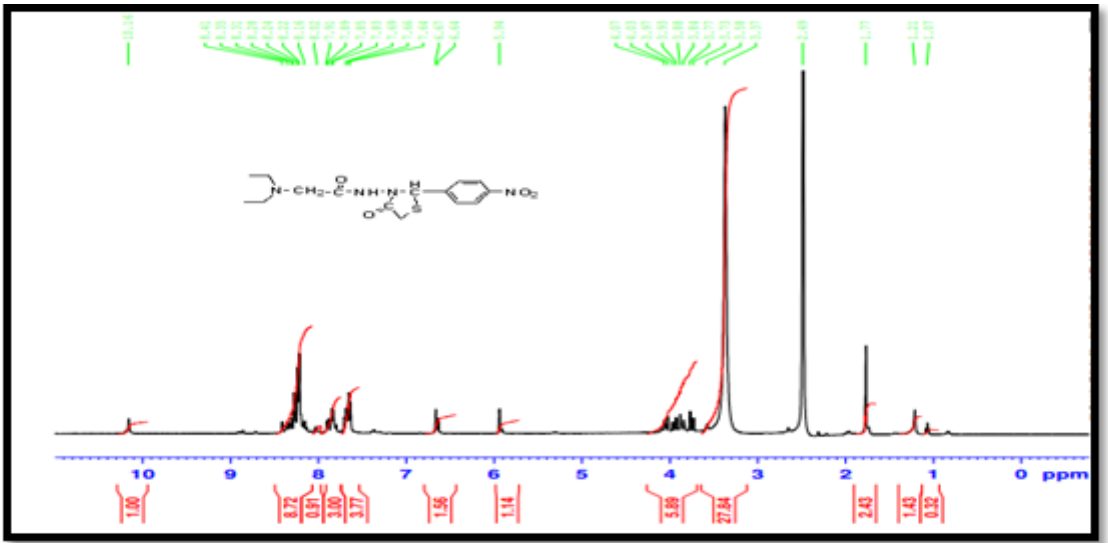


Fig. 10 : ^{1}H NMR spectrum of compound 2- (diethylamino)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)acetamide (8).

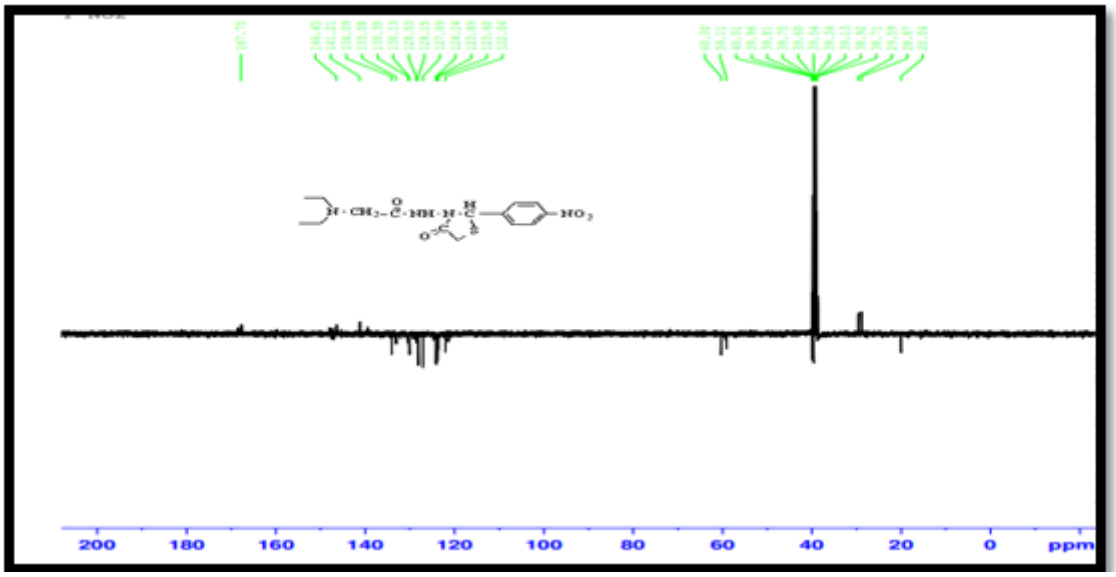


Fig. 11 : ^{1}H NMR spectrum of compound 2- (diethylamino)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)acetamide (8).

Table 1 : Percentage inhibition values of different cell lines treated with two prepared organic compounds at different concentrations. Inhibitions (%) values are expressed as Mean±SD.

