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Synthesis and Evaluation of the Antiproliferative Potency and Induced Biochemical Parameters of Novel Pyrazolones Derivatives Towards Hepatocellular Carcinoma

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Abstract

There is currently a great deal of interest in the chemistry of pyrazol-5-ones and their related compounds due to their wide range of biological and pharmacological activities. A variety of compounds having a pyrazol-5-one moiety as a structural unit have been synthesized and studied with interest centered on their potential pharmaceutical activity. Previous researches revealed that pyrazol-5-one moiety is an important pharmacophore which exhibits outstanding biological activities such as antioxidant, anti-fungal, antibacterial, antidepressant, anticonvulsant, anti-inflammatory, antipyretic and antitumor. The present work focused on studying the synthesis of novel pyrazoline derivatives and screening of some selected their antiproliferative potency of some selected derivatives namely compounds **1, 2, 3, 4, 5, 6, 7, 8** and **9** towards hepatocellular carcinoma cell line (HEPG2). Results indicated that the selected compounds **9**, **7** and **6** were the most active derivatives towards HEPG2 cell line with IC50 equals 3.74, 3.92 and 4.31µg/mL respectively. The newly prepared selected derivatives showed interesting antiproliferative potency in comparison to the traditional anticancer drugs: 5 Fluorouracil and doxorubicin. However, the potent compounds needs more pharmacological tests.

Keywords:

Pyrazolines, Hepatocellular Carcinoma (HCC), Cell Line (HEPG2), Induced Biochemical Parameter.

1. Introduction

The chemistry of pyrazolones and related compounds is particularly interesting because of their potential application in medicinal chemistry, as they have constituents of several intermediates for medicinal drugs [1].

Compounds with a pyrazoline structure have been found to possess anti-fungal [2], anti-depressant [3], anticonvulsant [4], anti-inflammatory [5],antibacterial [6-7], antipyretic [8] and antitumor [9-10] properties. Cancer is one of the most widespread serious diseases. It is characterized by uncontrolled growth of abnormal cells. The growth and metastasis of cancer cells are dependent on angiogenesis; therefore, affecting angiogenesis will be of great importance in inhibition of tumor growth, invasion and metastasis [11]. Hepatocellular carcinoma (HCC) is a worldwide problem, with epidemiological data varying from place to place [12]. HCC is the sixth most common cancer in the world and the fourth most common cancer in Egypt, respectively. Egypt is the third and fifteenth-most populous country in Africa and the world, respectively [13]. The present work focused on studying the synthesis of different novel pyrazoline derivatives, chemical characterization of the selected derivatives, namely compounds **1, 2, 3, 4, 5, 6, 7, 8** and **9**, that showed significance antiproliferative potency towards hepatocellular carcinoma cell line (HEPG2).

Materials and Methods

Chemical Synthesis

All the solvents and reagents were used as obtained from commercial sources without further purification. Melting points were determined on Gallen-Kamp melting point apparatus and are uncorrected. IR (KBr) spectra were recorded using a Mattson 5000 FT-IR spectrometer in KBr disks, microanalysis unit, Chemistry Department, Faculty of Science, Mansoura

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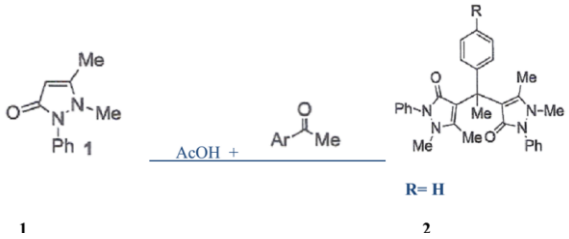
University. 1H NMR spectra in CDCl3 as solvent were measured on Bruker AC–400 MHz spectrophotometer using TMS as an internal reference at the Chemistry Department, Faculty of Science; Cairo University. Elemental analysis was carried out at the Microanalytical Unit, Faculty of Science; Cairo University. Mass spectra were recorded on a GC-MS QP –1000 EX Shimadzu instrument. The progress of the reaction was monitored by thin layer chromatography using pre-coated Merck silica gel 60 F254. The spots were visualized by UV.

General procedure: 1-1 Reaction of Antipyrine with Aryl Ketones (Compound 2):

The acid catalysed condensation of antipyrine (**1**) with acetophenone, 4 nitroacetophenone, 4-methoxyacetophenone, 4-hydroxyacetophenone, 4-phenylacetophenone and 1,4-diacetyl benzene afforded the corresponding bis-antipyrines:4,4'- (l-aryl)ethane-1,1-diyl)bis(l,5-dimethyl-2-phenyl-l ,2-d ihydro-3H-pyrazol-3-ones(**2**).

A solution of antipyrine 1 (1.88 g, 0.0 l mol) and acetophenone or p-nitroacetophenone or p-methoxy acetophenone or p-hydroxy acetophenone or 4-phenyl acetophenone or 1,4-diacetylbenzene (0.005 mol) in 20 mL glacial acetic acid \Vas heated over boiling water bath for 6 hrs. The product that obtained by addition of crushed ice, was filtered, washed with ethanol, dried and recrystallized from absolute ethanol to give (**2**) in Scheme1.



