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BIOCHEMICAL STUDY AND SYNTHESIS OF NEW PHARMACEUTICALS COMPOUNDS CONTAINING PHENOXAZINE MOIETIES

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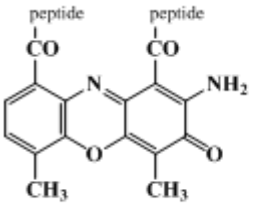
ABSTRACT : The current study includes synthesis of new derivatives of Phenoxazine , in order to obtain these derivatives; á,â-unsaturated ketone [5] was adopted to a Aldol condensation reaction between 10-ethyl-3-phenoxazin-3-yl aldehyde, that used with some different amine such as hydrazine hydrochloride, Hydrazinobenzene, Hydrazinecarbothioamide and Nitrinous acid to give pyrazolines [6,7,8,9,10] and oxazoline[11]. All prepared compounds have been characterized by Elemental-Analysis [CHN] and other spectroscopic methods such as FT-IR, NMR and Mass fragmentation. All pyrazolines and oxazoline were treated against bacterial and fungicidal and compare with pharmaceuticals and biological activity.

Key words : Pyrazol, antimicrobial activity, phenoxazine derivatives, oxazoline.

INTRODUCTION

Phenoxazine part is found in various organic extracts (Kadhim, 2015) such as active drugs like Actinomycin [1] and the prepared drugs such as Ragaglitazar [2], which has been developed as a drug for the treatment of hyperglycemia and hyperlipidemia, it represented an auspicious structural class of organic drugs depend on their biological activity to use in chemotherapy recently (Nielsen *et al*, 1998; Sawarkar *et al*, 2012).



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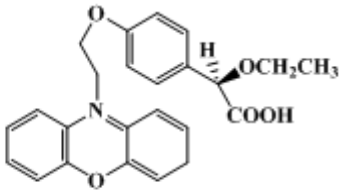

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Phenoxazine and its derivatives can exert steric inhibition effects on many viruses (Usdin and Efron, 1992) through its incorporation into oligonucleotide analogs which can bind tightly to RNA target locations (Wainwright *et al*, 1998) that are known to be recognition cites for proteins and to block processes that are essential for gene regulation. In a similar case phenoxazine and a certain group (Antonescu *et al*, 2010; Alwani *et al*, 2014) of its C- and N-substituted derivatives were found to inhibit phosphorylation of certain enzymes by competition with ATP for binding to the kinase (Satyanarayana *et al*, 2013).

Instruments and apparatus

1. Melting's points were recorded using Gall. Kamp melting's point apparatuses and are un correction.
2. Infrared spectroscopy were recorded on Fourier transform infrared Shimadzu (8300) (FT-IR) used as Potassium Bromide disc
3. Shimadzu UV-Visible spectrophotometer UV-160A were performed
4. Microanalytical of Elemental (C.H.N.O) were recorded on a Perkin–Elmer 240 B Analysis of elements (C.H.N.O) were performed at the laboratory of Chemistry Department, Tehran University.

Thin layer chromatography (T.L.C) was done on aluminum plate that coated silica-gel Ff254. Spots were detected within iodine vapors.

Experimental part

3-(10-ethyl-phenoxazin-3-yl)-1-(4-DMAP) prope-2-ene-1-one (5)

A mixture of (24 gram) of 10-ethyl-phenoxazin-3-carbaldehyde [3] (100 mmole) and 14.5 g from 4-dimethylaminoacetophenone [4] (100 mmole) in 250 cm^3 of ethanol were stirred and refluxed for 3hr at room temperature, 30% Sodium hydroxide aqueous solutions was add up to drop-wise. after stirring . The solid precipitates formed was collected and filtered and washed within 4% aqueous HCl and recrystallized from ethanol to give crystals compound [5] as a chalcone in 78 %

yield.

