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Synthesis and anticancer evaluation of new heterocyclic Compounds in cell line L20B *in-vitro*

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Abstract:

A new derivatives of phenytoin containing Oxazepines and Diazepines moiety has been synthesized. The reaction was achieved through reaction between of 4-(dimethylamino)benzaldehyde with *p*-phenylenediamine to yielded Schiff base compounds 1. Cyclization of this compound (1) with a variety of anhydride and phthalimide compounds yielded the Oxazepines and Diazepines compounds . in the final step, mannich reaction was used to prepare the target compounds through reaction of Oxazepines and Diazepines compounds with phenytion . The chemical structure of synthesized compounds were confirmed by FT-IR and ^{1}H-NMR, spectroscopy. Cell culture and cytotoxicity were tested for synthesized compounds .

Keywords : phenytoin, Schiff base, Oxazepines, . mannich reaction

1. INTRODUCTION:

Example of a popular five cyclic ring comprise a reactive cyclic urea core is hydantoin nucleus or imidazolidine-2,4-dione, This compound is existing in a broad varity of pharmaceutical active drug implicate antitumor(1) anticonvulsant(2-4) and antiarrhytmics,(5) agents. Among these agents, phenytoin, or 5,5-diphenylhydantoin, is a well-known therapeutic drug for the treatment of epileptic seizures.(6) Chemists explain that 5,5-diphenylhydantoin was efficient versus electrically induced seizures in the cat,(7) the phenytoin is yet the medicine of option for the therapy of generalized tonic–clonic seizures (so-called grand mal epilepsy) and focal motor seizures.(8) These days, 5,5-diphenylhydantoin has establish novel enforcement owing to the neuro-and cardioprotective characteristic.(9,10)

many heterocyclic compounds, for example oxazepine derivatives which containing oxygen and nitrogen atom were prepared by used Schiff bases method (11,12). A wide variety of biological activities such as antibacterial and anticonvulsant activity.(13,14) showed Oxazepine derivatives.

2. EXPERIMENTAL PART

2.1 General

Melting points of the synthesized compounds were determined by open capillary tubes and are uncorrected. The IR spectra were recorded on a SHIMADZU FT-IR 8400S spectrophotometer and ^{1}H-NMR spectra in DMSO-d_6 on Bruker 400MHz spectrometer using tetramethylsilane (TMS) as an internal standard(chemical shift in δ ppm).

2.2 Synthesis of Schiff base (1).

Equimolar amounts (0.01 mol) of appropriate aldehyde 4-(dimethylamino) benzaldehyde and the amine *p*-phenylenediamine) in EtOH 99% (15ml) with three drops of(CH_3COOH) was refluxed in water bath for 3 hours. The reaction mixture was then allowed to cool at room temperature, and the precipitate was filtered and dried, recrystallized from ethanol to give yellow crystal.

N^1-(4-(dimethylamino)benzylidene)benzene-1,4-diamine (1): yellow . m.p : 153-155 °C, yield 83% , IR (KBr, cm^{-1}): amine N- H_{str}(3422, 3322), aromtic C-H_{str}(3066), aliphatic C-H_{str} (2939, 2831), C=N_{str}(1618), aromatic C=C_{str} (1598, 1508 , 1473, 1424), C-N_{str} (1188), ^{1}HNMR (dmso-d_6) δ ppm : 3.52 (s , 6H , $2CH_3$), 7.18-7.7.3(m,8H,Ar-H) 8.9 (s, 1H, N=CH).

2.3 Synthesis of compounds (2,3,4).

Equimolar amounts (0.01) of Schiff base and (phthalic anhydride ,malic anhydride ,phthalic imide,)respectively in dry C_6H_6 was refluxed for (4-5) hours, the solvent was removed by roatory evaporater and resulting colored solid was recrystallized from dry dioxane to give the title compounds.