Scheme 1

4,4'-(1phenylethane-1l-diyl)bis(l,5-dimethyl-2phenyl-l2-dihydro-3H-pyrazol-3- one (2):

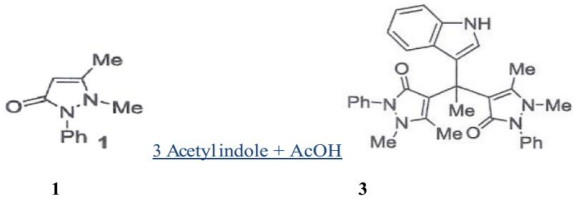
Mp. 75-7 °C. Yield 80 % (white crystals).

Analysis: $C_{30}H_{30}N_4O_2$ (478.60): calcd. C 75.29%, H 6.32%, N 11.71%. Found C 75.13%, H 6.21%, N 11.58%. IR(cm-$^{-1}$) (KBr): 1665 (C=O), 1582 (C=N), 1482, 1316, 769 cm-$^{-1}$

MS (EI, 70 eV): *m/z* (%): 480 (50) [M +2]$^+$, 479 (72) [M + 1]$^+$, 478 (78) [M]$^+$, 463 (72) [M - CH_3]$^+$, 447 (50) [M- (CH_3 + H)]$^+$, 419 (54)) [M+ H - (4CH_3)]$^+$, 371(69) [M- (Ph + 2CH_3)]$^+$, 341 (60) [M - (Ph + 4CH_3)]$^+$, 326 (65) [M - (Ph + 5CH_3)]$^+$, 324 (57) [M - 2Ph)]$^+$, 291(53) [M – (antipyrine ion)]$^+$, 187(68) [antipyrine ion]$^+$, 77(75) [Ph]$^+$. ^{1}H NMR (300MHz, CDCIJ, 25 °C, TMS): 1.72(s, 3H, CH_3), 2.23 (s, 6H, 2 CH_3), 3.05 (s, 6H, 2N- CH_3), 7.27-7.95 ppm (m, 15H, Ar-H) ppm.

1-2 Reaction of antipyrine with heterocyclic ketones (Compound 3):

In the present study, the synthesis of heterocyclic ternary systems incorporating two antipyrine units has been realized by treating antipyrine (**1**) with heterocyclic ketones. Therefore, the acid-catalyzed condensation of 1 with 4-acetylpyridine, 3- acetylindole and barbituic acid lead to the formation of (**3**) in Scheme 2.



Scheme 2

4,4'-(1(1H-Indol-3yl)ethane-1l-diyl)bis(l,5-dimethyl-2-phenyl-2-dihydro-3H-pyrazol-3-one)(3):

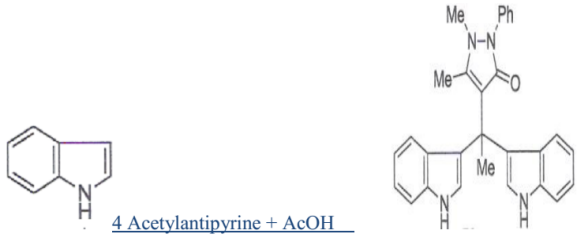
M. p. 150-3°C. Yield 60 % (white crystals).

Analysis: $C_{32}H_{31}N_5O_2$ (517.63): calcd. C 74.25%, H 6.04%, N 13.53%. Found C 74.11%, H 5.88%, N 13.38%.

IR(cm-$^{-1}$) (KBr): 3407 (NH), 1665 (C=O), 1574, 1482, 1411, 771, 616 cm-$^{-1}$. MS (EI, 70 eV): *m/z* (%): 457 (51) [M -4CH_3)+, 442 (51) [M -5CH_3)+, 425 (54) [M -(Ph + CH_3)+, 402 (48) [M - (indole ion)]$^+$, 380 (66) [M - (Ph + 4CH_3)]$^+$, 363 (58) [M - 2 Ph]$^+$, 315 (61) [M - (CH_3+ (antipyrine ion))]$^+$, 188(68) [antipyrine ion]$^+$, 174 (48) [pyrazolone ion]$^+$, 143 (58) [M - 2 (antipyrine ion)]$^+$. ^{1}H NMR (300MHz, CDCIJ, 25 °C, TMS): 1.62 (s, 3H, *CH_3)*, 2.57 (s, 6H, 2CH_3), 3.48 (s, 6H, *2N-CH_3)*, 7.29-7.44 (m, 13H, *Ar -H)*, 7.85 (d, H, *CH)*, 8.40 (s, H, *Ar -H)*, 8.81 (s, lH, *N H)* ppm.

1-3 Reaction of indole with acetylpyrazolones (Compound 4):

In the present study, the acid-catalyzed condensation of antipyrine (**1**) and its related compounds with indole was investigated as a possible route to some new heterocyclic systems containing both indole and pyrazoline moieties. In a preliminary sttdy, treatment of 4-acetylantipyrine with indole in acetic acid afforded **4** as in Scheme 3.



