

Anti-tumor and anti-oxidant activities of some Novel Synthetic Pyrazoline Derivatives

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ABSTRACT

Background: pyrazolines are used widely in the current decades due to their various biological and pharmacological activities **Aim:** The main objective of the present study is to evaluate the chemotherapeutic effects of some novel substituted pyrazoline compounds against animal carcinogenesis. **Materials & Methods:** the synthesized Pyrazoline derivatives were characterized by IR spectroscopy, and then their effects on Ehrlich Carcinoma were studied by evaluation their antitumor activity (viability of tumor cells, and life span prolongation), and estimation of antioxidants (catalase, superoxide dismutase, glutathione peroxidase activities, glutathione reduced content, and total antioxidant capacity). **Results:** Pyrazoline derivatives showed a significant reduction in the volume and count of Ehrlich cells. Also, these compounds suggested potential antioxidant activity by elevation the catalase, superoxide dismutase, glutathione peroxidase activities, and glutathione reduced content. **Conclusion:** the synthesized compounds have potent antitumor, antioxidant, and decrease the survival of cancer cells.

INTRODUCTION

Pyrazolines are well-known important nitrogen containing five membered heterocyclic bioorganic molecules. These heterocyclic compounds widely occur in nature in the form of alkaloids, vitamins, and pigment sand as constituents of plant and animal cell ⁽¹⁾. These pyrazolines are used widely in the current decades due to their various biological and pharmacological activities ⁽²⁾. 2-Pyrazolines seem to be the most frequently studied pyrazoline type compounds ⁽³⁾.

C5 atom is deviated from the almost planar system of the other four atoms of the heterocyclic ring ⁽⁴⁾. It plays a crucial role in the development of theory in heterocyclic chemistry and is also extensively used as useful synthons in organic synthesis ⁽⁵⁾. 2-pyrazoline is insoluble in water but soluble in propylene glycol because of its lipophilic character ⁽⁶⁾. Pyrazoline derivatives,

typical Intra-molecular Charge Transfer (ICT) compounds, an intra-molecular conjugated charge transfer process has been reported to exist in it in the excited state. In the conjugated part (–N1–N2–C3–) of the ring, the nitrogen atom at the 1-position and the carbon atom at the 3-position are respectively, electron donating and withdrawing moieties. The carbon atoms at 4-and 5-positions do not conjugate with the above conjugated part ⁽⁷⁾.

An especially popular procedure is based on the reaction of α , β -un saturated aldehydes and ketones with hydrazines ⁽⁸⁾. The combination of the hydrazono group with other functional groups leads to compounds with unique physical and chemical properties. Pyrazoline derivatives display various biological activities such as antitumor, antifungal, antiviral, anti-parasitic, anti-inflammatory and analgesic, anti-

mycobacterial, anticancer, antibacterial, insecticidal, anti-nociceptive, hypotensive, antidepressant, photoluminescence, anti-tubercular, anti-amoebic, amine oxidase inhibitory and antioxidant properties⁽⁹⁾.

This work aims to investigate the antitumor and antioxidant activities of some novel pyrazoline derivatives.

Materials and Methods:

Synthesis of Pyrazolines

Pyrazoline derivatives were synthesized according to scheme (1)⁽¹⁰⁾. The chalcones were condensed with phenylhydrazine in absolute ethanol in the presence of pyridine at reflux temperature (2 to 6 h). The solvent was completely evaporated and the residue was poured into ice cold water, which resulted in the formation of the corresponding 2-pyrazolines. Reaction completion was identified by thin layer chromatography (TLC) using silica gel-G. After completion of the reaction, the reaction mixture was poured into crushed ice with constant stirring. The separated solid was filtered and dried. It was purified by column chromatography on silica gel, using ethyl acetate and hexane mixture as the mobile phase. After purification the 2-pyrazolines were obtained as light or bright colored powders.