4-(4-aminophenyl)-3-(4-(dimethylamino)phenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione (2):
white . m.p : 210-212 °C, yield 76% , IR (KBr, cm^{-1}): amine N- H_{str}(3502, 3362), aromtic C-H_{str}(3099, 3023), aliphatic C-H_{str} (2932, 2892), ester C=O_{str} (1753), amide C=O_{str}(1705), aromatic C=C_{str} (1597, 1541 , 1438, 1439), C-N_{str} (1219, 1141,1099), C-O_{str} (1249), ^{1}H-NMR (DMSO-d_6) δ ppm : 3.54 (s , 6H , $2CH_3$), 5.44 (s , 2H , NH_2) 8.93 (s , 1H , -CH Oxazepines ring) 7.42-8.10 (m, 12H, Ar-H),

2-(4-aminophenyl)-3-(4-(dimethylamino)phenyl)-3,4-dihydro-1H-benzo[e][1,3]diazepine-1,5(2H)-dione (3):
red . m.p : 298-301 °C, yield 84% , IR (KBr, cm^{-1}): amine N- H_{str} (3233, 3192), amide N- H_{str}(3125), aromtic C-H_{str}(3073, 3007), aliphatic C-H_{str} (2913), amide C=O_{str}(1780, 1681), aromatic C=C_{str} (1570, 1539 , 1466, 1471), C-N_{str} (1212, 1153,1122), ^{1}H-NMR (DMSO-d_6) δ ppm : 3.54 (s , 6H , $2CH_3$), 5.56 (s , 2H , NH_2) 8.53 (s , 1H , -CH Diazepine ring) 6.72-8.39 (m, 12H, Ar-H), 6.30(s , 1H , CO-NH Diazepine ring),

-3-(4-(aminophenyl)-2-(4-(dimethylamino)phenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione (4):
yellow . m.p : 201-203 °C, yield 77% , IR (KBr, cm^{-1}): amine N- H_{str}(3522, 3363), aromtic C-H_{str}(3096, 3022), aliphatic C-H_{str} (2939, 2897), ester C=O_{str} (1756), amide C=O_{str}(1706), aromatic C=C_{str} (1638, 1597), C-N_{str} (1219, 1141), C-O_{str} (1249), ^{1}H-NMR (DMSO-d_6) δ ppm : 3.34 (s , 6H , $2CH_3$), 5.14 (s , 2H , NH_2) 8.73 (s , 1H , -CH Oxazepines ring) 7.42-8.10 (m, 12H, Ar-H)

