Synthesis, Characterization and Bio Study of New Oxazepine and Diazepine Derivatives

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Abstract: In this study, oxazepine and diazepine compounds were prepared, which are non-homogeneous seven-membered ring compounds. The oxazepane derivatives were prepared using diamine compounds with dicarboxylic compounds to produce anile compound that it used in preparation of oxazepane through cyclization reaction, then the product reacted with amine derivative to yield diazepine derivatives. Most of the seven-membered rings that were prepared had high effectiveness in pharmaceutical studies similar to what those rings contain of hybrid nitrogen atoms such as oxygen and nitrogen, which are medically known to have entered the fields of drug manufacturing and the field of antioxidant manufacturing, so researchers used them in several techniques such as polymeric drug carrier technology and others, Many researchers have succeeded in linking seven-ring molecules to produce anticancer drugs, the effectiveness of which has been demonstrated through molecular docking programs and other pharmaceutical programs that enhance practical studies, These compounds are characterized by high activity and efficacy, as demonstrated by studies using FT-IR, 1H-NMR, and 13C-NMR techniques. By verifying the chemical structure of these compounds, it was found that these derivatives have biological activity such anticancer activity and can be used as medical drugs against certain diseases, in addition to the use of their derivatives in polymers.

1 INTRODUCTION

Triazoles are five-membered rings, considered among the most active in pharmaceutical chemistry. They are also essential for many reactions, particularly coordination chemistry, due to their nitrogen donor content when forming coordination complexes. Imidazoles are no less important than other cyclic compounds, known for their pharmacological activity in the fields of anticancer drugs, anti-inflammatory drugs, and antibacterial drugs. Imine compounds are an active class of organic bonding, characterized by being intermediate compounds for heterocyclic compounds [1]-[3]. These compounds can be used in various fields due to their diverse structural properties, flexibility, and high selectivity, in addition to their distinction in structural design [4]. The azomethine group (C=N) present in these compounds is the active functional group, and the nitrogen atom is the donor atom in this group. The resulting compounds are characterized by stability due to the presence of this atom, as it possesses an electron pair capable of donating [5] it in hydrogen bonding. Most diazepine compounds are found in a variety of medications and drugs. These rings are also considered anesthetics because [6]-[9] they contain more than one nitrogen atom, making them capable of anesthetizing the nerves [10], [11]. Therefore, they are used as anesthetics in surgical procedures at controlled doses. Oxazepine is a pharmaceutical compound associated with several active groups, which gives the compounds high biological efficacy [12]-[15], especially in the field of anesthetic drugs [16], [17].

2 EXPERIMENTAL METHODS

2.1 Synthesis of Compound {1}

Cyclic compound {1} prepared from reaction of compound {A} that was prepared in 2 step refluxed with (0.002 mol) of malic anhydride with ethanol (50 ml) for cyclization step to formation oxazepane ring derivative, the creation of cyclic compounds, the product filtered, desiccated, purified by using (Eth.OH) as a solvent to yield cyclic compound {1} according to [12], [15].

2.2 Synthesis of Compound {2}

Cyclic compound {2} prepared from reaction of compound {B} with (0.001 mol) for triphthalic acid refluxed (7 hrs) with (4N HCl) with ethanol (50 ml), for cyclization step to formation Triazole, the creation of cyclic compounds, the product filtered, desiccated, purified by using (Eth.OH) as a solvent to yield cyclic compound {2}, according to [12], [15]

2.3 Synthesis of Compound {3}

Synthesis of Compound{3} from (0.001 mol) of Compound{2} refluxed (0.001 mol) of Semicarbazide refluxed (3 hrs) with ethanol and with (3 ml H2SO4) The linking step and the output formation Oxazole ring were completed ring derivative, product filtered, desiccated, purified by using (DMF) as a solvent to yield compound {3}, according to [11], [13].

2.4 Synthesis of Compound {4}

Synthesis of compound {4} from (0.001 mol) of Compound {3} with refluxed (3hrs) with (0.001 mol) of phthaldehyde with (3 drops) from Glacial acetic for, with ethanol (50 ml), the product filtered, desiccated, purified by using (DMF) as a solvent to yield compound {4} according to [12]

2.5 Synthesis of Compound {5}

Cyclic compound {5} prepared from reaction of compound {4} with (0.001 mol) of toluidine refluxed (4 hrs) with the reaction of cyclic compounds for cyclization step to formation Oxadiazol ring the creation of cyclic, the product filtered, desiccated, purified by using (Eth.OH) as a solvent to yield cyclic compound {5}, according to [12]

2.6 Synthesis of Compound {6}

Cyclic compound {6} prepared from reaction of compound {5} with refluxing (6 hrs) with (0.002 mol) of maliec anhydride with benzene solvent, the creation of cyclic compounds for cyclization step to formation oxazepane ring derivative, the product filtered, desiccated, purified by using (DMF) as a solvent to yield cyclic compound {6}, according to [15].

2.7 Synthesis of Compound {7}

Cyclic compound {7} prepared from reaction of compound {6} with (0.001 mol) for hydrazine refluxed (6 hrs) with ethanol (50 ml), with (2 ml) HCl the reaction of cyclic compounds for cyclization step to formation diazepine ring derivative, the product filtered, desiccated, purified by using (Eth.OH) as a solvent to yield cyclic compound {7}, according to [12], [15].

