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Regioselective synthesis and antitumor screening of some novel *N*-phenylpyrazole derivatives

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Abstract—The versatile, *hitherto* unreported 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) was prepared via the reaction of 2-(2-phenylhydrazono)-2-chloro-*N*-phenylacetamide with pentan-2,4-dione in the presence of sodium ethoxide. Reaction of **3** with dimethylformamide-dimethylacetal (DMF-DMA) furnished the corresponding 4-[(*E*)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**5**). The latter product underwent regioselective 1,3-dipolar cycloaddition with some nitrilimines to afford the non-isolable dihydropyrazole intermediates which then lose dimethylamine yielding the corresponding pyrazole derivatives. The preliminary screening for the antitumor activity of all newly synthesized compounds was carried out against *Ehrlich Ascites Carcinoma* tumor cells.

1. Introduction

N-Arylpyrazole derivatives are a very interesting class of heterocyclic compounds that have remarkable pharmacological activities as antibacterial-antifungal,[1] hypoglycemic,[2] tumor necrosis inhibitor,[3] antithromboembolic disorders,[4] antiangiogenic agent,[4] A_3 Adenosine receptor antagonist,[5] neuropeptide Y Y5 receptor antagonists,[6] kinase inhibitor for treatment of type 2 diabetes, hyperlipidemia, and obesity,[7] insecticides,[8] thrombopiotinmimetics,[9] and anti-inflammatory.[10,11]

Although many literatures discussed the antitumor activity of pyrazole derivatives,[12–20] recently few studies have discussed the activity of 3-carboxamide (Fig. 1)[21] and 5-carboxamidepyrazole derivatives.[22] This article describes our procedure to develop a facile, rapid, three-step reaction for the synthesis of bis-system of 3-phenylcarboxamidopyrazole derivatives in good yields and high purity, and test their inhibitory effect on tumor growth, in order to study part of structure-activity relationship (SAR) of this new antitumor class in order to get a new agent that could be optimized to be used as a potent antitumor agent.

2. Results and discussion

2.1. Chemistry

The versatile, *hitherto* unreported 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) was prepared via the reaction of 2-(2-phenylhydrazono)-2-chloro-*N*-phenylacetamide (**1**) with pentan-2,4-dione (**2**) in the presence of sodium ethoxide (Scheme 1). Reaction of **3** with dimethylformamide-dimethylacetal (DMF-DMA) (**4**) furnished the corresponding 4-[(*E*)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**5**) (Scheme 1).

The ^{1}H NMR spectrum of compound **3** displayed two singlet signals at δ 2.76 and at δ 2.43 characteristics for two methyl groups and broad D_2O-exchangeable signal at δ 9.01 characteristic for amide NH, in addition to multiplets

Keywords: Phenylcarbamoyl-*1H*-pyrazole; 1,3-Dipolar cycloaddition; Nitrilimines; Cytotoxicity; Antitumor agent; *N*-Phenylpyrazoles; Regioselective synthesis; Antitumor activity; Dimethylformamide-dimethylacetal (DMF-DMA); Enaminones; Structure–activity relationship (SAR).
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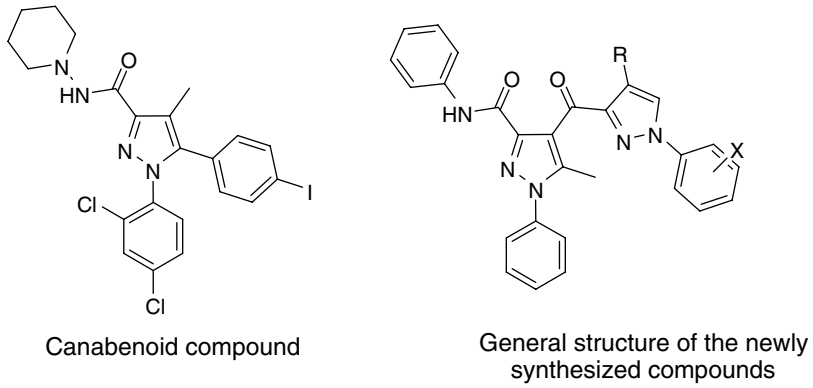
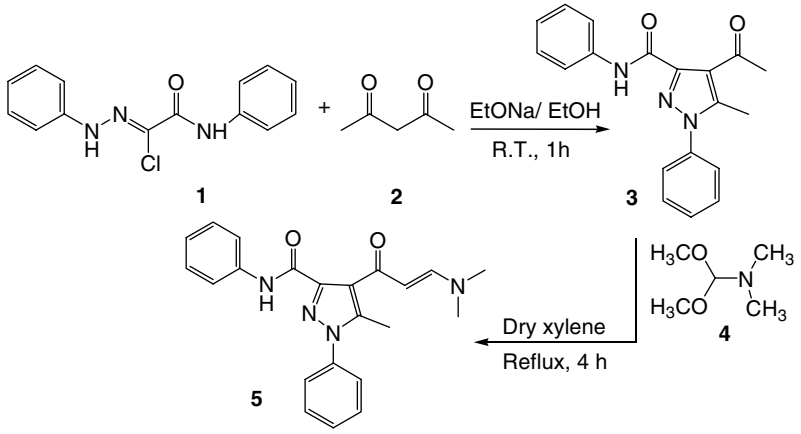