Elucidation of Chemical structure of Pyrazoline derivatives: the structure of the synthesized compounds illustrated in fig. (1). [compound 1: Phenyl-3methyl-4-(o-chlorophenyl(azo)hydrazono)-2-pyrazoline -5-one, compound 2: Phenyl-3methyl-4-(m-chlorophenyl(azo)hydrazono)-2-pyrazoline -5-one, compound 3: Phenyl-3methyl-4-(m-nitrophenyl(azo)hydrazono)-2-pyrazoline -5-one, and compound 4: Phenyl-3methyl-4-(o-methylphenyl(azo)hydrazono)-2-pyrazoline -5-one] were identified using IR according to **Laurence and Christopher** method⁽¹¹⁾.

Animals

Adult female Swiss albino mice weigh (20-25 g) were purchased Abo Rawash culture – Giza used throughout this study. The animals were housed in steel mesh cages (animal house, faculty of Science, Zagazig University) and maintained in controlled environment of temperature, humidity, light, and fed on a commercial standard diet and tap water *ad libitum*.

Ehrlich ascites carcinoma (EAC)

EAC cells were initially supplied from the National Cancer Institute, Cairo, Egypt (only for the first transplantation), and maintained in female Swiss albino mice through serial intraperitoneal (I.P.) inoculation at 8 or 10 day intervals in our laboratory in an ascites form.

Dose response curve

Dose response curve of compounds 1, 2, 3, and 4 in mice was determined according to method of Crump et al.,⁽¹²⁾.

Experimental design

Female Swiss albino mice were divided into the following groups (10 mice/ each group) as follows: *Group (I):* Negative Control: mice injected I.P. with sterile saline for 10 days (day after day). *Group (II):* Positive Control (EAC bearing group): mice injected with EAC cells, (2×10^6 cells/ 0.3 ml/mouse) by I.P. injection once. *Group (III) compound (1) treated group:* mice were injected I.P. with compound 1 (2.5 mg/Kg) after EAC injection (2×10^6 cells/mouse) for 10 days (day after day). *Group (IV) compound (2) treated group:* mice were injected I.P. with compound 2 (10 mg/Kg) after EAC injection (2×10^6 cells/mouse) for 10 days (day after day). *Group (V) compound (3) treated group:* mice were injected I.P. with compound 3 (2.5 mg/Kg) after EAC injection (2×10^6 cells/mouse) for 10 days (day after day). *Group (VI) compound (4) treated group:* mice were injected I.P. with compound 4 (5 mg/Kg) after EAC injection (2×10^6 cells/mouse) for 10 days (day after day).

Collection of Blood Samples and EAC Cells:

At the end of the experiment, the blood samples were collected from the retro-orbital venous plexus under light ether anesthesia. Plasma and serum were prepared and stored at -20 °C until biochemical analysis. EAC cells were harvest from each mouse in centrifuge tube containing heparinized saline. Note the volume of ascetic fluid in each mouse in each group. Each sample of cells were undergoes counting and viability of EAC cells in each group.

Viability and Counting of EAC cells

The viability of EAC cells was determined by the Trypan Blue Exclusion Method described by McLiman et al.,⁽¹³⁾.

Effect of compounds (1, 2, 3, 4, and 5) on life span prolongation:

Life span calculation was carried out according to the method described by Mazumdar *et al.*,⁽¹⁴⁾.

Antioxidant assays:

Catalase activity (CAT), Superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, Reduced glutathione (GSH) content, and Total Antioxidant Capacity (TAC) level were determined according to method of Aebi,⁽¹⁵⁾; Nishikimi *et al.*,⁽¹⁶⁾; Paglia, and Valentine⁽¹⁷⁾; Beutler *et al.*,⁽¹⁸⁾; Koracevic *et al.*,⁽¹⁹⁾; respectively.

Statistical Analysis:

All statistical analyses were done by a statistical for social science package "SPSS" 14.0 for Microsoft Windows, SPSS Inc. (Levesque,⁽²⁰⁾ and considered statistically significant at a two-sided $P < 0.05$.