1 4

Scheme 3

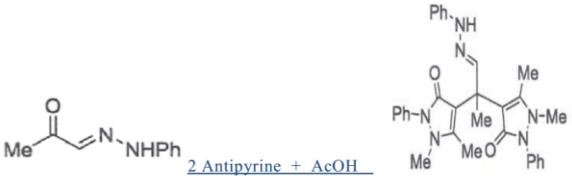
4-(1,1-Di(1H-indol-3-yl)ethyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (4):

M. p. 110 °C. Yield 60 % (black powder). Analysis: $C_{29}H_{26}N_{40}$ (446.55): calcd. C 78.00%, H 5.87%, N 12.55%. Found C 77.87%, H 5.72%, N 12.39%. IR (cm^{-1}) (KBr): 3408 (NH), 1627 (C=O), 1599, 1491 (Ph), 1414, 1100, 743 cm^{-1}. MS (EI, 70 eV): *m/z* (%): 447 (3) [M+1]$^+$, 446 (3) [M]$^+$, 355 (3) [M+H-(CH3+Ph)]$^+$, 332 (3) [M-(indole ion) +2H]+, 260 (1) [M-(antipyrine ion)]$^+$, 187 (3) [antipyrine ion]$^+$, 9 (48), 77 (6) [Ph]$^+$. ^{1}H NMR (300MHz, CDCIJ, 25 °C, TMS): 1.66 (s, 3H, *CH3)*, 2.23 (s, 3H, *CH3*), 3.06 (s, 3H, *N- CH3)*, 7.06 (s, 2H, *CH)*, 6.87-7.69 (m, 15H, Ar-*H)*, 7.27 (s, 2H, 2N*H)* ppm.

1-4 Synthesis of Antipyrinyl Formazans (Compound 5):

In order to prepare formazans bearing three antipyrine units, the reaction of pyruvaldehyde phenylhydrazone with two equivalents of antipyrine afforded the corresponding bis-adduct 4 which underwent coupling with 4-antipyrinyl diazonium chloride in pyridine medium to access the formazan 68 efficiently.

A mixture of antipyrine (1.88 g, 0.01 mol) and pyruvaldhyde-1-phenyl hydrazine (0.81 g, 0.005 mol) in glacial acetic acid (30 mL) was heated on water bath for 6 hrs. The product obtained on cooling, basification with sodium hydroxide and washing with water was recrystallized from ethanol to give (**5**) as in Scheme 4 .



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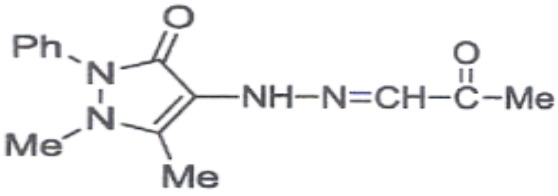
Scheme 4

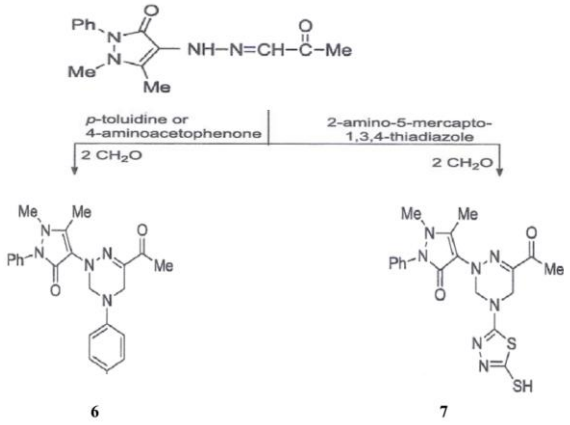
4,4'-(1(2-Phenylhydrazono)propane-2,2-diyl)bis(1,5-dimethyl-2-phenyl-1,2-dihydro-3 H-pyrazol-3-one) (**5**):

M. p. 96-8 °c. Yield 60 % (yellow powder). Analysis: $C_{31}H_{32}N_6O_2$ (520.64): calcd. C 71.52%, H 6.20%, N 16.14%. Found C 71.43%, H 6.11%, N 16.06%. IR(cm^{-1}) (KBr): 3249 (NH), 2974 (CH_3), 1648 (C=O), 1601, 1494 (Ph), 1242, 1172, 684 cm^{-1}. MS (EI, 70 eV): *m/z* (%): 523 (56) [M + 3]$^+$, 522 (89) [M + 2]$^+$, 521 (56) [M + 1]$^+$, 520 (100) [M]$^+$, 505 (56) [M - (CH_3 + H)]$^+$, 491 (86) [M + H - (2CH_3)]$^+$, 443 (99) [M - Ph]$^+$, 397 (72) [M - (Ph + 3CH_3 + H)]$^+$, 305 (69) [M - (2Ph + 4CH_3 + H)]$^+$, 461 (55) [M + H - 4CH_3]$^+$, 445 (56) [M - (5CH_3 + H)]$^+$, 368 (55) [M - (Ph + 5CH_3 + H)]$^+$, 289 (53) [M - 3Ph]$^+$, 77 (82) [Ph]$^+$. ^{1}H NMR (300MHz, CDCIJ, 25 °C, TMS): 1.33 (s, 3H, C*H3)*, 2.27 (s, 6H, 2C*H3*), 3.45 (s, 6H, *2N-CH3)*, 6.98-7.34 (m, 15H, Ar-*H*). 7.94 (s, I H, *CH)*, 12.16 (s, 1H, N*H*) ppm.

1-5 Synthesis of Antipyrinyl 1,2,4-Triazines (Compounds 6-7):

A solution of pyruvaldhyde-1-(4-antipyrinyl)hydrazone (2.72 g, 0.01 mol) in absolute ethanol (30 mL) was treated with p-toluidine (1.07 g, 0.012 mol) or *p*-aminoacetophenone (1.35 g, 0.012 mol) or 5-amino-1,3,4-thiadiazole -2-thiol and formalin 37% (0.022 mol), the mixture heated for 2 hrs on water bath. The product obtained on cooling was recrystallized from ethanol to give **6 and 7** in Scheme 5.