Figure 1.



Scheme 1.

at 7.12–7.70 due to aromatic protons. Whereas, its ^{13}C NMR spectrum revealed fifteen carbon types, the most important signals being displayed at δ 192 and δ 154 characteristics for acetyl and amide carbonyl carbons, respectively. These two carbonyl groups have absorption bands on the IR spectrum at 1751 and 1666 cm^{-1}, respectively. Another important band on IR spectrum appeared at 3259 cm^{-1} characteristic for amide NH.

The ^1H NMR spectrum of enaminone **5** displayed three singlet signals at δ 2.43, at δ 2.88, and at δ 3.11 characteristics for three methyl groups, two doublets at δ 5.55 and at δ 7.30 (J = 12.5 Hz) due to olefinic protons, in addition to an aromatic multiplet in the region of δ 7.25–7.45. The value of the coupling constant for the ethylenic protons indicates that the enaminone **5** exists exclusively in the *E*-configuration. Its ^{13}C NMR spectrum revealed 18 carbon types, the conjugation in enaminone **5** makes its carbonyl's carbons displayed at δ 186 (instead of 192 in compound **3**), the other important signals being displayed at δ 159, 45, and 37 corresponding to amide carbonyl carbon and two *N*-methyl carbons, respectively. IR spectrum revealed three important bands at 3421, 1681, and 1627 cm^{-1} characteristics for amide NH, ketonic, and amidic carbonyl groups, respectively.

In spite of enormous literature on the uses of enaminones in heterocyclic synthesis,[23–28] a little attention has been paid to their utility as dienophiles in 1,3-dipolar cycloaddition reactions.[23,29] We report here on the 1,3-cycloadditions of some nitrilimines to the versatile, *hitherto* unreported 4-[(*E*)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenyl-carbamoyl-*1H*-pyrazole (**5**).

The double bond in the enaminone **5** can be looked on as an electron-rich one that may enter 1,3-dipolar cycloaddition reactions. Thus, reaction of nitrilimines **7a–d** [generated, in situ, by action of triethylamine on *N*-aryl-2-oxo-*N*-arylpropanehydrazonyl chlorides **6a–d**] with the enaminone **5** in refluxing benzene afforded, in each case, only one isolable product.

The reaction products were assigned the pyrazole structures **9a–d** which were assumed to be formed via 1,3-dipolar cycloaddition of the nitrilimines **7a–d** to the activated double bond in the enaminone **5** to afford the non-isolable dihydropyrazole intermediates **8a–d** which then lose dimethylamine yielding the corresponding pyrazole derivatives **9a–d**. The other possible isomeric structure **11** was excluded on the basis of spectral data of the isolated products. For example, in the pyrazole ring system C-4 is the most electron-rich carbon, thus, H-4 is expected to appear at a higher field, typically at δ 6.31. On the other hand, H-5 is linked to the carbon attached to the nitrogen atom and thus it is deshielded to appear typically in the region δ 7.5–8.5.