Comp No.	Cell line	Concentrations (µg/ml)						
		Inhibition (%)						
		15.1	31.2	62.5	125	250	400	500
9	WRL68 (control)	0c	0c	0c	0c	13.7±2.6b	20.2±2.5a	22.3±2.6a
	HepG2	10.5±5.6c	22.1±3.9c	47.7±2.6b	50.6±3.3a	51.2±3.3a	62.3±3.7a	64.5±3.7a
	SK-GT2	8.6±0.1d	26.6±0.2c	28.8±0.1c	29.8±1.2c	62.9±1.1b	67.8±0.2b	86.4±0.2a
7	WRL68 (control)	0c	0c	0c	0c	0c	0c	8.4±1.3a
	HepG2	5.4±4.2d	8.5±3.8d	13.6±4.9d	26.2±6.5c	51±4.1b	59.5±4.3b	60.1±3.9a
	SK-GT2	6.8±1.2c	9±1.8c	10±2.4c	21.3±3.6b	26.7±2.2b	59.3±2.4a	63.2±3.6a

The characterization details of all other synthesized compounds has been described in experimental section.

Biological part

The cytotoxicity assay was carried out using the crystal violate stain according to the method of Freshney (2012). In brief, the synthesized organic compounds were dissolved in DMSO and diluted by serum free media (SFM) to prepare different concentrations range of (15.1, 31.2, 62.5, 125, 250, 400 and 500) µg/ml. Three types of cell lines were used in this test included human liver cancer ($HepG_2$), humanesophagealcancer (SK-GT2) and normal human liver (WRL-68) cell lines. The tumor cells (1 x 10^5 cell/ml) were seeded in 96-well microplate and incubated for 24 hrs at 37°C, then old media was changed with a new serum-free medium (SFM) containing serial concentrations of each compound. Plate was incubated for 24 hrs in humidified incubator at 37°C containing 5% CO_2. After incubation period, the culture medium was discarded and 100 ìl of crystal violate dye was added into each well and re-incubated 20 min at 37°C. Then, the wells were washed with phosphate buffered saline (PBS) and left for 15 min at R.T. The absorbance was measured Microplate reader (company) at 492 nm. The inhibition percentage was calculated by the following formula:

Inhibition (%) = (A-B/A) × 100

Where,

A = Absorbance of the control

B = Absorbance of the sample

Cytotoxicity effect toward HepG2 and SK-GT2 cell line

Cancer cell lines HepG2 andHepG2 were seeded as (1 × 10^5 cell/ml) in 96-well microplate and incubated for 24 hrs at 37°C. when the cells become confluent monolayer, they were exposed to the compound's concentrations at (15.1, 31.2, 62.5, 125, 250, 400 and 500) µg/ml and incubated in 37! for 24 h, then stained with crystal violate dye and calculated the inhibition rate (%) for each compound. Two of synthesized compounds were

Table 2 : The *in vitro* cytotoxicity effect of prepared organic compounds on different cell lines at 15.1 and 500 µg/ml after 24 hr incubation at 37^{0}C.

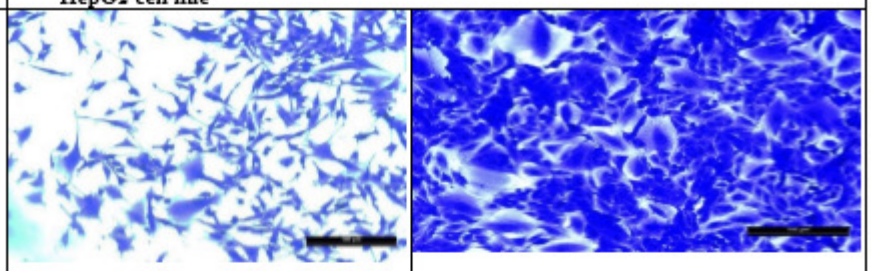
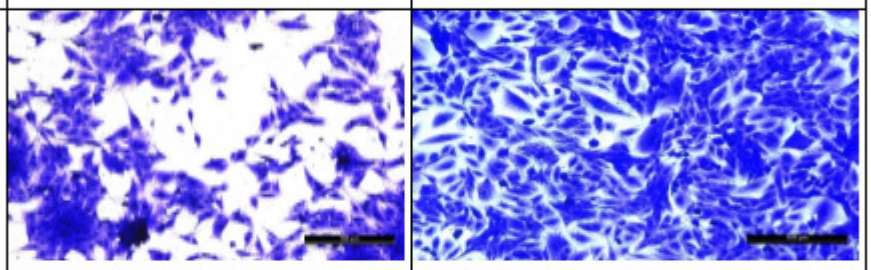
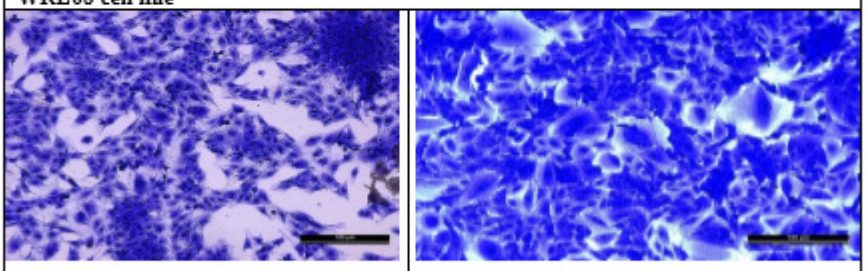
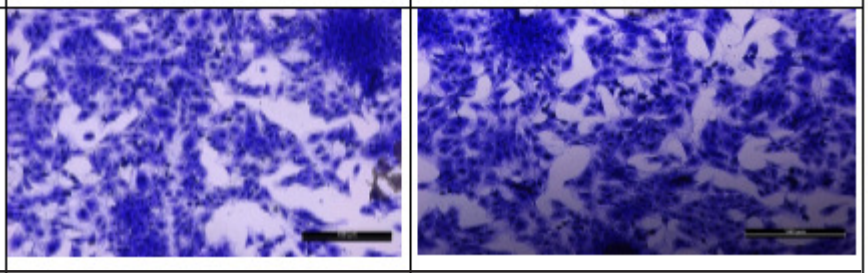
Compound	Concentration (µg/ml)	
	500	15.1
	HepG2 cell line	
9		
7		
	WRL68 cell line	
9		
7		