Results

Infrared spectral study:

Fig. (2) illustrated IR spectra of compounds (1, 2, 3, and 4; respectively). In compound (1) the absorption bands at 1252, 3447 and 1496 for OCH₃, NH and N=N respectively. N=C, C-O and C=O groups give bands at 2366, 1152 and 1655. C-H aromatic and CH₃ aliphatic appear at 3030 and 2922. The characteristic bands of compound (1) were still present in compounds (2 to 4) except the disappear of band character of OCH₃ at 1252, and showed three new peaks at 1556, 1072, 1047 attributed to NO₂, Cl in meta position and Cl at ortho positions respectively.

Dose response curve:

The most effective doses were found to be "2.5 mg/kg", "10 mg/kg", "15 mg/kg" and "5 mg/kg" for compounds 1, 2, 3, and 4; respectively (Fig.3).

Viability and life span prolongation

The mean values of EAC volume and count were found to be 4.96 ± 0.185 (ml) and 208.4 ± 3.97 ($\times 10^6$ cells/ml) in EAC bearing tumor group. While, cpd1, cpd2, cpd3 and cpd4 treated groups were demonstrated a significant decrease in EAC volume to 1.43 ± 0.07 , 0.45 ± 0.02 , 3.7 ± 0.14 & 2.55 ± 0.11 by 71.1%, 90.9%, 25.4%, & 48.6% and significant reduction in EAC cells count to 64.3 ± 3.22 , 21.0 ± 0.71 , 124.2 ± 1.55 , & 93.40 ± 1.96 by 69.1%, 89.9%, 40.4%, & 55.1%; respectively compared to positive control group (EAC bearing tumor), ($p < 0.001$), table (1).

The mean life span in the positive control group was found to be 16 days. cpd1, cpd2, cpd3 and cpd4 treated groups showed a significant increase in the life span prolongation to 23 days by 43.75% (T/C ratio= 143.75%), 29 days by 81.25% (T/C ratio= 181.25%), 21 days by 31.25% (T/C ratio = 131.25%), and 25 days by 56.25% (T/C ratio= 156.25%); respectively compared to the positive control group, table (2).

Antioxidant assay:

CAT, SOD and GPx activities were found to be reduced significantly from 235.9 ± 4.05 (U/ml), 210.4 ± 2.48 (U/L), and 202.4 ± 1.34 (mU/ml) in negative control group to 146.3 ± 1.78 , 100.94 ± 1.76 , and 99.6 ± 1.4 in EAC bearing tumor group by 37.98%, 52%, and 50.8%; respectively, ($p < 0.001$). Meanwhile, in cpd1, cpd2, cpd3 and cpd4 treated groups; their activities were significantly increased to (496.3 ± 6.33 , 849.12 ± 28.3 , 207.6 ± 4.6 , and 374 ± 5.8), to (661.6 ± 21.7 , 1089.1 ± 48.8 , 199.2 ± 1.57 , 369.7 ± 10.45) & to (523.1 ± 16.94 , 724.6 ± 11.26 , 191.4 ± 3.32 , 327.8 ± 8.5) by (98.9%, 259.%, 11.99%; and 58.5%), by (214.4%, 417.6%, 5.3%, and 75.7%) & by (158.4%, 258%, 5.6% & 61.9%); respectively, ($p < 0.001$) compared to EAC group. On the other hand; GSH content was significantly decreased to be 4.19 ± 0.11 (nmol/L) by 71.4% in EAC bearing tumor group, ($p < 0.001$). While in cpd1, cpd2, cpd3 and cpd4 treated groups, this content was significantly elevated to 17.5 ± 0.19 by 19%, 26.9 ± 1.21 by 82.9%, 7.9 ± 0.19 by 46.2%, and to 13.1 ± 0.45 by 10.88% respectively; ($p < 0.001$).

TAC concentration was found to be decreased from 0.42 ± 0.017 in negative control group to 0.155 ± 0.016 by 64.2% in EAC bearing tumor group. In cpd1, cpd2, cpd3 and cpd4 treated groups TAC concentration showed significant increased compared to 2.27 ± 0.09 , 4.47 ± 0.15 , 0.203 ± 0.007 , and 1.25 ± 0.114 , by 440.4%, 964.2%, 51.6%, and 197.6%; respectively, compared to EAC bearing tumor group ($p < 0.001$), table (3).