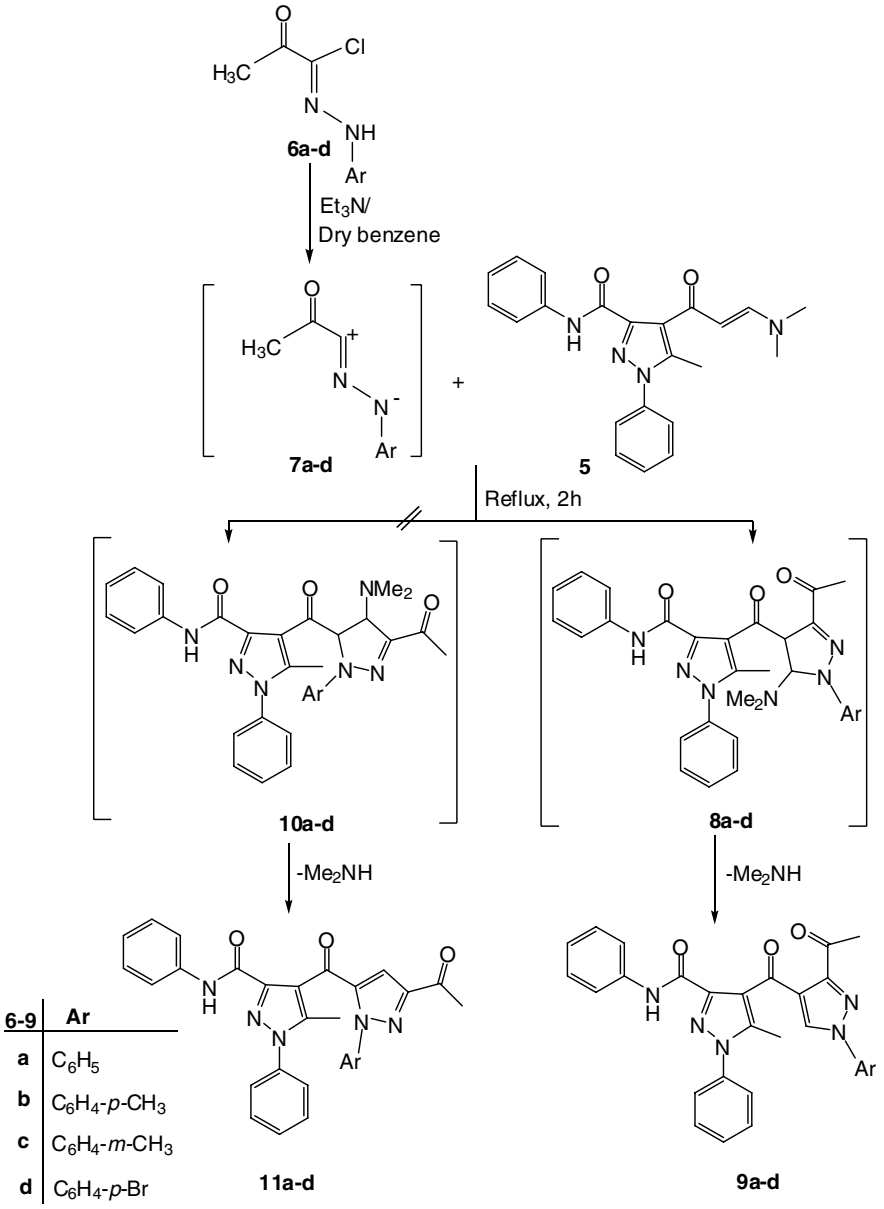
The ^{1}H NMR spectra of the isolated reaction products revealed, in each case, a singlet signal in the region of 7.68–8.87 which indicates the presence of the pyrazole H-5 rather than H-4 in the structure of the isolated products (Scheme 2).

Aliphatic methyl groups of compounds **9a–d** appear on the ^{1}H NMR spectra as singlet signals between δ 2.35 and δ 2.52. The ketonic carbonyl groups appear on the IR spectra as absorption bands at frequencies lying between 1670 and 1797 cm^{-1}.