2.8 Synthesis of Compound {8}

Cyclic compound {8} prepared from reaction of compound {6} with refluxed with (0.001 mol) of thiourea with ethanol (50 ml) for cyclization step to formation thio diazepine ring derivative, the creation of cyclic compounds, the product filtered, desiccated, purified by using (Eth.OH) as a solvent to yield cyclic compound {8}, Figures 1, 2, 3 according to references [12], [15].

2.9 Cell Viability and Cytotoxicity Assays (MTT)

The main purpose of the assay (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT)), which is basically a colorimetric test, is to infer and detect the toxicity of the compounds on healthy and infected cells that are being tested. outside the body of the organism and determine the extent of toxicity by calculating the percentage of live cells (cells viability) according to [12]. That is why it was possible to use these prepared compounds as a treatment for various types of this disease in the event that healthy cells were not affected and showed toxicity towards these infected cells, as the living cells work to change the color of the (MTT) dye from yellow to the infected cells, As the living cells change the color of the pigment (MTT) from yellow to blue, and the more blue color spreads, this means the increase in the number of living cells

2.10 Initialization of the Cancer Cell Line

The cell line (MCF-7) and the cell line (MCF-10A) were grown in (95%) of (RPMI-1640) supplemented with (PBS 10%), the cells were suspended and incubated in (37˚C) in a 5CO_2% incubator. The suspended cells were centrifuged at (250gm) for (10min) and the supernatant was removed, the cells were suspended in freezing medium, then placed in a beaker (-70°C) for (1-3) days, according to [15], the beakers were transferred from the Standard freeze to liquid N_2 container.

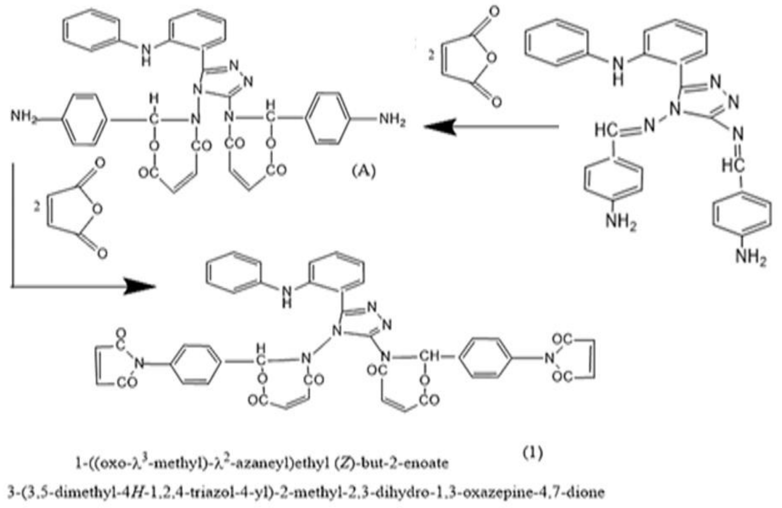

Figure 1: The synthesized compound {1}.

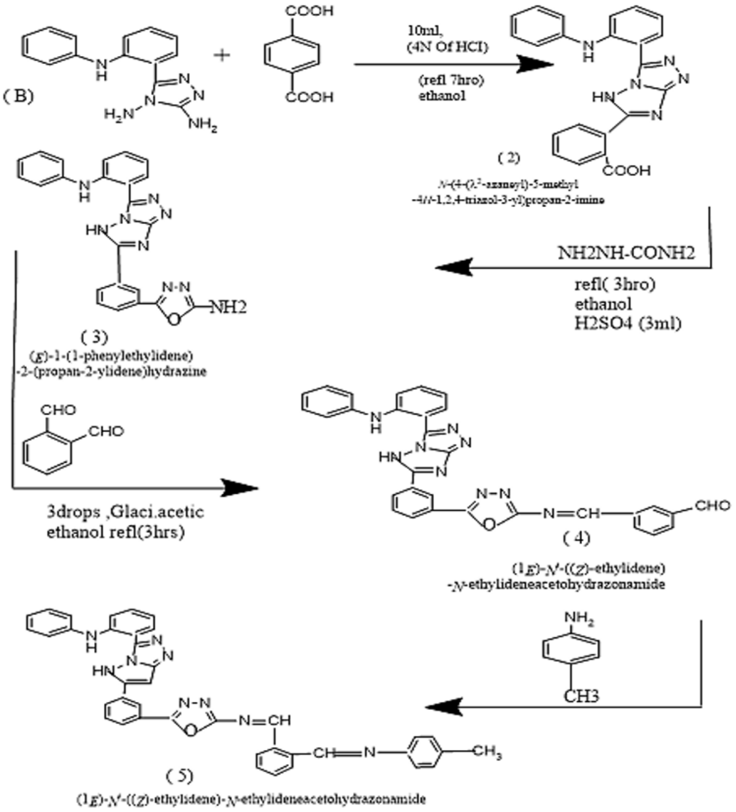

Figure 2: The synthesized compounds {3, 4, 5}.