Scheme 5

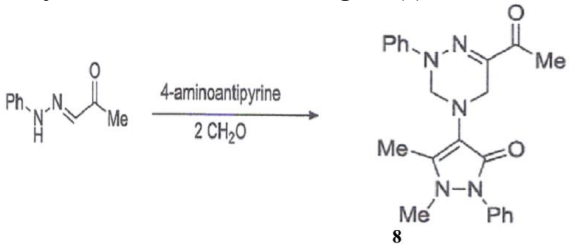
4-(6-Acetyl-4-(p-tolyl)-4,5-dihydro-1,2,4-triazin-2(3H)-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one) (6):

M. p. 127-9 °C. Yield 58 % (brown powder). Analysis: $C_{23}H_{25}N_5O_2$ (403.49): calcd. C 68.47%, H 6.25%, N 17.36%. Found C 68.38%, H 6.17 %, N 17.24%. IR(cm-$^{-1}$) (KBr):1748, 1666 (C=O), 1598, 1549, 1515 (Ph), 1359, 1239, 1167, 831cm-$^{-1}$. MS (EI, 70 eV): *m/z* (%): 404 (1) $[M+1]^+$, 403 (1) $[M]^+$, 373 (1) $[M-2CH_3]^+$, 358 (1) $[M-3CH_3]^+$, 261 (I) $[M-COCH_3]^+$, 216 (1) [M - (antipyrine ion)], 107 (14) [*p*-toluidine]$^+$, 91 (93) $[C_6HsCH_3]^+$, 85(39), 77 (60) $[Ph]^+$.

4-(6-Acetyl-4-(5-mercapto-1,3,4-thiadiazol-2-yl)-4,5-dihydro-1,2,4-triazin-2(3H)-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one(7):

M. p. 250 °C. Yield 75 % (white powder). Analysis: $C_{18}H_{19}N_7O_2S_2$ (429.?2): calcd. C 50.34%, H 4.46%, N 22.83%. Found C 50.22%, H 4.37%, N 22.74 %. IR (cm-$^{-1}$) (KBr): 1669 (C=O), 1598, 1513 (Ph), 1359, 1267, 820cm-$^{-1}$. MS (EI, 70 eV): *m/z* (%): 417 (2) $[M-CH_3]^+$, 401 (1) $[M-2CH_3]^+$, 243 (1) [M - (antipyrine ion)], 145 (100), 77 (35) $[Ph]^+$.

Moreover, a solution of pyruvaldehyde-1-phenylhydrazone (1.62g, 0.01mol) in absolute ethanol (30 mL) was treated with of 1,0 (1g, 0.005 mol) and formalin 37% (0.022 mol). The mixture heated for 2 hrs. The product obtained on cooling was recrystallized from ethanol to give (**8**) in Scheme 6.



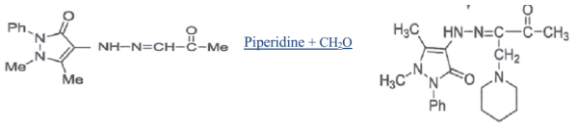
Scheme 6

4-(6-Acetyl-2-phenyl-2,5-dihydro-1,2,4-triazin-4(3H)-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one(8)

M. p.75 °C. Yield 75 % (yellow powder). Analysis: $C_{22}H_{23}N_5O_2$ (389.46): calcd. C 67.85 %, H 5.95%, N 17.98%. Found C 67.74 %, H 5.86%, N 17.89%. IR (cm-$^{-1}$) (KBr): 304, 1659 (C=O), 1497 (Ph), 1268, 1153, 757, cm-$^{-1}$. MS (EI, 70 eV): *m/z* (%): 390 (1) $[M+1]^+$, 389 (2) $[M]^+$, 347(3) $[M-COCH_3]^+$, 241 (22), 203 (2) [M-(antipyrine ion)]$^+$, 77 (39) $[Ph]^+$, 56 (100).

1-6 Synthesis of Mannich bases (Compound 9):

In different scenario, performing Mannich reaction of the hydrazone with secondary amines such as piperidine and piperazine in a solution of (2.72 g, 0.01 mol), formalin (37%, 0.01 mol) and piperidine (0.85 g, 0.01 mol) in absolute ethanol (50 mL) was heated for 2 hrs, the corresponding (**9**) is obtained in good yields in Scheme 7.



Scheme 7

1,5-Dimethyl-4-(2-(3-oxo-1-(piperidin-1-yl)but an-2-ylidene)hydrazinyl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (9)

M.p.90 °C. Yield 78% (yellow powder).