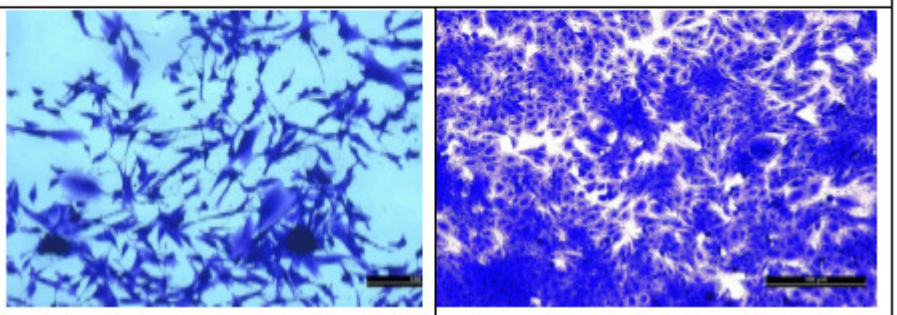
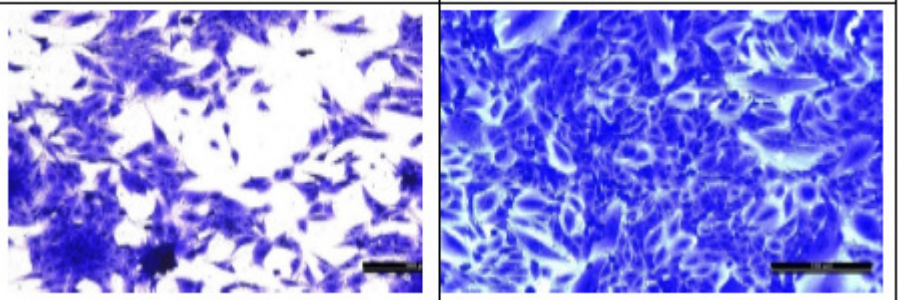
Table 2 continued...

screened against two cancer cell lines. The *in vitro* anticancer activity of these compounds was expressed in the form of inhibitory concentration as Mean±SD. The different two compounds**,** N-(2-(2-bromophenyl)-4-oxothiazolidin-3-yl)-2- (diethylamino)acetamide (**9**) and N'-((1H-indol-3- yl)methylene)-2-(diethylamino)acetohydrazide (**7**) showed broad range of viability against HepG2 and SK-GT2cell lines. Compounds **9** showed moderate to very good cytotoxicity against HepG2 and SK-GT2cell lines.having Inhibition (%) of 500(µg/ml 64.5±3.7a) and Inhibition (%) of 500 (µg/ml 86.4±0.2a). The compound 7 showed moderate cytotoxicity against HepG2 and SK-GT2 cell lines having Inhibition (%) of 500 (µg/ml 60.1±3.9a) and Inhibition (%) of 500 (µg/ml (63.2±3.6a) (Table 1).

CONCLUSION

A series of derivatives of 4-thiazolidinones (**8-11**) were synthesized, by a linear strategy, and characterized. two of synthesized compounds were tested in vitro for their cytotoxic effect against **HepG2** and **SK-GT2 cell line**. These compounds have showed good cytotoxicity and also appear to be safe to WRL68 control cell line . Compounds were characterized by IR, ^{1}H NMR, ^{13}C

Table 2 continued...

Compound	Concentration (µg/ml)	
	500	15.1
	SK-GT2 cell line	
9		
7		

NMR and APT^{13}C NMR spectroscopic. Schiff's base compounds were synthesized with four different aldehyde, 4-nitro benzyaldehyde, 4-chloro benzyaldehyde, 2-bromo benzyaldehyde and 1H-indole-3-carbaldehyde. Final compounds were obtained by cyclocondensation step withthioglycolic acid to form 4-thiazolidinones heterocyclic ring in different yield.

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