2.4 Synthesis of hybrid phenytion (5,6,7).

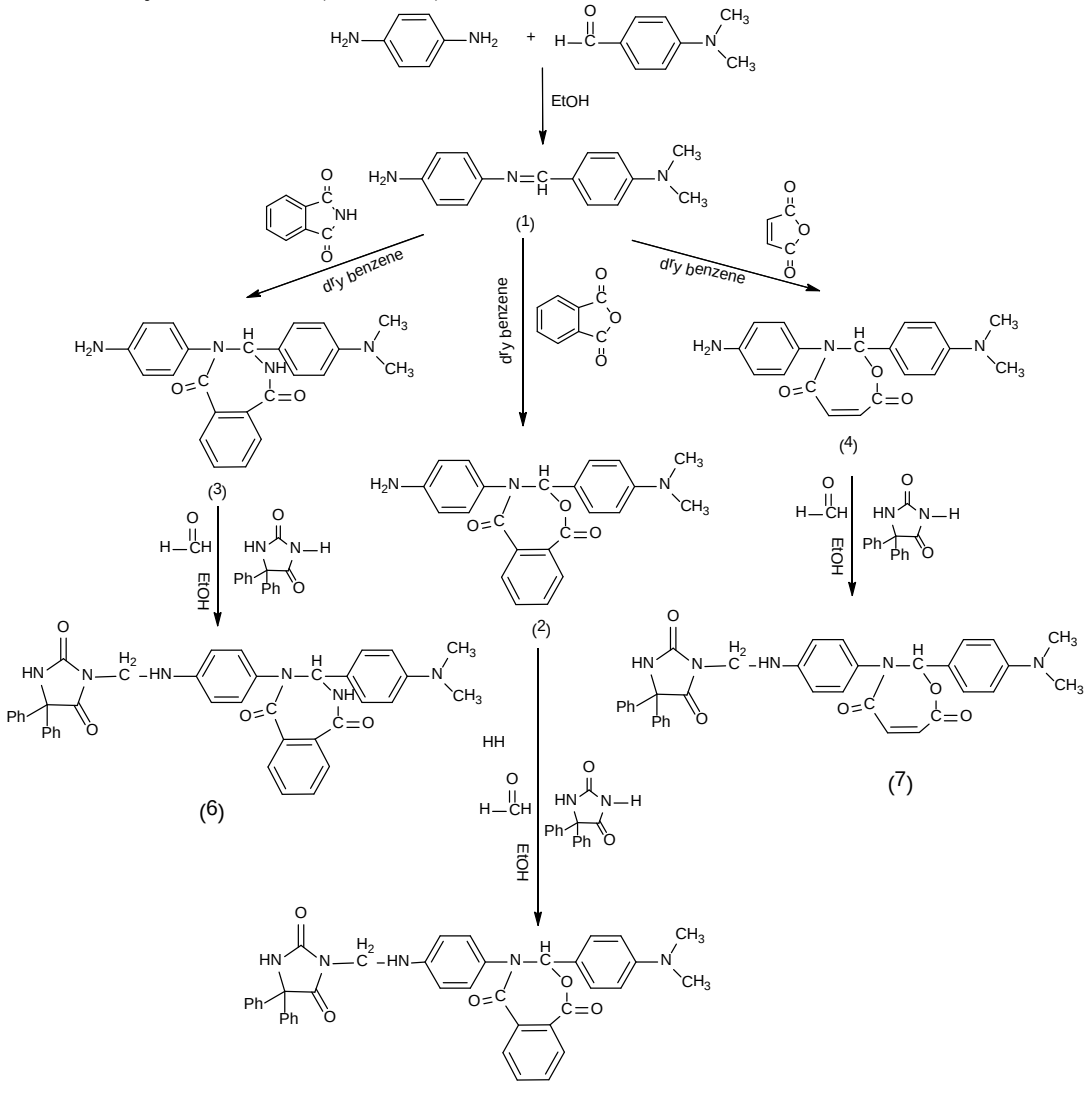
(0.05 mol) of **phenytion** was dissolved in absolute ethanol (15 ml) with Oxazepines , Diazepine (0.05 mol) and

paraformaldehyde(0.05 mol) arespectively in round flask (50 ml) and reflux condenser. The mixture was refluxed for (4-7 hours) and filtered directly to get colored products was cooled and recrystallized from ethanol. Table (1) shows the physical properties of the prepared compounds.

4-(4-(((2,5(dioxo-4,4-diphenylimidazolidin-1-yl)methyl)amino)phenyl)-3-(4-(triazan-2-yl)phenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione (5): white . m.p : 244-246 °C, yield 73% , IR (KBr, cm^{-1}): amine N-H_{str}(3335), amide N- H_{str}(3263), aromtic C-H_{str}(3092,3062), aliphatic C-H_{str} (2910), ester C=O_{str} (1739 Oxazepines ring), amide C=O_{str}(1695 Oxazepines ring), imidazolidine C=O_{str}(1661) aromatic C=C_{str} (1589, 1546 , 1473,1427), C-N_{str} (1226, 1184,1134), C-O_{str} (1265), ^{1}H-NMR (DMSO-d_6) δ ppm : 3.72 (s , 6H , $2CH_3$), 8.94 (s , 1H , NH-CH),5.42 (s , 1H , NH) , 4.23(s , 1H , -CH Oxazepines ring) 7.56-7.86 (m, 22H, Ar-H), 9.37 (s , 1H ,imidazolidine NH),