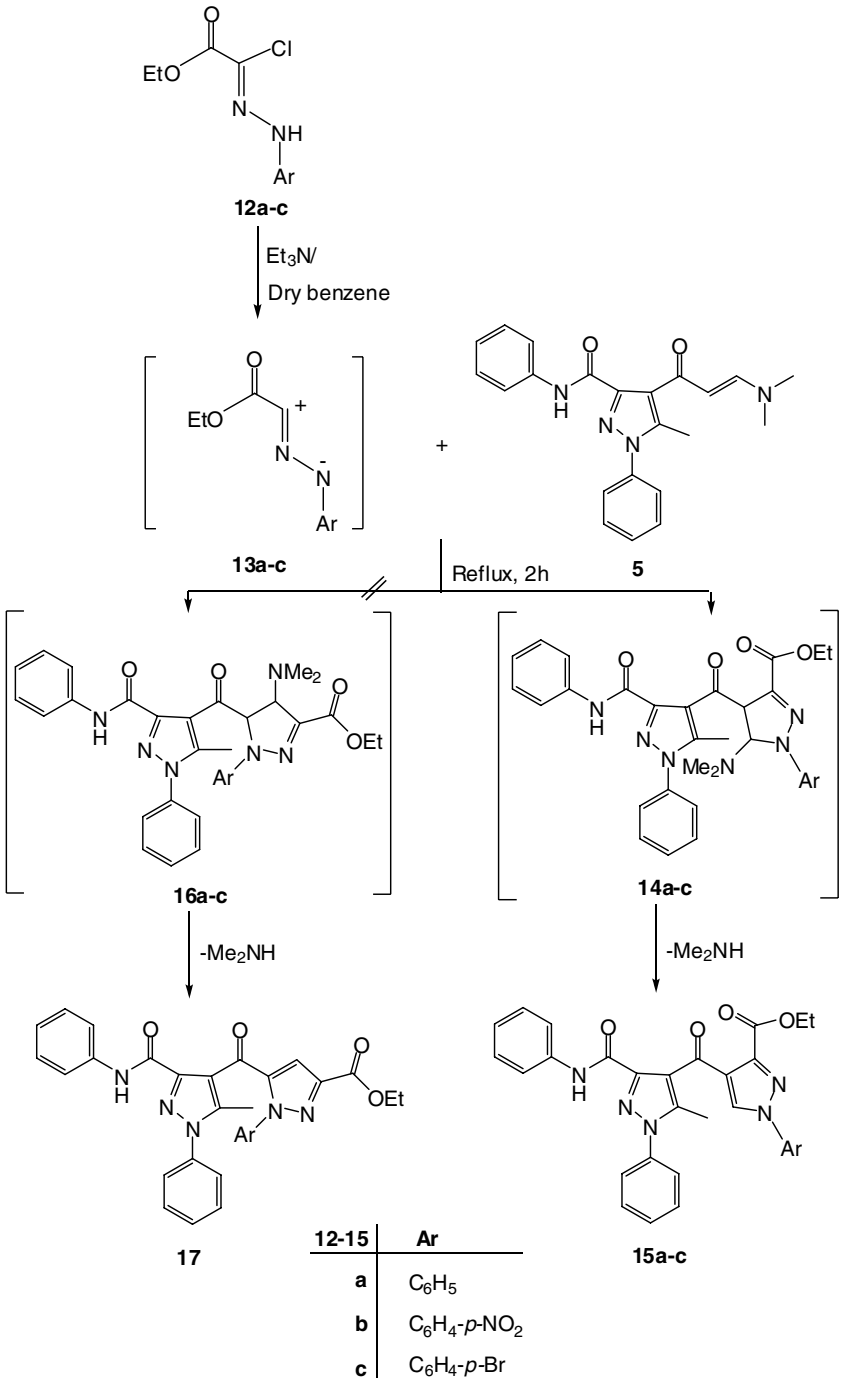
The ^{13}C NMR spectrum of compounds **9a–c** revealed two ketonic carbonyl carbons around δ 183.71 and 192.97, amide carbonyl carbons were displayed downfield at δ 160.34–160.38. While the aliphatic methyl groups appeared in the region between δ 11.97 and δ 27.39 (see Section 4).

In the same manner, the enaminone **5** reacts with *C*-1-(ethoxycarbonyl)-*N*-4-arylnitrilimine **13a–c** to afford products that may be formulated as the pyrazole structures **15a–c** or their regioisomers **17** (Scheme 3).

Structure **17** was excluded on the basis of the spectroscopic data of the isolated products. For example, ^{1}H NMR spectra of the reaction products revealed, in each case, the pyrazole CH-5 in the region between δ 8.96 and 9.15 which is in accordance with structures **15a–c**. Their IR spectra showed, in each case, a carbonyl adsorption band around 1735 cm^{-1}, due to carbonyl ester, the latter structures were further established on the basis of the ^{1}H NMR spectra of the reaction products which revealed, in each case, the presence of ethyl protons as triplet signals at δ 1.16, quartet signals at δ 4.212, and H-5 pyrazole protons at δ 9.1. The ^{13}C NMR spectrum of compound, **15b** and**15c** revealed



Scheme 2.