Compound[5] in 78% yield. IR (KBr): 3053 (C**H**$_{olef.}$), 1635.5 (**C=O**$_{withC=C}$) cm^{-1}; ^{1}H NMR (300MH$_z$, DMSO$_{solvent}$-d6): 3.8 (s, 6H$_{amine}$, Arom-N(C**H**$_3$)$_2$), 7.14-7.16 (multi, 2H, Aromat-H), 7.28-7.31 (multi, 4H, Aromat-H), 7.52-7.557 (multi, 2H, Aromat-H), 7.69(dd, 1H, J Value = 13.85 H$_z$, (carbonylC=O)conjugated(á,â-unsaturated CH=C), 7.978 (doublet ,1H , J$_{coupling}$ = 12.88 H$_z$ (carbonyl-C=O)conjugated(C=CH), 8.13-8.38 (multi, 1H$_{Arom}$, Aromat-H). Analytical calculations for **C$_{25}$H$_{24}$N$_2$O$_2$**: C(78.13); H(6.25); N(7.3). Found: C (77.75); H(5.88);N(7.22).

3-(4,5-dihydro-3-(4-DMAP)- 1H-pyrazol-5-yl)- 10-ethyl-phenoxazine [6]

To a stirred mixture of (4 gram) of á,â-unsaturated ketone [5] (10 mmole) in 55 cm^3 of C_2H_5OH, 6 cm^3 of acetic acid and 1.2 g of $NH_2NH_2.H_2O$ (80%) (10 mmole) were added and refluxed for 12 hr. and left 12hr. The precipitate separated out was filtered and recrystallization from C_2H_5OH to produced compounds **[6]** as colored crystals in 70 % yield.

Compound [6] as yellow crystals in 70% yield; FT-IR (K-Br) disk: 3178 (N-H$_{pyrazol}$), 1632 (C=N$_{pyrazol}$) .cm^{-1}; ^{1}H NMR (300MH$_z$, DMSO$_{solvent}$-d6): ä. = 1.87 (multi, 1H$_{AMX}$), 2.69 (multi, 1H$_{AMX}$), 3.49 (multi, 1H$_{AMX}$), 3.88 (s, 6H$_{amine}$, -N(CH$_3$)$_2$), 6.66-8.68 (m, 11H$_{arom}$, Aromat-H), 8.95 (s, D$_2$O-exchangeable, 1H$_{pyrazoline}$, NH$_{pyrazoline}$), Analytical calculations for $C_{25}H_{26}N_4O$: C(75.38); H(6.53); N(14.07). Foundi. C(74.62); H(6.35); N(13.95).

3-(4,5,-dihydrogen-3-(4-DMAP)-1-phenyl-1H-pyrazol-5-yl)- 10-ethyl-phenoxazine [7]

To a stirred mixture of (4 gram) of chalcones[5] (100 mmole) in 55 cm^3 of ethanol, 7 cm^3 of CH_3COOH and 1.1 gm of Hydrazinobenzene (10 mmole) were added to a mixture , all component in round was reflux for 12 hrs. and still 12 hrs. The precipitate was separated and washes with ethanol and recrystallized to give compound [7] as colored crystals in 72% yield.

Derivative [7] as brown crystals in 72% yield , mp 152-154ºC; FT-IR (K-Br): 3228 (NH$_{pyrazol}$) 3038 (CH$_{pyrazol}$), 1638 (C=N$_{pyrazol}$) cm^{-1}; ^{1}H NMR (300MH$_z$, DMSO$_{solvent}$-d6): ä = 2.18 (multi, 1H$_{AMX}$), 2.58 (multi, 1H$_{AMX}$), 3.18 (multi, 1H$_{AMX}$), 3.38 (s, 6H$_{amine}$, -N(CH$_3$)$_2$), 6.88-7.08 (multi, 2H$_{Arom}$, Aromat-H), 7.22-7.28 (multi, 2H$_{Arom}$, Aromat-H), 7.25-7.29 (multi, 1H$_{Arom}$, Aromat-H), 7.29-7.33 (multi, 4H$_{Arom}$, Aromat-H), 7.45-7.55 (multi, 2H$_{rom}$, Aromat-H), 8.38 (s, 1H$_{Arom}$, Aromat-H); Analytical calculations for $C_{31}H_{30}N_4O$: C(78.48); H(6.33); N(11.82). Foundi: C(78.88); H(5.98); N(11.45).