Analysis: $C_{20}H_{27}N_5O_2$ (369.47): calcd. C 65.02%, H 7.37%, N 18.96%. Found C 64.90%, H 7.26%, N 18.84%. IR(cm-$^{-1}$) (KBr): 3199 (NH), 2933 (CH_2 aliphatic), 1670 (C=O), 1601, 1566, 1526 (Ph), 1304, 1167, 873, cm-$^{-1}$. MS (EI, 70 eV): *m/z* (%): 370 (1) $[M+1]^+$, 354 (1) $[M-CH_3)^+$, 339 (1) $[M-2CH_3)^+$, 277 (1) $[M-(Ph+CH_3)]^+$, 147(31), 119 (100), 91(90.76), 98 (16), 77 (37) $[Ph]^+$. ^{1}H NMR (400MHz, CDCIJ, 25 °C, TMS): 1.51 (m, 2H, CH_2 piperidine), 1.62 (m, 4H, 2CH_2 piperidine), 2.42 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 2.70 (t, 4H, 2CH_2 piperidine), 3.27 (s, 6H, 2*N*-CH_3), 3.68 (s, 2H, CH_2), 7.21 (s, 1H, N*H*), 7.51-8.04 (m, 5H, *Ar-H*) ppm.

1- Pharmacological Screening

2-1 Measurement of Potential Cytotoxicity by Sulforhodamine B (SRB) Assay:

The selected derivatives (compounds **1, 2, 3, 4, 5, 6, 7, 8 and 9**), were subjected to a screening system for evaluation of their antitumor activity against liver HEPG2 cancer cell lines in comparison to the known anticancer drugs: 5-FU and DOX. Potential cytotoxicity of the compounds in this study was investigated using the method of Skehan *et al*. [14].

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. Cultures were then fixed with trichloroacetic acid and stained for 30 min with 0.4% (*w*/*v*) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered tris base (tris hydroxymethyl aminomethane, Sigma-Aldrich , Taufkirchen, Germany) for determination of optical density in a computer-interfaced, 96-well micro titer plate reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

2-2 Biochemical Analysis:

Male albino mice weighing 18–20 g were used in the present study. Mice were divided into three main groups as follows: Untreated or control group (5 mice each), the second group is, divided into two subgroups (5 mice for each subgroup) and treated with 5-FU or DOX as reference anticancer drugs and the third group is divided into nine subgroups (5 mice for each subgroup) which was treated with the selected derivatives. In the control group each mouse was given a single intraperiotneal (i.p.) injection of 0.1 mL DMSO while the second and the third groups were given a single i.p. injection of 0.1 mL containing 12 mg/kg body weight of the standard or tested compounds. 5-FU or DOX was dissolved in sterile water and the synthesized compounds were dissolved in DMSO. Blood was collected after 7 days from all mice groups. The biochemical effects of selected compounds which shows antiporoliferative potency, on some liver enzymes such as aspartate, alanine aminotransferases (AST and ALT) [15] and alkaline phosphatase (ALP) [16], were analyzed using a blood auto analyzer (Olympus AV 400, Tokyo, Japan). Moreover, albumin [17], globulins [18], creatinine [19], total lipids [20], cholesterol [21], triglycerides and bilirubin [22] in serum of mice were evaluated in comparison to 5-FU and DOX. Statistical analysis of the results was performed using Chi-square values (SPSS computer program, IBM Corporation , New York , United States).

Results and Discussion

3-1 Reaction of Antipyrine with Aryl Ketones (Compound 2):

The analytical, IR, ^{1}H NMR and mass spectral data are consistent with the structure proposed for compounds 2a – 2f. The main characteristic features of the ^{1}H NMR spectrum of **2**, are a singlet at δ = 1.72 assignable to (CH_3), two singlets at 2.23 (2 x C-Me of antipyrine), 3.05 (2 x N-Me of antipyrine). The mass spectra of **2** showed cleavage at the branched carbon atom with elimination of antipyrine ion leads to the peak at *m/z* = 291 and a very intense peak at *m/z* = 478 (78 %).

3-2 Reaction of antipyrine with heterocyclic ketones (Compound 3):

The analytical, IR, H NMR and mass spectral data are consistent with the structure proposed for compounds **3**. The main characteristic features of the H NMR spectrum of **3**, revealed the presence of a singlet at δ = 1.62 assignable to (CH_3), three singlets at 2.57 (C-Me of antipyrine), 3.48 (N-Me of antipyrine) and 8.81 (NH). The mass spectra of 3 showed cleavage at the branched carbon atom with elimination of antipyrine ion leads to the base peak at *m/z* = 315.

3-3 Reaction of indole with acetylpyrazolones (Compound 4):

It has been reported earlier that the acid-catalyzed condensation of indole with carbonyl compounds led to the formation of diindolyl products [24 - 25]. In the present study, the acid-catalyzed condensation of antipyrine and its related with indole was investigated as a possible route to some new heterocyclic systems containing both indole and pyrazoline moieties such as treatment of 4-acetylpyrine with indole in acetic acid afforded compound **4**. The analytical, IR, H NMR and mass spectral data are consistent with the structure proposed for compound. The main characteristic features of the H NMR spectrum of **4**, revealed the presence of two singlets at δ = 1.66 and 2.23 assignable to (CH_3), two singlet at 3.06 (N-Me of antipyrine) and 7.27 (NH).

3-4 Synthesis of Antipyrinyl Formazans (Compound 5):

The analytical, IR, ^{1}H NMR and mass spectral data are in agreement with the proposed structure of compound **5**. For instance, the ^{1}H NMR spectrum of 67, showed singlets at δ = 7.94 assignable to (CH), 1.33 (CH_3), 2.27 (2 x C-Me of antipyrine), 3.45 (2 x N-Me of antipyrine) and 12.14 (NH). Moreover, the mass spectra of 67 indicated the molecular ion peak at *m/z* = 523 $[M+3]^+$.