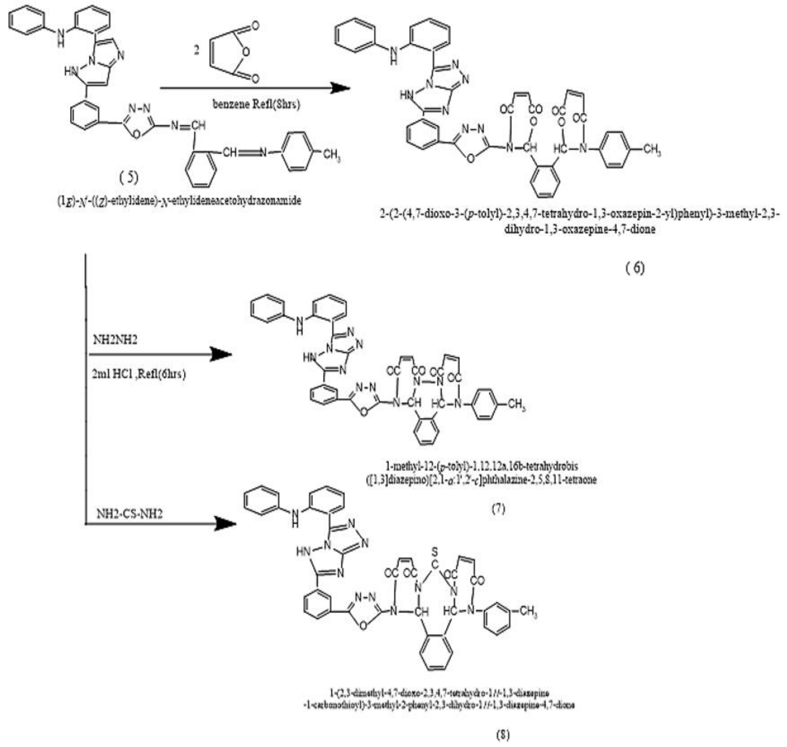

Figure 3: Synthesis of derivatives {6, 7, 8}.

3 RESULTS AND DISCUSSION

A new and different group of cyclic compounds was created by forming new seven-membered heterocyclic rings containing oxygen and nitrogen atoms, namely the oxazepine and diazepine rings, which are new Schiff bases resulting from the condensation reaction resulting from the use of the basic compounds used and the intermediate compounds, where the focus in this reaction is on the carbonyl groups present in these compounds and using an acidic medium in which different acids are used in the presence of ethanol in order to produce derivatives of new Schiff bases according to the resulting synthetic methods, where these compounds were analyzed according to the spectral studies used in analyzing and studying the purity of the materials well, and the results were different for these compounds depending on the difference in their groups present in their structural structure.

3.1 Evidences of Compounds via Spectral Identification

FT.IR spectrum: The spectrum showed absorption bands for carbonyl of lactone (CO-O) at (1710) and carbonyl of lactam at (1685) in compound {1}, while in compound {2} appeared bands at (3200) for amine group (NH) of triazole in compound {2}, also the (NH2) group showed at (3420, 3453), which belong to the amide group in compound{3}, which disappeared in compounds {2-8} as a result of the formation of cyclic compounds made of two rings, one with six members and the other with five, seven members. All frequencies were identified and explained according to reference [13]. Many other spectra appeared, such as (C=N) that behaved at {(1648), (1670), (1652), (1668), (1641), (1633), (1660), (1635)} respectively in compounds {1-8}.

Meanwhile, other spectra appeared in the spectrum such as NH spectrum {1,2,3,4,5,6,7,8} {(3311), (3394), (3200), (3369), (3421), (3201,3346), (3207,3414), (3388,3439)} respectively for compounds {1,2,3,4,5,6,7,8}. Another spectrum appeared in the spectrum such as that of the amide compound (CO-N) for compound {1}: at (1678) and other functional groups in some form in Figures 4-11 and Table 1.

3.2 1H-NMR Spectra

The compounds from {1-8} were diagnosed using proton NMR spectroscopy with DMSO-d6 as a solvent , where several signals indicating the validity of the prepared compounds were observed. Compound {1} gave signal was observed at (1.23, 1.47) for methyl groups while same groups (methyl) observed at (1.23, 1.57), (0.86, 1.56), (1.21, 1.84), (1.7, 1.20), (1.10, 1.27), (1.10, 1.28), (1.11, 1.31), (1.23, 1.45), (1.12, 1.23) respectively in compounds {2-8} of the CH3 group, or the appearance of specific signals for the protons of aromatic ring groups in all compounds {1-8}observed respective ranging from (6.62 - 7.68), (6.53 - 7.83), (6.55 - 7.85), (6.77 - 7.83), (6.64 - 7.69), (6.86 - 7.90), (6.62 - 7.68), (6.68 - 7.85), (6.68 - 7.35), (6.70 - 7.56) parts per million. Additionally, the NH signal for the amine group protons was observed at (4.90), (4.23), (4.34), (4.25), (3.45), (4.21), (4.21), (4.34), (4.96), (5.10) ppm, while for the DMSO-d6 solvent, its signal appeared at (2.5) parts per million. Furthermore, other group signals appear in Table 2 and Figures 12-19.