Preparation of compounds [8, 9]

In a similar manner as prepare compound [6] that mentioned in synthesis compound [8,9] and using's CH_3CO_2H instead of CH_3OH to prepared [8] and $CH_3CH_2CO_2H$ to prepared [9].

1-(4,5-dihydro-5-(10-ethyl-phenoxazin-3–yl)-3-(4-DMAP)pyrazol-1-yl)ethanone [8]

A mixture of (4 gram) of compound [5] (100 mmole) in 55 cm^3 of ethanol, 5 cm^3 of Ethanoic acids and 1.1. grams of Hydrazinobenzene (100 mmole) were mixed. The reactions mixtures were refluxed overnight and left to still for 12 hrs. The precipitate will separate out was filtered and recrystallized from C_2H_5OH to give compounds [8] as colored crystals in 72% yields**.**

Derivative 8 as a yellow crystals, mp 148-149°C, yield 63.82%; FT-IR (K-Br): 3028 (C-H$_{pyrazol}$), 1621 (-C=N$_{pyrazol}$) cm^{-1}; 1688 (C=O$_{acetyl}$) ^{1}H NMR (300MH$_z$, DMSO$_{solvent}$-d6): ä, = 1.96 (multi, 1HH), 2.27 (s, 3H,C**H**$_3$, -methyl), 2.42 (multi, 1H$_{AMX}$), 3.38 (s, 6H$_{amine}$, -N(CH$_3$)$_2$), 3.87 (multi, 1H$_{AMX}$), 7.12-7.26 (multi, 4H$_{Arom}$, Aromat-H), 7.23-7.47 (multi, 2H$_{Arom}$, Aromat-H), 7.91-7.98 (multi, 1H$_{Arom}$, Aromat-H), 8.17-8.33 (multi, 2H$_{Arom}$, Aromat-H), Analytical calculations for $C_{27}H_{28}N_4O_2$: C(73.64); H(6.36); N(12.73). Found: C(74.77); H(7.56); N(12.72).

1-(4,5-dihydroge-5-(10-ethyl- phenoxazin-3 –yl)-3—(4-DMAP)pyrazol-1-yl)propan-1-one [9]

A mixture of (4 gram) of á,â-unsaturated ketone [5] (100 mmole) in 50 cm^3 of C_2H_5OH, 5 cm^3 of CH_3CH_2COOH and 1.1 gram of Hydrazinobenzene (100 mmole) was added. The reaction mixture takes over night and refluxed and then left 12 hrs. The precipitate was wash and filtered and recrystallized from C_2H_5OH to give compound **[9]** as colored crystals in 72% yield.

Derivative [9] as yellow crystals , mp 144-145°C, yield 68.51%; FT-IR (K-Br): 3017 (CH$_{pyrazol}$), 1674 (C=O$_{propyl}$) cm^{-1}; ^{1}H NMR (300MH$_z$, DMSO$_{solvent}$-d6): äs = 1.7-2.3 (t, 3H,C**H**$_3$, -methyl), 2.11 (multi, 1H$_{AMX}$), 2.75 (multi, 1H$_{AMX}$), 2.62-2.78 (q, 2H,**CH**$_2$, -methlene), 3.46 (multi, 1H$_{AMX}$), 3.75 (s, 6H$_{amine}$, 6.83-6.89(multi, 1H$_{Arom}$ Aromat-H),6.91-6.95 (multi, 2H, Aromat-H), 7.17-7.28 (multi, 4H$_{Arom}$, Aromat-H), 7.42-7.51 (multi, 2H$_{Arom}$, Aromat-H) Analytical calculations for $C_{28}H_{30}N_4O_2$: C(74.00); H(6.61); N(12.33). Found: C(74.43); H(6.92); N(12.33).