Discussion

Cancer is a group of diseases that cause cells in the body to change and grow out of control⁽²¹⁾. Pyrazolines have been exhibiting various pharmacological activities, such as analgesic⁽²²⁾, anti-inflammatory⁽²³⁾, antimicrobial⁽²⁴⁾, anti-

amoebic⁽²⁵⁾, antitubercular⁽²⁶⁾, hypoglycemic⁽²⁷⁾, anticoagulant⁽²⁸⁾, antidepressant⁽²⁹⁾, pesticide⁽³⁰⁾, fungicide⁽³¹⁾, antibacterial⁽³²⁾, and anticonvulsant activities⁽³³⁾. Recent report shows some new pyrazoline-substituted thiazolone-based compounds that exhibit anticancer activity⁽³⁴⁾. pyrazolines are also extensively used as synthons in organic synthesis⁽³⁵⁾, as optical brightening agents for textiles, paper, and fabrics, and as a hole-conveying medium in photoconductive materials⁽³⁶⁾. On a hand, our tested compounds (Pyrazoline derivatives) reduced the volume and count of cancer cells, and increased the life span of mice. On the other hand, these compounds have anti-oxidant activity by increasing the CAT, SOD, and GPx activities. Compound 2 [Phenyl-3methyl-4-(m-chlorophenyl(azo)hydrazono)-2-pyrazoline -5-one] showed the greatest anti-tumor and anti-oxidant activities than other tested compounds. Compounds with electron withdrawing groups (halogens) on the aromatic ring favor antitumor, analgesic, and anti-inflammatory activities⁽³⁷⁾. Also, 1, 2- pyrazoline nitrogen mustards with a phenyl and hydroxyphenyl group at second and fifth position was found to be effective in the inhibition of cancer growth⁽³⁸⁾.

These compounds exhibited antioxidant activity, this is due to the substitution in the aromatic ring system with halogens like chlorine sharply enhanced the antioxidant potency, as chlorine atom has lone pair electron as well as its electronegative power enhanced the formation and subsequent stabilization of the nitrogen-ring radical through intervening aromatic system property, it might have enhanced the power to absorb free radicals, especially reactive oxygen and reactive nitrogen species (ROS and RNS)⁽³⁹⁾. Also, Compounds with a methyl substituent at the para position of the phenyl ring showed good activity; while compounds with a nitro substituent at the para and meta positions, respectively, exhibited moderate activity⁽⁴⁰⁾. Erhan Palaska et al.,⁽⁴¹⁾ reported that 4-chloro and 4-methoxy substituents on the phenyl ring at position 3 of the pyrazoline ring (Meta position) increased the antitumor activity; the replacement of these groups by Nitro and methyl substituents decreased activity in mice. In general, (substitution by a halogen atom particularly, a

chlorine atom) in the 'meta' position of the phenyl ring led to a better anti-tumor effect than that observed in the absence of any substituent⁽⁴²⁾. These findings were in a line with many authors, *Alka et al.*,⁽⁴³⁾ who, reported that Pyrazoline ring containing compounds with alkylating groups can act as antitumor agents, further alkoxy groups and halogen atoms as substituent groups on the aromatic ring of the molecules can show significant anti-cancerous activity. *Metwally et al.*,⁽⁴⁴⁾ achieved pyrazolotriazines derivatives by refluxing aryl hydrazone derivatives in acetic acid investigated their cytotoxic activity against different cell lines (HepG2, WI 38, VERO and MCF-7) and antioxidant activity.

Conclusion:

Pyrazoline derivatives showed anticancer and anti-oxidant activities. Compound (2) Phenyl-3methyl-4-(m-chlorophenyl(azo)hydrazono)-2-pyrazoline -5-one has a strong effect of the synthesized compounds, as its halogen substitution in the meta position.