3.3 13C-NMR-Spectra

The compounds {1-8} were diagnosed via 13C-NMR spectroscopy, starting with DMSO-d6 as a solvent. It was noted that several signals appeared indicating the validity of the synthesized compounds' structures, with signals for the carbon atom of the methyl group of the ten compounds observed at (12.95 - 18.99), (12.5 - 21.5), (12.4 - 15.6), (15.6 - 17.2), (15.21 - 17.11), (13.28 - 19.41), (6.601 - 18.8), (18.99 - 15.00), (18.0 - 20.0), (14.0 - 20.0) ppm. Additionally, a series of signals corresponding to the carbon atoms of the aromatic ring appeared at (124 - 135), (113 - 136), (113 - 129), (114 - 147), (115-131), (114 - 136), (114 - 136), (124 - 140), (120 - 140), (118 - 135) ppm. Other signals corresponding to various groups were also observed as detailed in the referenced Table 3 and Figures 20-27. As for the DMSO-d6 solvent signal, it appeared at (40.0).

3.4 Anticancer-Assay

Four compounds were selected for anticancer evaluation (breast cancer) represented by Diazepine Compound [8] and other cyclic compound [2], Cyclic compound [6], and last one compound [3] by MTT-Test of two types of Cells (MCF-7) as Cancer cell and (MCF- 10 A) as healthy cell according to studies [11], [13].

Through results of Cancer studies, we noted that the compound {8} has compound {2}, then other compound [6] and last one in activity of inhibition represented by compound [3], the first compound [8] has higher anticancer activity due to its structure that is containing (imidazole ring incorporated with triazole, also two ring of diazepine with thion group) in addition to it involved nitrogen and sulfur atoms more than other compound, all data from physical properties in Table 4,while bio data in Figures 28, 29 and Tables 5, 6.

Table 1: Compounds via spectral identification FT.IR- spectra.

Com	(CH) aliph (CH) arom	(C=N) endocycle	NH	NH2	(CO-N) Amide	Other bands cm^{-1}
1	2995 3080	(1648)	(3311)	/	1678	(C-N):(1303), (CO- N)Lactam:1678 (CO-O) Lactone:(1718) (CH = CH) alkene:(3100)
2	2935 3080	(1670)	(3394)	/	/	(C-N):(1303),(CO-O) carboxyl 1712 (OH) carboxyl:(2600-3100)
3	2920 3000	(1652)	(3200)	3420 3453	/	(C-N):(1332), (C-O-C) oxadiazole:1153
4	2998 3047	(1668)	(3369)	/	/	(C-N):((1323), (C-O-C) oxadiazole:1170 (CO-H)aldehyde 1707 (CH = N)Imine 1627
5	2916 3014	(1641)	(3421)	/	/	(C-N):((1319), (CH = N)Imine (1618) (C-O-C) oxadiazole:1147
6	2900 3093	(1633)	3201 3346	/	/	(CO- N) Lactam:1668,(CO-O) Lactone: (1720) (CH = CH),alkene):(3149)
7	2945 3000	1660	3207 3414	/	/	(CO- N) Lactam: 1676,1691 (C-O-C) oxadiazole:1149 (CH = CH) alkene:(3100)
	2920 3064	1635	3388 3439	/	/	(CO- N) Lactam:1658,1697 (CH = CH) alkene):(3150),(C = S) thion: 1232

Table 2: Compounds via spectral identification ^{1}H- NMR - spec.

Com	(CH3) methyl	(NH) amine	Aromatic protons	Other peaks
1	1.23,1.47	5.10	(6.67-7.68)	(O-CH- N) oxazepine:2.54 (CO-CH= CH- CO) oxazepine:(5.98,5.95) (CO-CH= CH- CO) cycle Imide (6.03,6.08)
2	1.23, 1.57	4.90	(7.13-7.50)	(COOH) protons of carboxyl (12.0) (NH) Triazole:(3.44)
3	0.86, 1.56	5.10	(6.56-7.60)	(NH) Triazole:(5.41), (NH2) amine: 4.56
4	1.21, 1.84	4.91	(6.61-7.89)	(NH) Triazole:(5.20), (CH=N) Imine: (8.60) (CO-H) aldehyde: (12.50)
5	1.15, 1.67	3.34	(6.61-7.94)	((NH) Triazole:(4.62), (CH=N) Imine: (8.13,8.16) (CH3) methyl group:(1.09)
6	1.22, 1.68	5.33	(6.54-7.71)	(CH3):(1.06), (NH) cycle: (5.66) (CO-CH= CH- CO) alkene, (6.08, 6.10) (N - CH- O) OXazepine:(3.39)
7	1.23, 1.86	5.00	(6.56-7.59)	(CH3):(1.00), (NH) cycle: (5.40) (CO-CH= CH- CO) alkene (6.02, 6.08) (N - CH- N) Diazepine:(2.31)
8	1.22, 1.31	5.00	(6.58-7.86)	(CH3):(0.90), (NH) cycle: (5.88), (CO-CH= CH- CO) alkene (6.03, 6.07),(N - CH- N) Diazepine:(2.34)

Table 3: Compounds via spectral identification ^{13}C- NMR -spectra.