2-(4-(((2,5-dioxo-4,4-diphenylimidazolidin-1-yl)methyl)amino)phenyl)-3-(4-(triazan-2-yl)phenyl)-3,4-dihydro-1H-benzo[e][1,3]diazepine-1,5(2H)-dione (6): red. m.p : 315-317 °C, yield 73% , IR (KBr, cm^{-1}): amine N- H_{str}(3229), amide N- H_{str}(3175,3131), aromtic C-H_{str}(3094), aliphatic C-H_{str} (2950), amide C=O_{str}(1773,1716), phenytoin C=O_{str}(1634) aromatic C=C_{str} (1585, 1500 , 1456,), C-N_{str} (1222, 1165,1134), ^{1}H-NMR (DMSO-d_6) δ ppm : 3.66 (s , 6H , $2CH_3$), 8.93 (s , 1H , NH-CH),5.47 (s , 1H , NH) , 4.23 (s , 1H , -CH diazepine ring) 7.56-8.16 (m, 22H, Ar-H), 9.27 (s , 1H ,imidazolidine NH),

2-(4-(dimethylamino)phenyl)-3-(4-(((2,5-dioxo-4,4-diphenylimidazolidin-1-yl)methyl)amino)phenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione (7): dark yellow . m.p : 273-276 °C, yield 71% , IR (KBr, cm^{-1}): amine N-H_{str}(3235), amide N- H_{str}(3173), aromtic C-H_{str}(3073,3029), aliphatic C-H_{str} (2970,2810), ester C=O_{str} (1777 Oxazepine ring), amide C=O_{str}(1715 Oxazepine ring), imidazolidine C=O_{str}(1655,1626) aromatic C=C_{str} (1562, 1546 , 1454,1400), C-N_{str} (1222, 1168,1103), C-O_{str} (1253), ^{1}H-NMR (DMSO-d_6) δ ppm : 3.44 (s , 6H , $2CH_3$), 8.73 (s , 1H , NH-CH),5.35 (s , 1H , NH) , 7.01 (s , 1H , -CH Oxazepines ring) 7.56-7.86 (m, 22H, Ar-H), 9.33 (s , 1H ,imidazolidine NH).



Scheme 1: The synthesis of target compounds

Table 1. Properties of the prepared compounds.

Comp no .	M . Formula Formula	M.p (°C)	M . wt (g/mole) /mole)	Color	Yield %
1	$C_{15}H_{17}N_3$	165- 167	239.32	yellow	80%
2	$C_{23}H_{21}N_3O_3$	212-214	387.43	white	72%
3	$C_{23}H_{22}N_4O_2$	291-293	386.45	white	88%
4	$C_{19}H_{19}N_3O_3$	277-279	337.37	dark yellow	75%
5	$C_{37}H_{31}N_7O_5$	285-287	653.69	white	77%
6	$C_{37}H_{32}N_8O_4$	310-312	652.70	dark yellow	83%
7	$C_{35}H_{31}N_5O_5$	297-299	601.65	dark yellow	81%

Table 2. Growth Inhibition (GI) % of L20B cell line by new synthetic compounds

Concentration mg/ml	Growth Inhibition (GI) (%)						
	C1	C2	C3	C4	C5	C6	C7
0.04	9.3	22.4	19.4	0.0	11.07	0.0	0.0
0.02	16.1	18.7	14.4	10.06	4.3	6.04	-17.4

3. RESULTS AND DISCUSSION

The target compounds (5,6,7) were synthesized by three steps procedure as depicted in scheme 1. The synthesis of title compounds started from Schiff base reaction of the 4-(dimethylamino)benzaldehyde with a *p*-phenylenediamine. The IR spectra of compound (1) exhibited absorption bands, at (3066 cm^{-1} and 2939 cm^{-1}) which attributed to the C-H Aromatic and aliphatic group respectively . While the bands at (1598, 1508 , 1473, 1424cm^{-1}) and (1618 cm^{-1}) are attributed to C=C and C=N stretching frequency respectively. The IR spectrum of this compound showed bands at (3422, 3322) which corresponding to NH_2. compound (1) showed in ^{1}H-NMR signal at (δ 3.52 ppm) which assigned to(2 CH_3) methyl protons. and signal at (8.9 ppm) for (N=CH) imine group. The Oxazepines, Diazepine compounds (2,3,4) were synthesized by the reaction of compound (1) with corresponding (phthalic anhydride , malic anhydride and phthalimide) in the presence of dry benzene . The structure of all compounds (2,3,4) was confirmed by its FT-IR and ^{1}H-NMR spectroscopy, The FT-IR spectra of prepared compounds exhibited absorption bands at (3099-3007)cm^{-1} was attributed to the C-H aromatic group and the bands at (2939-2913) cm^{-1} was attributed to the C-H aliphatic group . The infrared spectra of synthesized compounds showed disappeared absorption band at (1600 cm^{-1}) which attributed to C=N imine stretching frequency of compounds (1) which indicate the success of the cyclization reaction. Also, appeared the absorption bands at (1780-1681 cm^{-1}) which assigned to C=O (ester and amid) stretching frequency indicated the occurs the cyclization reaction. The FT-IR spectra of compound (3) exhibited bands at (3125)cm^{-1} which correspondin to the N-H stretching frequency