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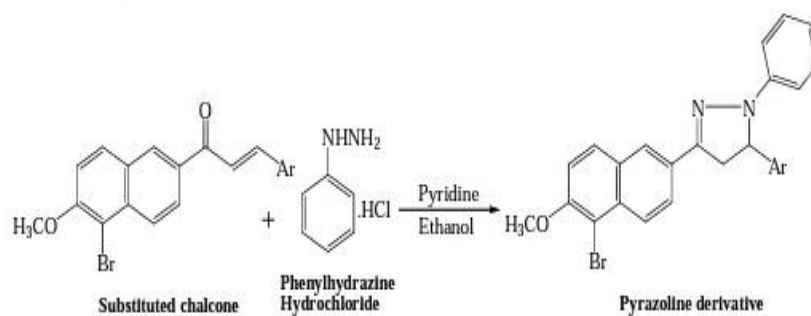
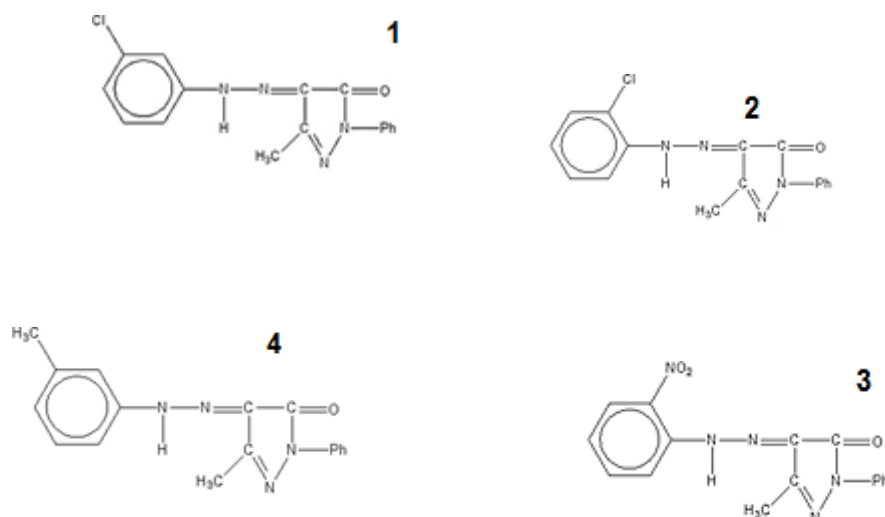
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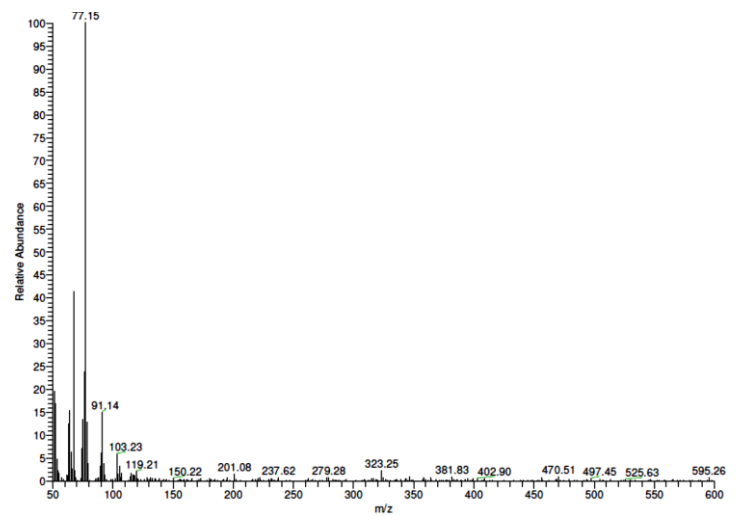
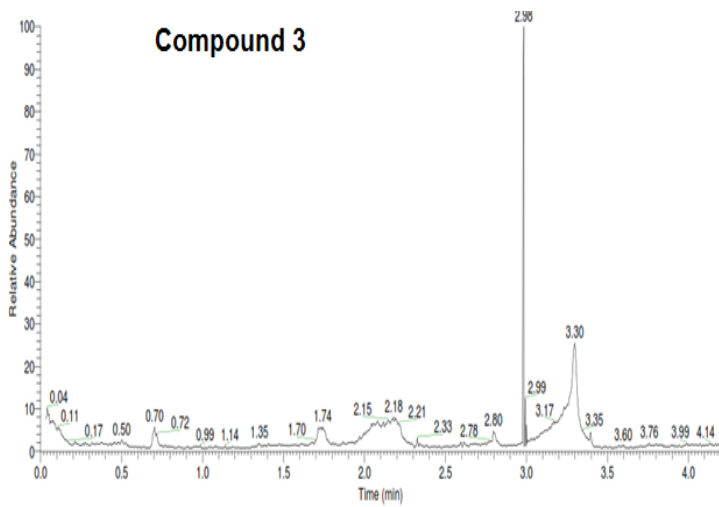