Com	(CH3) carbon of methyl	Aromatic carbon Atoms	Other peaks
1	15.5,20.0	(115-146)	(CH=CH): (100,106), (CO-N):(162.5,166.5) (CO-O) Lactone:(175.0), (N - CH- O): (61.8)
2	18.00, 20.0	(120-138)	(CO-OH) Carbon Atom of carboxyl:(178.0)
3	15.0, 18.0	(125-145)	/
4	12.0, 15.0	(119-133)	(CH=N) Imine:153.17, (CH=O) Carbonyl of aldehyde: (190.0)
5	14.0, 18.0	(125-140)	(CH=N) Imine:152.0, (CH3): (10.0)
6	15.0, 20.0	(118-140)	(CH=CH) Carbon Atoms of Alkene (100,104), (CO-N) Lactam amide:(160.0,164.0) (CO-O) Lactone:ester:(170.0,172.0), (CH3): (12.0)
7	15.0, 20.0	(118-141)	(CH3): (10.0), (CO-N) Lactam amide: (160,165) (CO-O) Lactone: ester:(170,172), (CH = CH) alkene (100,104), (N - CH- N) Diazepine:(50.0)
8	12.0, 21.0	(113-136)	(CH=CH) Carbon Atoms of Alkene:(104,106), (CO-N) Lactam amide:(160, 163,165), (N - CS- N):(169.0), (CH3):(0.50)

Table 4: Physical and chemical properties of compounds {1-8}.

Comps	M.F	M.W g/mole	m.p C° ±2	Color	R.$_F$ (Ethanol: Benzene)	Yield %
Comp {1}	$C_{59}H_{84}N_8O_{10}$	1065.37	212	Yellowish Red	0.66	68
Comp {2}	$C_{27}H_{35}N_6O_2$	475.62	188	Yellowish Orane	0.64	80
Comp {3}	$C_{31}H_{45}N_9$ O	559.76	202	Golden Yellow	0.74	72
Comp {4}	$C_{39}H_{50}N_9$ O	676.89	216	Deep Yellow	0.66	74
Comp {5}	$C_{54}H_{83}N_9O$	875.33	228	Bill Orange	0.70	72
Comp {6}	$C_{56}H_{67}N_{10}O_7$	992.52	218	Yellowish Orane	0.64	68
Comp {7}	$C_{56}H_{67}N_{12}O_5$	988.23	230	Reddish Yellow	0.64	82
Comp {8}	$C_{59}H_{76}N_{12}O_5S$	1065.40	246	Yellowish Red	0.62	76

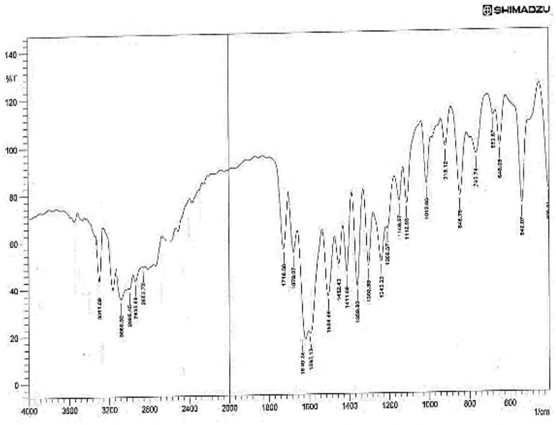

Figure 4: I.R of compound {1}.

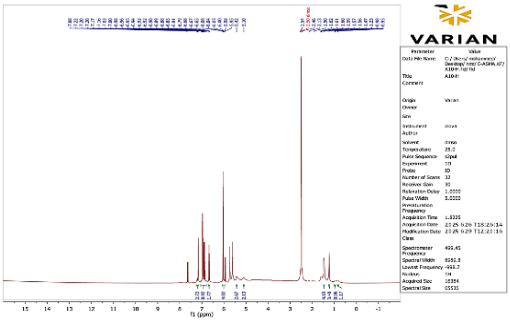

Figure 5: ^{1}HNMR spectrum of compound {1}.

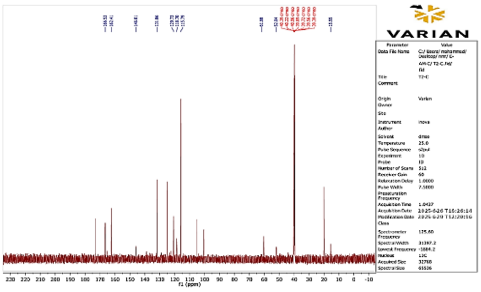

Figure 6: ^{13}CNMR spectrum of compound {1}.

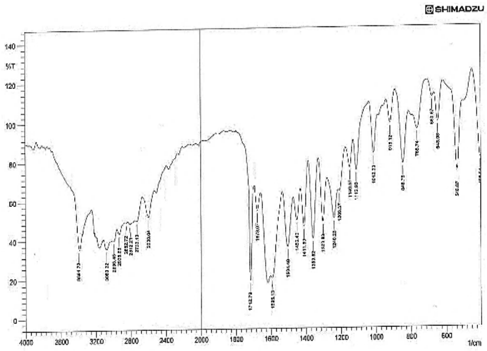

Figure 7: I.R of compound {2}.

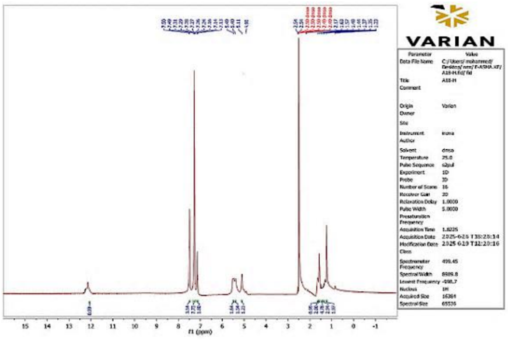

Figure 8: ^{1}HNMR spectrum of compound {2}.