3-5 Synthesis of Antipyrinyl 1, 2,4-Triazines (Compounds 6 - 8):

In the presence of formalin and different amines namely; *p*-toluidine, 4-amino-acetophenone and 2-amino-5-mercapto-1,3,4-thiadiazole, the

hydrazone underwent double Mannich reaction to give various antipyrinyl 1, 2,4-Triazines derivatives (**6, 7 and 8**). The analytical, IR, ^{1}H NMR and mass spectral data are consistent with the structure proposed for compounds **6 - 8**.

3-6 Synthesis of Mannich bases (Compound 9):
In the present investigation, preforming Mannich reaction of the hydrazone compound 69 with secondary amines such as piperidine and piperazine, compound **9** were obtained in good yields. The mass spectral data of compound **9** include peaks of their molecular ions, and fragmentation patterns which supported their structures. The mass spectrum of **9** showed a molecular peak at *m/z* = 372 $[M+3]^+$, the basic side chain can be identified by two peaks at *m/z* = 84 (11 %) for piperidine ion, and 98 (16 %) due to *N*-piperidinomethyl ion, which undergo further fragmentation to give the peak at *m/z* = 57 (4 %).

4-Pharmacological Screening of the selected derivatives

4-1 ***Antiporoliferative potency***

It is well known that chemotherapy aims to destroy the cancer cells with various types of chemicals. The substances used are supposed to target mainly the cancer cells and doses are calculated to minimize the collateral damage to surrounding tissues, which nevertheless occurs [23]. This kind of treatment increases the entropy of the organism, suppresses the immune system, and forms a toxic cell environment which may destroy surrounding healthy cells [24], so it is important to minimize curing doses to the least amount possible to minimize the side effects of these drugs. The antiproliferative activity of the selected new compounds **1-9** were assessed against the HEPG2 cancer cell line in comparison to the traditional anticancer drugs: 5-flurouracil (5-FU) and doxorubicin (DOX). Regarding the antitumor activity results, all of the selected compounds showed reasonable antitumor activity in comparison to 5-FU and DOX in concentration ranging from 3.92–9.38 µg/mL. Table 1 shows the cytotoxic activity of the selected newly synthesized derivatives, where compounds **9, 7** and **6** were the most active derivatives with IC_{50} equals 3.74, 3.92 and 4.31 µg/mL respectively.

Table 1. The antiproliferative effects of different pyrazolines derivatives against hepatic carcinoma tumor cell line (HEPG2).

Compound	IC_{50} (µg mL^{-1})
5-Flurouracil	5.00
Doxorubicin	3.56
2	6.59
3	7.43
4	6.53
5	9.38
6	4.31
7	3.92
8	8.48
9	3.74

4-2 ***Induced Biochemical Parameters***

The biochemical effects of the selected compounds which shows antiporoliferative potency, on some enzymes such as alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatase (ALP), in addition to total lipids, cholesterol, triglycerides, bilirubin, albumin, globulin and creatinine in serum of mice were investigated. The study of the induced biochemical parameters of most of the tested compounds in mice showed insignificant differences relative to the control group which indicates a moderate safety margin for the selected compounds as shown in Tables 2–4.

Table 2. Biochemical effects of treatment with 5-flurouracil (5-FU), doxorubicin (DOX), and the different pyrazolines derivatives on serum ALT, AST, and ALP in mice.

Compounds	ALT (IU/mL)	AST (IU/mL)	ALP (k.k./dL)
2	60.80 ± 9.20 *	157.28 ± 20.30 *	43.25 ± 7.08 *
3	39.10 ± 8.40 ***	126.20 ± 12.10 **	18.76 ± 6.43 ***
4	38.53 ± 6.50 ***	111.59 ± 12.80 **	19.99 ± 4.39 ***
5	45.51 ± 4.23 ***	108.66 ± 4.61 ***	21.77 ± 3.50 **
6	48.07 ± 6.16 ***	112.09 ± 8.83 ***	17.79 ± 3.06 ***
7	54.2 ± 11.05 *	146.50 ± 28.90 *	46.47 ± 10.87 *
8	51.88 ± 11.50 **	113.00 ± 9.52 **	21.09 ± 3.48 **
9	67.39 ± 11.00 *	146.40 ± 28.10 *	36.90 ± 9.80 *
Control	43.50 ± 2.03	108.32 ± 4.19	17.70 ± 1.10
5-Flurouracil	51.47 ± 9.02 *	130.43 ± 8.92 *	25.49 + 6.03 *
Doxorubicin	59.26 ± 12.03 *	147.23 ± 16.34 *	30.32 ± 5.14 *

* $p < 0.001$: Highly significant; ** $p < 0.01$: Significant; *** n.s.: Non significant; ALT: Alanine amino transferase; AST: Aspartate amino transferase; and ALP: Alkaline phosphatase.

Table 3. Biochemical effects of treatment with 5-FU, DOX, and different pyrazolines derivatives on total lipids, cholesterol, triglycerides, and bilirubin in mice.