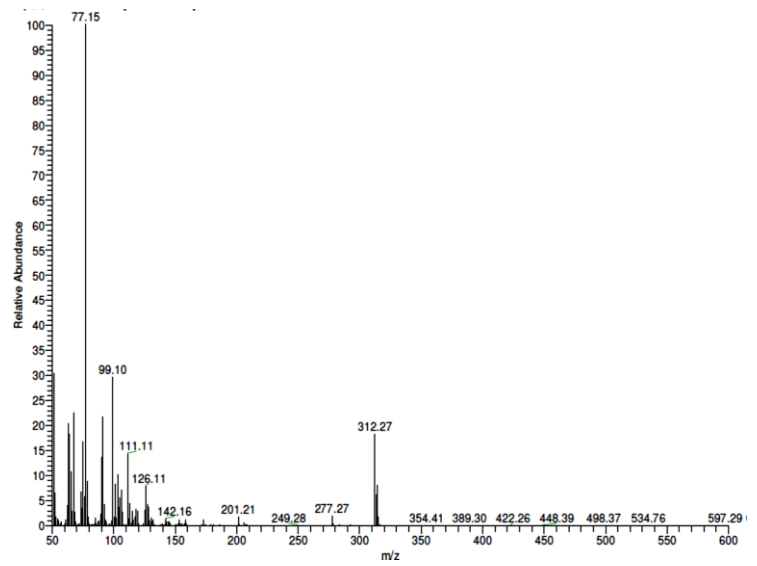
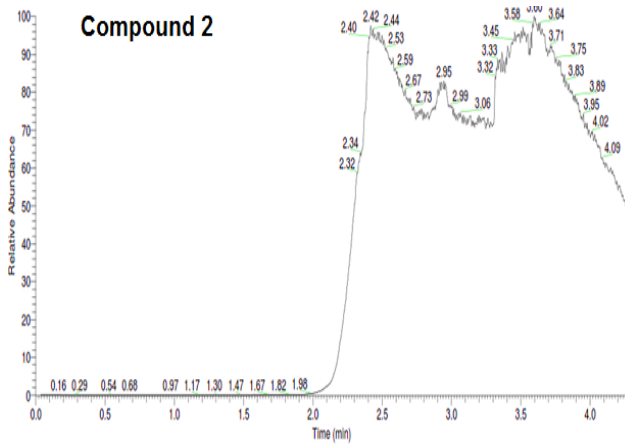
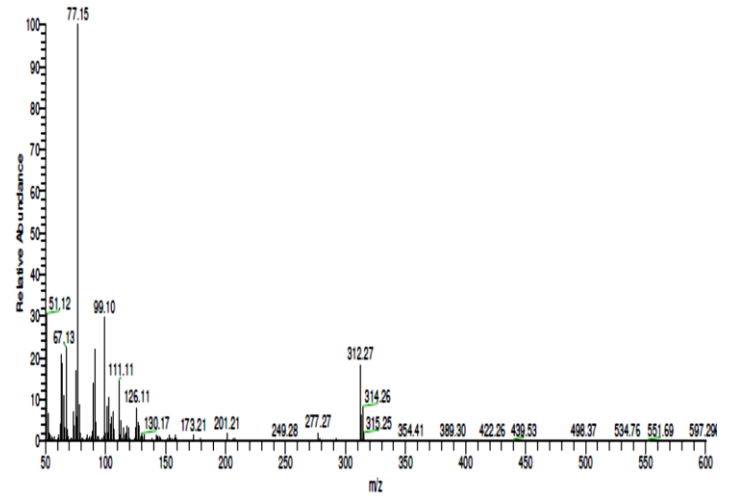
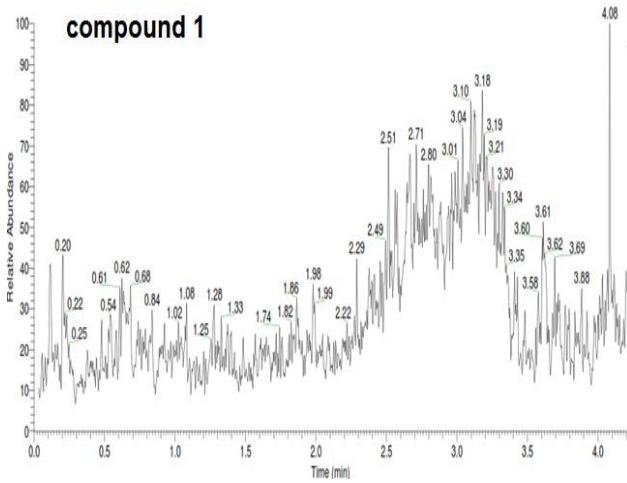
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REACTION:**Scheme (1): synthesis of pyrazoline derivatives**