In final step , Mannish reaction was used to synthesis compounds(5,6,7). Through reaction of compounds (2,3,4) with phenytion compound. The structure of compounds (5.6.7) was estblished by FT-IR and ^{1}H-NMR spectroscopy,, The FT-IR spectra of prepared compounds exhibited absorption bands at (3094-3073)cm^{-1} was attributed to the C-H aromatic group and the bands at (2970-2910) cm^{-1} was attributed to the C-H aliphatic group The FT-IR spectra of compounds 5,6,7 Also, appeared the absorption bands at (1773-1634 cm^{-1}) which assigned to C=O (imidazolidine ,ester and amid) stretching frequency indicated the occurs the hybrid reaction.. In ^{1}H-NMR spectra of compounds (5,6,7) exhibited signal at (δ 3.72, 3.66 and 3.44 ppm) which assigned to($2CH_3$) methyl protons respectively. and signals at (9.37 , 9.27 and 9.33 ppm) for (N-H) imidazolidine group respectively . . The reaction proceeds according to the Scheme 1.

4. CELL CULTURE AND CYTOTOXICITY

The Cell culture and Cytotoxicity of this study were carrying out in tissue culture unit of Biotechnology Research Center / Al-Nahrin University, L20B Cell Line was used. In 10% inactivated fetal calf serum (FCS), RPMI-1640 medium, antibiotic penicillin, and streptomycin (100 U/ml) and (100 mg/ml) respectively, the cells were grown. The cytotoxicity of new formed compounds was tested by method of (15). Each compound was dissolved to give concentrations 0.04, and 0.02 mg/ml, the cells were grown in tissue culture flasks containing growth medium at 37°C in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator.

Cells were plated in 96 multi well plate for 24 hours in a CO_2 incubator at 37°C. The concentration of the tested substance (0.04, and 0.02 mg /ml) were added to the cells (tow replicate wells for each concentration) and reincubated for 48 hrs. Control cultures containing RPMI-1640 medium were tested as control cytotoxicity. Crystal violate stain were added to the wells (50 µl), then, plates were incubated in a CO_2 incubator for 30 minutes at 37 °C. Spots were washed with tap water for three times and reader at 492 nm. The inhibitory rate of cell growth was calculated as following formula (16).

Growth Inhibition (GI) % = [(Optical density of control wells - Optical density of test wells) / Optical density of control wells] * 100 .

RESULTS

The effects of treating L20B cell line by the new compounds are shown in (Table 1). GI% rate in tow concentrations (0.04, and 0.02 mg /ml) were observed in L20B cell line. The best GI% rate for new compounds (C1-C7) on L20B cell line at 0.04 mg/ml was 22.4% for compound C2, and the best GI% at 0.02 mg/ml was 18.7 %

to the same compound (C2). New compounds C4, C6, and C7 had shown no effect on growth cell of L20B, at 0.04 mg/ml concentration. The C7 at 0.02 mg/ml concentration shown increasing in growth of L20B cell line.

CONCLUSION:

A new series of phenytion derivatives containing Oxazepines , Diazepines and Quinazolin ring were prepared . Some of the newly synthesized compounds exhibit importance anticancer evaluation.

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