Compounds	Total Lipids (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Bilirubin (mg/dL)
2	379.20 ± 37.80 *	127.50 ± 25.10 *	136.10 ± 27.09 *	0.97 ± 0.05 *
3	329.60 ± 14.40 ***	97.40 ± 18.60 ***	114.90 ± 10.70 ***	0.64 ± 0.02 ***
4	336.40 ± 19.10 ***	97.20 ± 9.90 ***	117.90 ± 18.40 **	0.67 ± 0.03 ***
5	313.70 ± 31.20 ***	94.80 ± 18.60 ***	117.31 ± 21.60 ***	0.55 ± 0.06 **
6	328.50 ± 22.70 ***	96.39 ± 17.10 ***	115.51 ± 18.10 ***	0.54 ± 0.05 **
7	374.60 ± 36.80 *	111.40 ± 16.50 **	97.40 ± 9.60 ***	1.09 ± 0.60 *
8	369.30 ± 26.30 *	109.90 ± 18.60 **	114.20 ± 18.40 **	0.73 ± 0.16 **
9	321.70 ± 18.90 ***	92.80 ± 14.30 ***	115.40 ± 8.70 ***	0.63 ± 0.05 ***
Control	323.41 ± 27.10	94.32 ± 13.50	108.70 ± 16.80	0.63 ± 0.04
5-Flurouracil	378.20 ± 31.40 *	105.90 ± 11.70 *	126.50 ± 19.40 *	0.75 ± 0.10 *
Doxorubicin	366.70 ± 6.10 *	109.30 ± 14.20 *	137.80 ± 17.10 *	0.81 ± 0.19 *

* $p < 0.001$: Highly significant; ** $p < 0.01$: Significant; and *** n.s.: Non significant.

Table 4. Biochemical effects of treatment with 5-FU, DOX, and different pyrazolines derivatives on serum albumin, globulin and creatinine in mice.

Biochemical Parameters	Albumin (mg/dL)	Globulin (mg/dL)	A/G Ratio	Creatinine (mg/dL)
2	6.43 ± 0.44 **	6.46 ± 0.8 **	1.001 *	0.73 ± 0.06 **
3	5.92 ± 0.86 ***	4.73 ± 0.87 ***	1.15 ***	0.85 ± 0.08 **
4	5.96 ± 0.4 ***	4.3 ± 0.69 ***	1.46 ***	0.62 ± 0.08 ***
5	5.95 ± 0.78 ***	5.16 ± 0.7 ***	1.15 ***	0.72 ± 0.08 ***
6	5.53 ± 0.71 ***	4.88 ± 1.01 ***	1.13 ***	0.68 ± 0.04 ***
7	6.81 ± 0.47 **	6.79 ± 0.7 **	1.02 *	1.62 ± 0.07 *
8	7.4 ± 0.59 **	6.65 ± 0.81 **	1.006 *	0.8 ± 0.09 **
9	5.65 ± 0.69 ***	4.67 ± 1.09 ***	1.13 ***	0.66 ± 0.07 ***
Control	5.63 ± 0.51	4.32 ± 0.9	1.3	0.69 ± 0.03
5-FU	6.49 ± 0.92 **	5.75 ± 0.8 **	1.13 **	0.81 ± 0.06 **
DOX	6.37 ± 0.85 **	5.91 ± 0.63 **	1.078 **	0.78 ± 0.04 **

* $p < 0.001$: Highly significant; ** $p < 0.01$: Significant; and *** n.s.: Non significant.

Data in Table 2 present the liver enzymatic activities (ALT, AST and ALP) in serum of control and treated groups of mice. The results showed that the values recorded for AST and ALT were significantly higher ($p < 0.001$) with 5-FU and DOX treated groups of mice than the control. On the other hand, treatment with the new compounds tested, caused inverse effects, where some values recorded for AST and ALT were non significant (n.s.) or slightly higher ($p < 0.01$) in comparison to control. Moreover, the recorded data showed that ALP activities were significantly increased ($p < 0.001$) with the treatment with 5-FU and DOX, while there were no significant changes in ALP activities upon treatment with some of the compounds.

Data listed in Table 3 demonstrate the comparison between the levels of total lipids, cholesterol, triglycerides and bilirubin in serum of treated mice and the control group. It can be deduced from the present data that 5-FU and DOX caused a significant increase in the level of these parameters while treatment with the new derivatives tested showed moderate or no significant changes.

Table 4 represents a comparison between the levels of albumin, globulins and creatinine in serum of control and treated groups of mice. It is clear from these results that there was a slight increase in the level of albumin and creatinine and globulins in the 5-FU and DOX treated groups of mice while there were moderate or non significant changes in the other treated groups.

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Conclusion

Based on the present findings in the present work, some of the newly synthesized pyrazolines derivatives would have better biological activity as anti-proliferative agents with less toxic side effects. However, these potent compounds needs more pharmacological and preclinical tests.

Conflicts of interest: There are no conflicts to declare.

References

[1] Zhao Z., Dai X., Li C.; *et al*., Pyrazolone structural motif in medicinal chemistry: Retrospect and prospect. *Eur J Med Chem.* **15** (186), 111893 (2020).

[2] Pathade S.S., Adole V.A. and Jagdale B.S., PEG-400 mediated synthesis, computational, antibacterial and antifungal studies of fluorinated pyrazolines. *Current Research in Green and Sustainable Chemistry*, **4**, 100172 (2021).

[3] Patil A., Jadhav R., Raundal H., Bobade V., Sharma L. and Badgujar R., Synergistic antifungal activity of pyrazole thio-oxadiazole derivatives. *Indian J. Chem*., **54B**, 1128-1133 (2015).