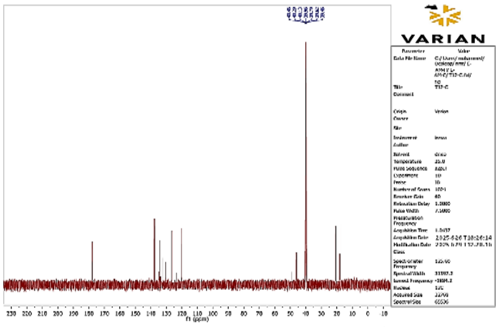

Figure 9: ^{13}CNMR spectrum of compound {2}.

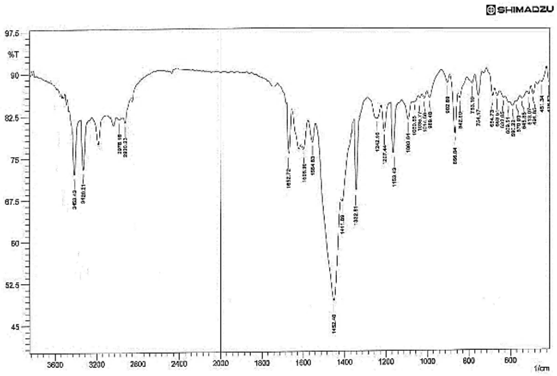

Figure 10: I.R of compound {3}.

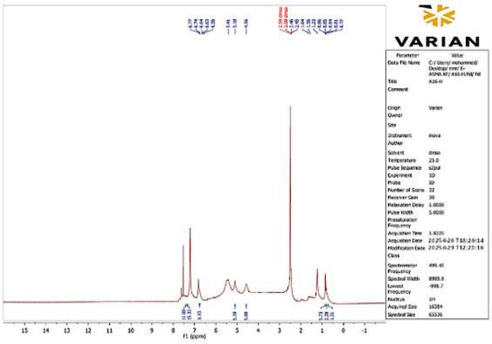

Figure 11: ^{1}HNMR spectrum of compound {3}.

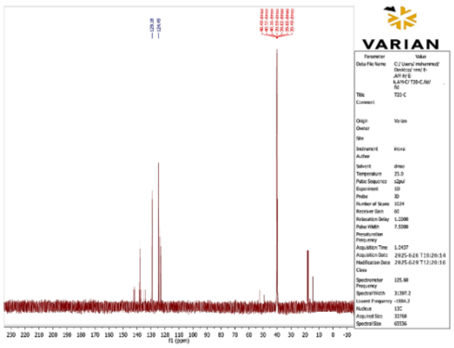

Figure 12: ^{13}CNMR spectrum of compound {3}.

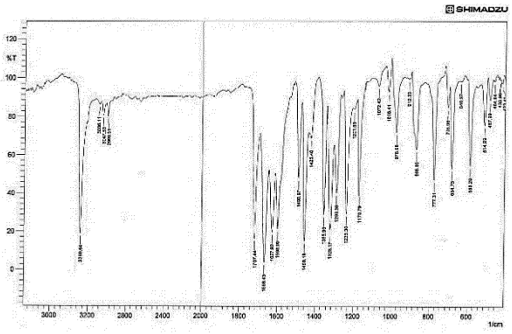

Figure 13: I.R of compound {4}.

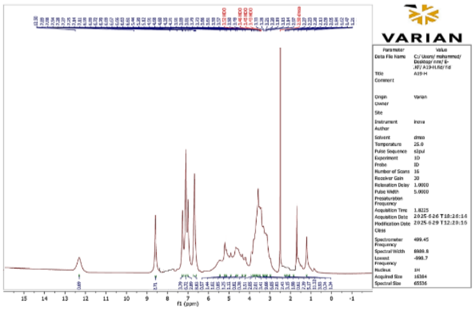

Figure 14: ^{1}HNMR spectrum of compound {4}.

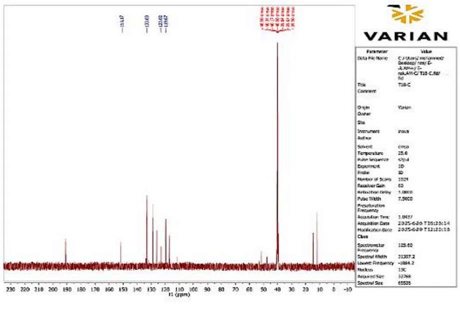

Figure 15: ^{13}CNMR spectrum of compound {4}.

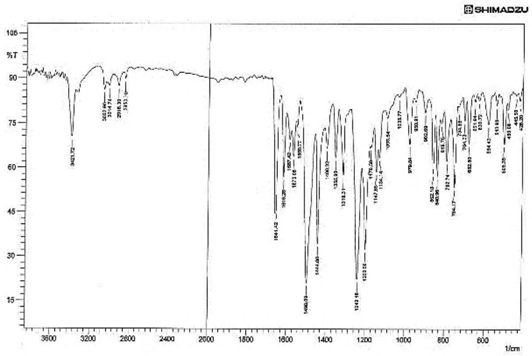

Figure 16:IR of compound {5}.