- 1) Phenyl-3methyl-4-(o-chlorophenyl(azo)hydrazono)-2-pyrazoline -5-one
- 2) Phenyl-3methyl-4-(m-chlorophenyl(azo)hydrazono)-2-pyrazoline -5-on
- 3) Phenyl-3methyl-4-(m-nitrophenyl(azo)hydrazono)-2-pyrazoline -5-one
- 4) Phenyl-3methyl-4-(o-methylphenyl(azo)hydrazono)-2-pyrazoline -5-one

Fig. (1): Structure of synthesized pyrazoline derivatives



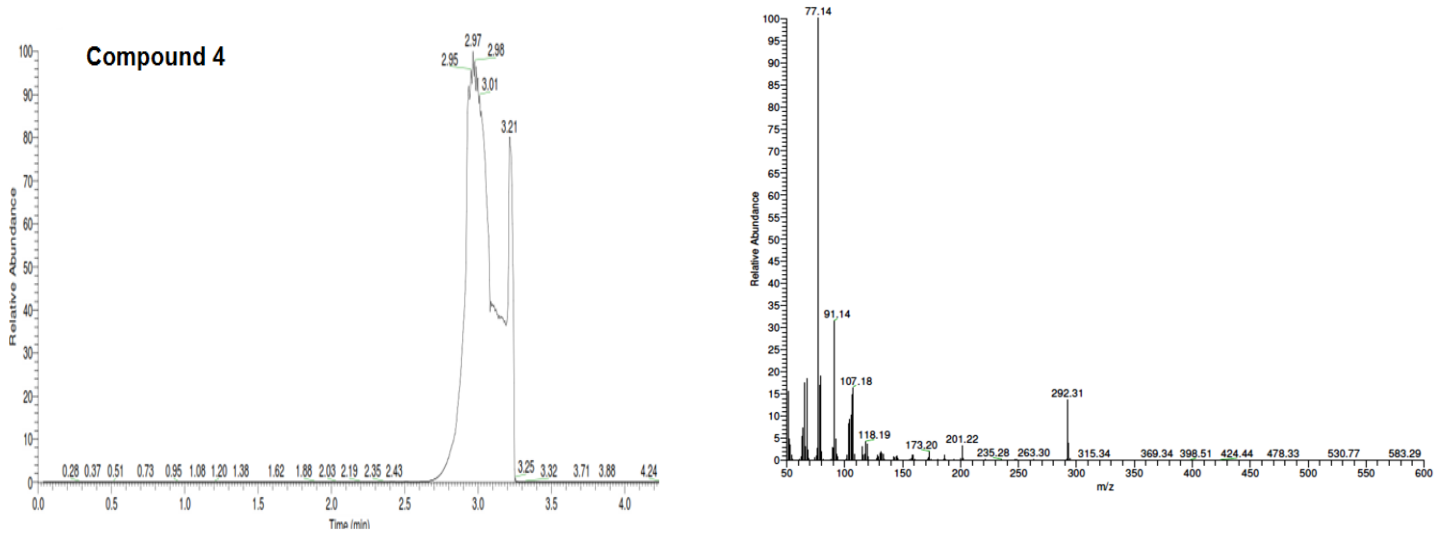


Fig. (2): IR spectra of synthesized pyrazoline derivatives

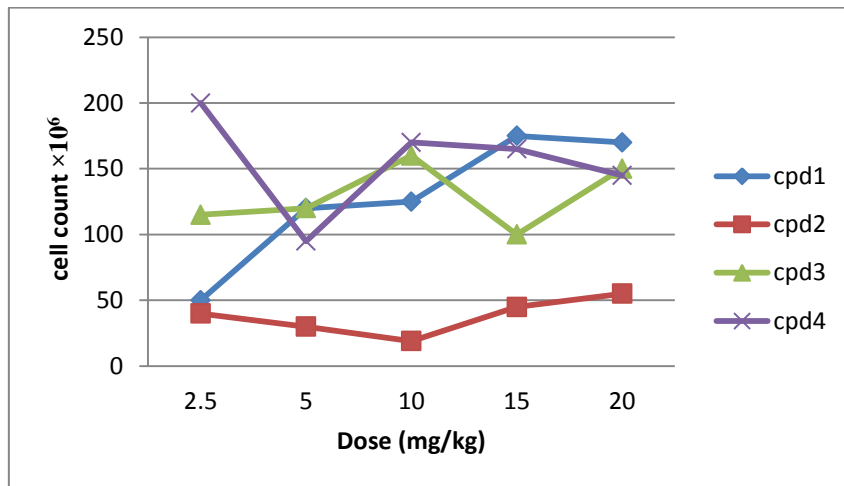


Fig. (3): Dose response curve for compounds of study

Table (1): Effect of pyrazoline derivatives compounds (1, 2, 3, and 4) on life span prolongation

Group	Positive Control Group	Compound (1) Treated Group	Compound (2) Treated Group	Compound (3) Treated Group	Compound (4) Treated Group
Days	16	23	29	21	25
% Change	-----	43.75	81.25	31.25	56.25

Table (2): Effect of compounds on Non-enzymatic and Enzymatic anti-oxidants in all studied group