4,5-dihydroge-5-(10-ethyl- phenoxazin -3 –yl)-3—(N, N-dimethylamino phenyl) pyrazole-1-carbothioamide [10]

A mixture of (4 gram) of á,â-unsaturated ketone [5] (100 mmole) in 50 cm^3 of C_2H_5OH, 1.0 gram of NaOH

(25 mmole) and 1.2 gram of Hydrazinecarbothioamide (12 mmole) were mixed , the collection refluxedover night and then left to still to cool for 12 hrs, The precipitate was wash and separate and recrystallized from C_2H_5OH to give compound **[10]** as colored crystals in 72 % yield

Derivative [10] as crystals mp 152-153 °C; FT-IR (K-Br): 3224 (**thioamide**NH_2), 1616 (C=$N_{\textbf{pyrazole}}$) , 1255 C=$S_{\textbf{thioamide}}$) cm^{-1}; ^1H NMR (300MH_z, $DMSO_{solvent}$-d6): ä = 2.24 (multi, $1H_{AMX}$), 2.588 (multi, $1H_{AMX}$), 3.38 (multi, $1H_{AMX}$,), 3.81 (s, $6H_{amine}$), 7.12-7.21 (multi, $2H_{Arom}$, Aromat-H), 7.23-7.44 (multi, $4H_{Arom}$, Aromat-H), 7.83-8.18 (multi, $2H_{Arom.}$, Aromat-H),8.24-8.31(multi,$1H_{Arom}$, Aromat-H), 11.22 (s, D2O-exchangeable, $2H_{amine}$, -NH_2), Analytical calculations for $C_{26}H_{27}N_5SO$: C(68.27); H(5.91); N(12.25). Found: C(68.18); H(6.26); N(11.98)

3-(4,5-dihydro-3-(4-DMAP)isoxazol-5-yl)- 10-ethyl-phenoxazine [11]

A mixture of (4 gram) of á, â-unsaturated ketone [5] (100 mmole), 0.8 gram of Nitrinous acid hydrochloride (10 mmole) and 1.5 gram of potassium carbonate (10 mmole) in (55 cm^3) of C_2H_5OH were refluxed overnight and the left for 12 hrs and then cool. The precipitate was separate and wash and re crysllized in C_2H_5OH to give afford isoxazole compound**[11]** as colored product in 72% yield**.**

Isoxazole derivative [11]

In 72% yield as yellow crystals, mp 156-157 ºC; FT-IR (K-Br): 3046 (-$C_{\textbf{isoxazole}}$), 1645 (-C=$N_{\textbf{isoxazole}}$) cm^{-1}; ^1H NMR (300MH_z, $DMSO_{solvent}$-d6): ä, = 2.16 (m, $1H_{\textbf{isoxazole}}$), 2.57 (multi, $1H_{AMX}$), 3.46 (multi, $1H_{AMX}$) 3.76 (s, $6H_{amine}$, 7.18-7.16 (multi, $2H_{Arom}$, Aromat-H), 7.16-7.25 (multi, $4H_{Arom}$, Aromat-H), 7.46-7.51 (m,$2H_{Arom}$, Aromat-H), 8.24-8.36 (m,1H, Aromat-H) , Analytical calculations for $C_{25}H_{25}N_3O_2$: C(75.01); H(6.25); N(10.5). Found: C(76.11); H(6.85); N(10.32).

RESULTS AND DISCUSSION

á, â-unsaturated ketone [5] in this study was formed from the reaction of 10-ethyl-phenoxazin-3-carbaldehyde [3] with N,N-dimethyl amino acetophenone **[4]** in ethanoic sodium hydroxide solution . the structure of á,â-unsaturated ketone [5] was identified by FT-IR spectrums that showed a various peaks for the determination of á,â-unsaturated conjugate carbonyls groups at 1633.5 cm^{-1}; and also by proton Nuclear magnetic resonance (Pavia *et al*, 2009), which gave signals at ä= 7.52 (doublet, 1H, *J* = 12.7 H_z (-carbonylC=O)(-alkeneCH=C). Reaction of á,â-unsaturated ketone[5] with either hydrazine hydrochloride or Hydrazinobenzene gives the analogues pyrazoline compounds [**6,7]** respectively. Structures of 6 and 7 were determined by their (FT-IR and ^{1}HNMR) Spectrums. Boths showed imine (Schiff base) protracting vibrations in a region 1620-1638 cm^{-1} and pyrazole **[6]** showed an extra vibration frequency at 3215.9 cm^{-1} that refer for a amineNH in pyrazole ring.