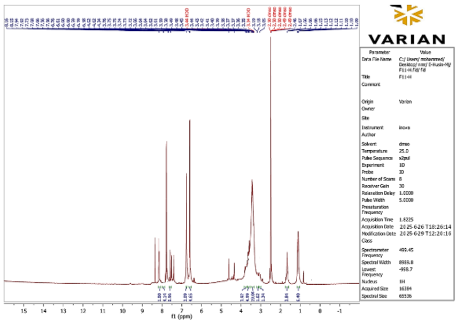

Figure 17: ^{1}HNMR spectrum of compound {5}.

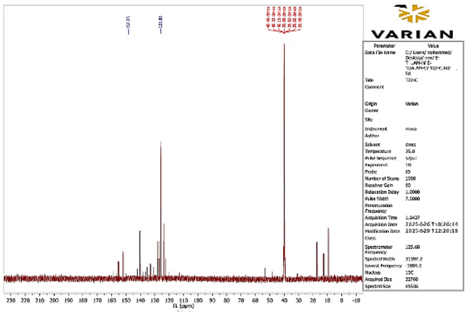

Figure 18: ^{13}CNMR spectrum of compound {5}.

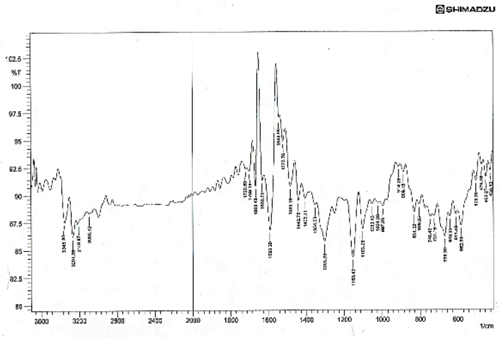

Figure 19: I.R of compound {6}.

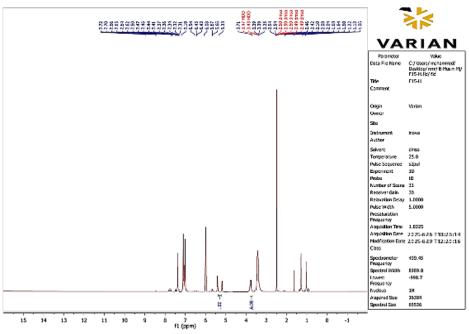

Figure 20: ^{1}HNMR spectrum of compound {6}.

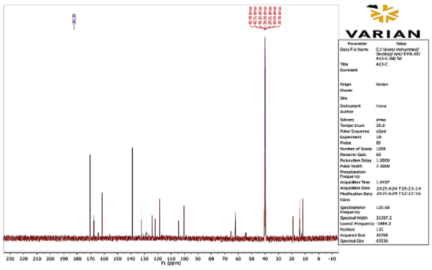

Figure 21: ^{13}CNMR spectrum of compound {6}.

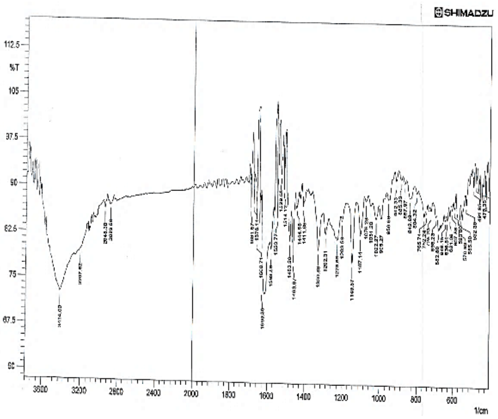

Figure 22: I.R of compound {7}.

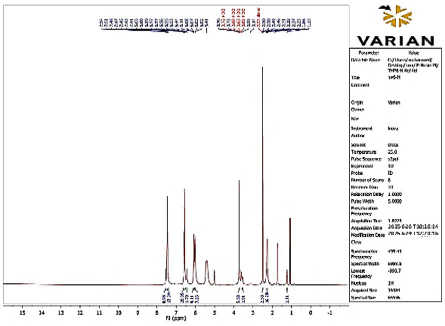

Figure 23: ^{1}HNMR spectrum of compound {7}.

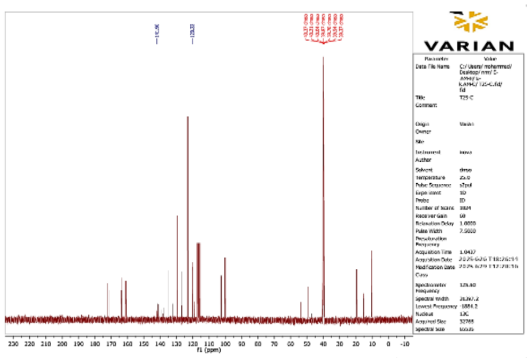

Figure 24: ^{13}CNMR spectrum of compound {7}.

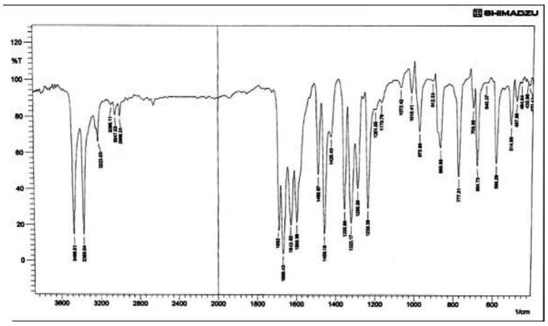

Figure 25: I.R of compound {8}.