Variables	Negative control group		EAC bearing tumor		Treated Cpd1		Treated Cpd2		Treated Cpd3		Treated Cpd4	
	Mean± SD.	% Change	Mean± SD.	% Change	Mean± SD.	% Change	Mean± SD.	% Change	Mean± SD.	% Change	Mean ± SD.	% Change
CAT (U/L)	235.9±4.05	-----	146.3±1.78	-37.98%	496.3±6.33	98.9%	849.12±28.3	259.9%	207.6±4.6	-11.99%	374±5.8	58.5%
SOD (U/mL)	210.4±2.48	-----	100.94±1.76	-52%	661.6±21.7	214.4%	1089.1±48.8	417.6%	199.2±1.57	-5.3%	369.7±10.45	75.7%
GSH (nmol/L)	14.7±0.14	-----	4.19±0.11	-71.4%	17.5±0.19	19%	26.9±1.21	82.9%	7.9±0.19	-46.2%	13.1±0.45	-10.88%
GPx (mU/ml)	202.4±1.34	-----	99.6±1.4	-50.8%	523.1±16.94	158.4%	724.6±11.26	258%	191.4±3.32	-5.6%	327.8±8.5	61.9%
TAC (mM/L)	0.42±0.017	-----	0.155±0.016	-64.2%	2.27±0.09	440.4%	4.47±0.15	964.2%	0.203±0.007	-51.6%	1.25±0.114	197.6%

Significance at *P < 0.05, **P < 0.01, ***P < 0.001