Proton Nuclear magnetic spectrums in both compounds [6 and 7] showed threes a multiples at ä, 2.10, 2.7 and 3.52 ppm that result from the A.M.X style interaction by two a diastereotopic interaction protons at C-4 (H_a and H_b) and only one protons (H_c) at C-5 (Scheme 1).

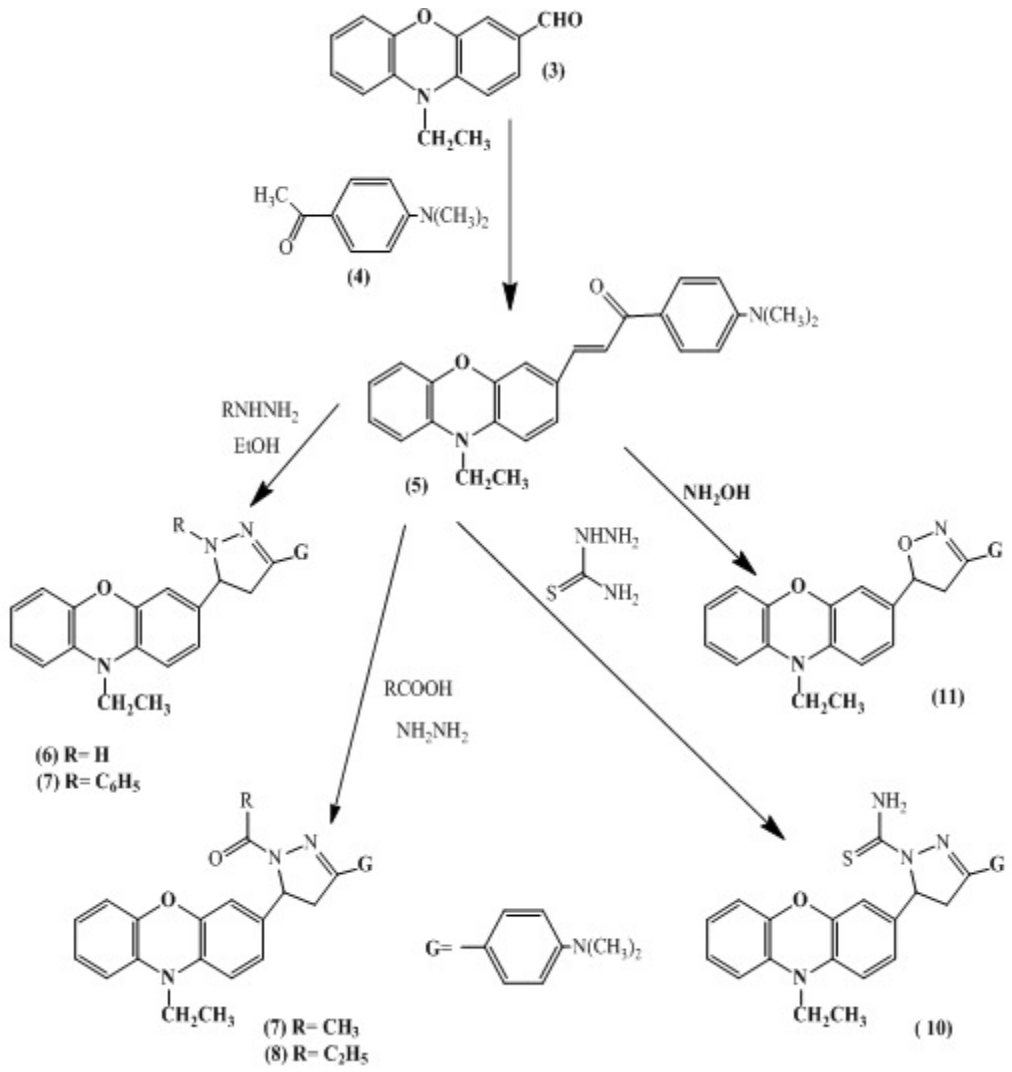
In the present study, the acyl derivatives [8,9]was designed either by treating [6] compound within the identical acids chlorides, or by reaction a chalcones [5] with the hydrazinehydrochloride on the alteration the reacting mediums from C_2H_5OH to ethanoic acid or propanioic acid, as respectively. Structures of **[8,9]**were formed by their FT-IR spectrums whereas that non characteristics bands for weak bond of amine that discovered on (3215.5 cm^{-1}). The 1-HNMRe of compound **[8]** display a singlet ats ä 2.24ppm (acetyls -methyl protons), whilst 9 showed a triplets at ä 2.0 (methyl group in ethyl C$\mathbf{H_3}CH_2$) and a quartets at ä 2.68(-metheneCH_3C$\mathbf{H_2}$) for each signal, respectively.