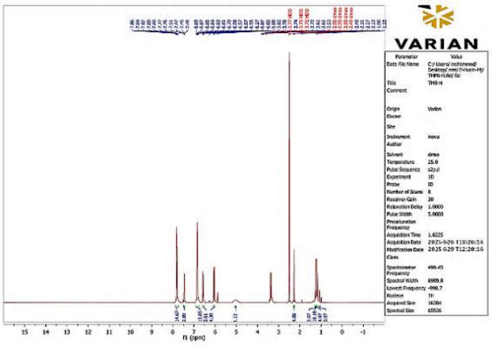

Figure 26: ^{1}HNMR spectrum of compound {8}.

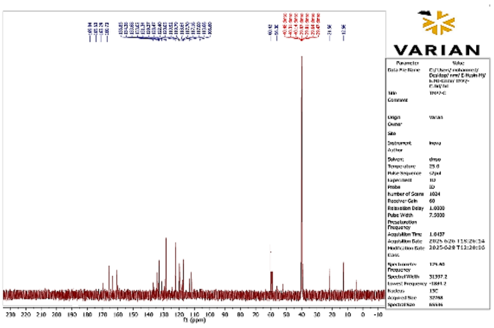

Figure 27: ^{1}HNMR spectrum of compound {8}.

Table 5: Cytotoxic activity of compound {6} on breast cancer cells line (MCF-7) and healthy cells (MCF-10A) at the same concentration using 24 hrs., MTT test 37^{0}c.

Con. (µg. mL^{-1})		Mean Percentage (%) for each cell line		
		MCF-7 / IC50= 32.63		MCF-10A / IC50 = 168.94
Compound {6}		Cancerous line cells of MCF-7		Normal line cells of MCF-10A
	Cell Viability	Cell Inhibition	Cell Viability	Cell Inhibition
31. 25	84.66	15.34	93.04	6.96
62. 5	77.91	22.09	94.23	5.77
125.0	64.58	35.42	94.36	5.64
250	48.37	51.63	95.41	4.59
500	42.97	57.03	96.30	3.7
Control	100	0.00	90.40	9.59

Table 6: Cytotoxic activity of compound {8} on breast cancer cells line (MCF-7) and healthy cells (MCF-10A) at the same concentration using 24 hrs., MTT test 370c.

Con. (µg. mL^{-1})		Mean Percentage (%) for each cell line		
		MCF-7 / IC50= 16.78		MCF-10A / IC50 = 196.49
Compound {8}		Cancerous line cells of MCF-7		Normal line cells of MCF-10A
	Cell Viability	Cell Inhibition	Cell Viability	Cell Inhibition
31. 25	84.32	15.68	87.59	12.41
62. 5	80.33	19.67	90.64	9.36
125.0	74.61	25.39	92.34	7.66
250	47.01	52.99	93.71	6.29
500	42.78	57.22	94.12	5.88
Control	100	0.00	90.84	9.16

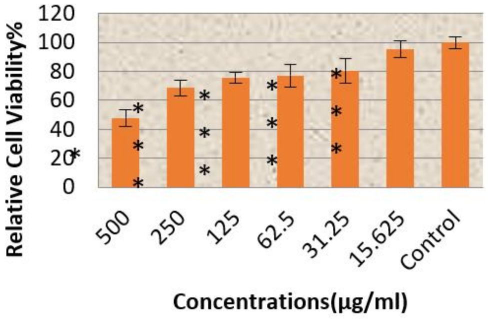

Figure 28: IC50 for cancer cells MCF-7 for compound {6}.

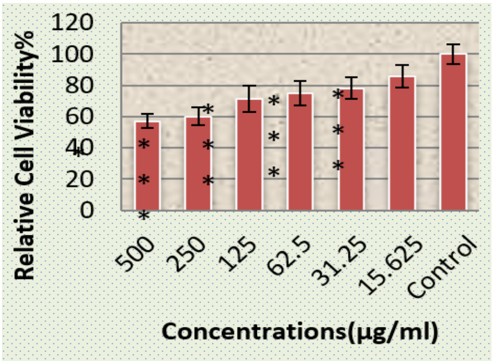

Figure 29: IC50 for cancer cells MCF-7 for compound {8}.

4 CONCLUSIONS

In this study, a series of novel heterocyclic compounds containing oxazepine and diazepine frameworks were successfully synthesized and structurally characterized. The formation of these seven-membered ring systems was confirmed through comprehensive spectroscopic analyses, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR techniques. The disappearance and appearance of characteristic functional group signals clearly supported the successful cyclization and structural transformation throughout the synthetic pathway.

The biological evaluation of selected compounds demonstrated promising anticancer activity against the MCF-7 breast cancer cell line, with comparatively lower cytotoxic effects on the normal MCF-10A cell line. Among the tested derivatives, compounds containing multiple heteroatoms (N, O, and S) and fused heterocyclic systems exhibited the highest inhibitory activity, indicating a strong structure–activity relationship. In particular, the enhanced biological performance of these compounds may be attributed to their increased ability to interact with biological targets involved in cancer cell proliferation.

Overall, the obtained results suggest that oxazepine and diazepine derivatives represent a valuable class of bioactive heterocycles with potential applications in anticancer drug development. However, further studies, including in vivo evaluation and molecular docking investigations, are recommended to better understand their mechanism of action and to optimize their pharmacological potential.

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