[4] Revanasiddappa B., Kumar, M. V. and Kumar, H., Synthesis and Antidepressant Activity of Pyrazoline Derivatives. *Dhaka University Journal of Pharmaceutical Sciences*, 19(2), 179–184 (2020).

[5] Gao M., Qu K., Zhang W. and Wang X., Pharmacological Activity of Pyrazole Derivatives as an Anticonvulsant for Benefit against Epilepsy. *Neuroimmunomodulation*, **28**, 90-98 (2021).

[6] Mantzanidou M., E. Pontiki E. and Hadjipavlou-Litina D., Pyrazoles and Pyrazolines as Anti-Inflammatory Agents. *Molecules*, **26**(11):3439 (2021).

[7] Rajeena C.H.A., Kamat V., Patil V.B. *et al*., Synthesis and antibacterial evaluation of pyrazolines carrying (benzyloxy)benzaldehyde moiety. *J. IRAN CHEM SOC* (2021). https://doi.org/10.1007/s13738-021-02403-9.

[8] Farahi M., Karami B., Z. Banaki Z., Rastgoo F. and Eskandari K., TSA-catalyzed regioselective synthesis of medicinally important 4-aryl-substituted dihydropyrimidine derivatives fused to pyrazole and triazole scaffolds via an efficient and green domino reaction, *Monatsh Chem*., **148**, 1469-1475 (2017).

[9] Li Q.S., Shen B.N., Zhang Z., Luo S. and Ruan B.F., Discovery of Anticancer Agents from 2-Pyrazoline-Based Compounds. *Curr Med Chem*. **28**(5), 940-962 (2021).

[10] Rana M., Arif R., Khan F. I., *et al*., Pyrazoline analogs as potential anticancer agents and their apoptosis, molecular docking, MD simulation, DNA binding and antioxidant studies,
Bioorganic Chemistry., **108**, 104665 **(2021)**.

[11] Lugassy C., Vermeulen P.B., Ribatti D. *et al*., Vessel co-option and angiotropic extravascular migratory metastasis: a continuum of tumour growth and spread?. *Br J Cancer* . https://doi.org/10.1038/s41416-021-01686-2, (2022).

[12] Okasha H., Nasr S.M. and Samir S., Recombinant Expression of Cec-B Peptide in Escherichia coli with a Significant Anticancer Effect on Hepatocellular Carcinoma, *Current Pharmaceutical Biotechnology*, **22**(9),1235-1245 (2021).

[13] Rashed W.M., Kandeil M.A.M., Mahmoud M.O. and Ezzat S., Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview, *Journal of the Egyptian National Cancer Institute* **32**(1), 1-11 (2020).

[14] Skehan, P.; Storeng, R., Scudiero, D., *et al*., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst*., **829**, 1107–1112 (1990).

[15] Ahur V.M., Agada P.O. **and** Saganuwan S.A**., Estimating the Plasma and Serum Activity Levels of Aspartate Aminotransferase and Alanine Aminotransferase, in Live Animals Using Regression Model.** *Trends in Applied Sciences Research*, **7**, 748-757 (2012).

[16] Kumar D. and Banerjee D., Methods of albumin estimation in clinical biochemistry: Past, present, and future. *Clin Chm Acta*., **469**, 150-160 (2017).

[17] Pandey A. S., Pandey A., Shreevastava N. K a Neupane D. P., Experiment-07 Estimation of

Serum Total Protein and Determination of Albumin to Globulin Ratio, In book: *Biochemistry Laboratory Manual*., pages: 4. DOI: 10.5005/jp/books/12493_8 (2015).

[18] Lala V., Goyal A., Bansal P., *et al*., Liver Function Tests. [Updated 2021 Aug 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482489, (2021).

[19] Bernert J.T., Turner W. E., Patterson D. G. and Needham L. L., Calculation of serum "total lipid" concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples, *Chemosphere*., **68**, 824-831 (2007).

[20] Syal K., Banerjee D. and Srinivasan A., Creatinine estimation and interference. *Indian J Clin Biochem*. **28**(2), 210-211 (2013).

[21] Bariyah S., Hamid S. and Omar I., Determination of Total Cholesterol in Serum by Cholesterol Oxidase and Cholesterol Esterase Co-immobilized on to Cellulose Acetate Membrane. *Pakistan Journal of Biological Sciences*, **6**, 1340-1344 (2003).

[22] Sullivan D. R., Kruijswijk Z., West C. E., Kohlmeier M. and Katan M. B., Determination of serum triglycerides by an accurate enzymatic method not affected by free glycerol., *Clinical Chemistry*., **31**, 1227–1228 (1985).

[23] Kumar V. and Gill K. D., To Estimate Total and Direct Bilirubin in Serum",in: "Basic Concepts in Clinical Biochemistry: A Practical Guide", ,"Springer Singapore", pages: 97-101 (2018).

[24] Wati F.A., Santoso M., Moussa Z., Fatmawati S., Fadlana A. Judeh M. A., Chemistry of trisindolines: natural occurrence, synthesis and bioactivity. *RSC Adv.*, , **11**, 25381-25421 (2021).

[25] Stockert J.C., Horobin R.W., Colombo L.L. and Blázquez-Castro A., Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. *Acta Histochem*., **120**(3),159-167 (2018).