The structures of a compound **[10]** were achievement the refluxed á,â-unsaturated ketone [5] within Hydrazine carbothioamide and NaOH in acid media. This structures were confirms by FT-IR at on the absorptions peakes were appear in region at (α3316 cm^{-1} and α 3227 cm^{-1}), characteristics to -NH_2 and -amineNH, on respectively, and it is protonH NMR (Silverstein *et al*, 2005) was display in two singlets at ä = 11.11ppm (D2O-commutable, 2H, NH_2) and 11.60 (D2O- commutable, 1H, phenoxazine-NH).

For compound (oxazolines **[11]** were formed from refluxed á,â-unsaturated ketone [5] with the Nitrinous acid in CH_3OH, where by spectral data it is structure was ascertained, where as its FT-IR spectrum in two peaks at a 3388 and 1645, characteristics to all -amineNH and- imine C=N, on respectivily. The proton ^{1}H- NMR showed a singlets at a ä = 12.67 (D2O- commutable, 1H, phenoxazine NH). The MS (Mass Spect.) revealed it is a peak at m/z 399 (M+) that referee to molecular parent ions as shown in the Scheme (1).

Biological activity

The new synthesized compounds that include [6,7,8,9,10] and [11] were performed and screened according to the disk diffusion method *in vitro* (Gaina *et al*, 2002; Atiya *et al*, 2019) for their biological activity against two strain contain Gram-negative bacterial -ve



Scheme 1 : represented synthesis of compound 4-9.

Table 1 : Fungicidal activity and Bactericidal of some of the new Phenoxazine derivatives and compare with drugs activity of ciprofloxacin and nystin.

Comp	*Candida albicans*	*Aspergillus niger*	*Fusarium*	*Pseudomonas aeroginosa*	*Escherichia coli*	*Staphylococcus aureus*
6	(16)++	-	(31)+++	(17)+++	-	(32)+++
7	(18)++	-	(16)+++	(20)+++	-	(21)+++
8	(20)++	-	(27)+++	(19)+++	-	(30)+++
9	(22)++	-	-	(17)+++	-	(18)+++
10	-	-	-	(27)+++	-	(15)+++
11	(20)++	-	(32)+++	(29)+++	-	(34)+++
Ciprofloxacin	-	-	-	++++	-	++++
Nystin	++++	++++	++++	-	-	-

(*Escherichia-Coli* and *Pseudomonas-aeruginosa*) and Gram-positive +ve bacterial (*Staaphylococcus-aureus*) (Kadhim *et al*, 2019; Jabbar and Al-Azawi, 2020) and used the fungicidal activity against *Candida albicans* and *Fusarium*, *Aspergillus niger* (Table 1). These measurements were carried out in the Quality Control and Propagation of plants unit, Departments of Environment, college of sciences, University of Al-Qadisiyah. All prepared compounds show highest or medical or pharmaceutical activity to *Pseudomonas aeruginosa* and *Staphylococcus aureus* compared with that of ciprofloxacin Compounds [6,7,8,9] showed a very perfect a fungicidal activity, compare with that of nystatin, against *Fusarium*. All prepared compounds except for

[10] was found to have moderate fungicideal activity versus *Canidida albicanes*.

CONCLUSION

The Aldol reaction between 10-ethyl-phenoxazin-3-carbaldehyde and 4-dimethylamino-acetophenone give the chalcone[5] that used to produced isoxazoles, and pyrazolines. Derivatives that linked to a phenoxazine parts was synthesized and this founds to have determining the biological acivity and compare with commercial common drugs.

Abbreviate 4-DMAP = N,N-dimethylamino phenyl

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