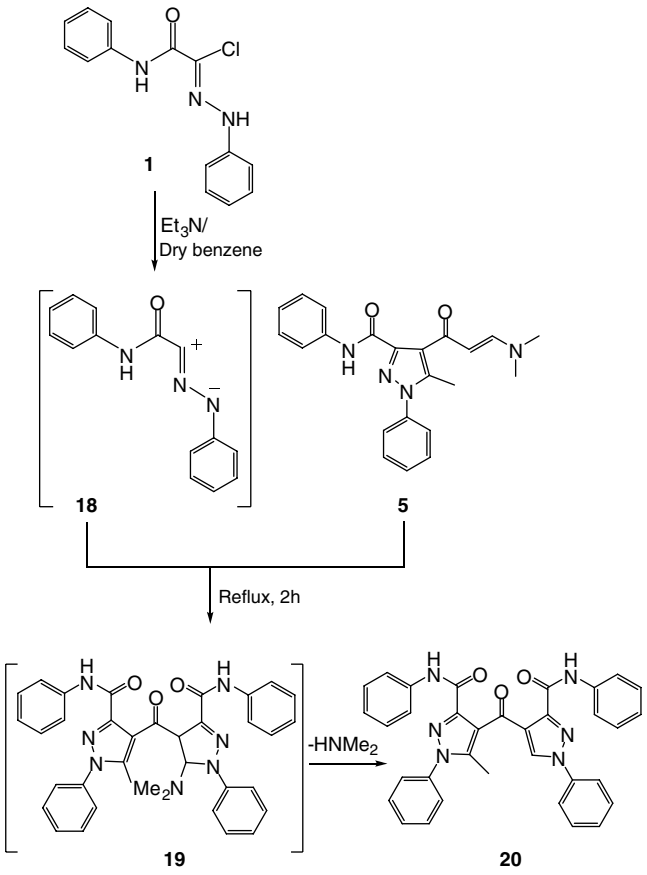


Scheme 3.

twenty-four carbon types. Carbonyl carbons of ketonic, ester, and amide groups are displayed at δ 182.6, 160, and 159.95, respectively. Other important signals are displayed at δ 13 and δ 61 characteristics for ethyl carbons, while C-5 pyrazole was displayed downfield at δ 147.5.

The enaminone **5** reacts also with *C*-phenylcarbamoyl-*N*-phenylnitrilimine **18** under the same experimental conditions to afford the corresponding pyrazole derivative **20** in a good yield (Scheme 4).

The ^{1}H NMR spectrum of compound **20** revealed a singlet signal at δ 9.06 corresponding to pyrazole H-5 proton and two singlet signals at δ 10.23 and at δ 10.76 (D_2O exchangeable) due to two NH protons. These two amide NH groups have two bands on the IR spectrum at 3228 and 3386 cm^{-1}. Its IR spectrum shows an intense band at 1662 cm^{-1} which revealed overlapping of the two carbonyl groups. These two carbonyl carbons were displayed very closely on ^{13}C NMR spectrum at δ 159.837 and at δ 159.395, other ketonic carbon was displayed at δ 184.05.



Scheme 4.

Antitumor screening result showed a strong correlation between cytotoxic activity and substituted carbonyl carbon which occupies position 3 from pyrazole ring B (Fig. 2). The cytotoxic result showed that compound **20** that carries carboxamido group has the highest activity, while compounds **9** and **15** showed weak activities (Table 1). In order to confirm this assumption, we synthesized compound **24** which has no carbonyl carbon at this position, that is, we removed the carboxamido moiety occupying position 3 from pyrazole ring B.

Compound **5** reacts with *C*-phenyl-*N*-phenylnitrilimine **19** under the same experimental conditions to afford the pyrazole derivative **24** (Scheme 5).

The ^{1}H NMR spectrum of the latter product revealed a singlet signal at δ 8.91 assignable to pyrazole CH-5 proton. Its ^{13}C NMR spectrum revealed twenty-five carbon types. Carbonyl carbons of ketonic and amide are displayed at δ 183.76 and 160.13, respectively. Other important signals are displayed at δ 152.50 and 11.53 characteristics for pyrazole C-5 and methyl attached to position 5 in other pyrazole ring.

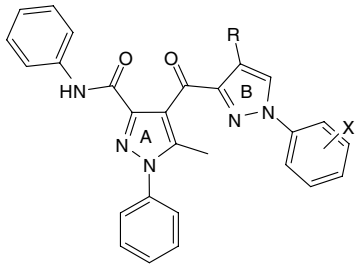


Figure 2.

Table 1. In vitro cytotoxic activity of newly synthesized compounds and doxorubicin

Compound	Non-viable cells (%)			IC_{50} (μg/ml)*
	Concentration (μg/ml)			
	100	50	25	
3	0	0	0	0
5	10	5	0	>100
9a	20	5	0	>100
9b	10	5	0	>100
9c	20	10	0	>100
9d	25	15	0	>100
15a	10	0	0	>100
15b	0	0	0	0
15c	20	10	0	>100
20	35	20	5	70
24	0	0	0	0
Control	0	0	0	—
Doxorubicin	100	55	20	35

*IC_{50} > 100 (μg/ml) is considered to be inactive.

The antitumor screening result confirms our pervious assumption, since compound **24** is devoid of any cytotoxic activity.

2.2. Pharmacology

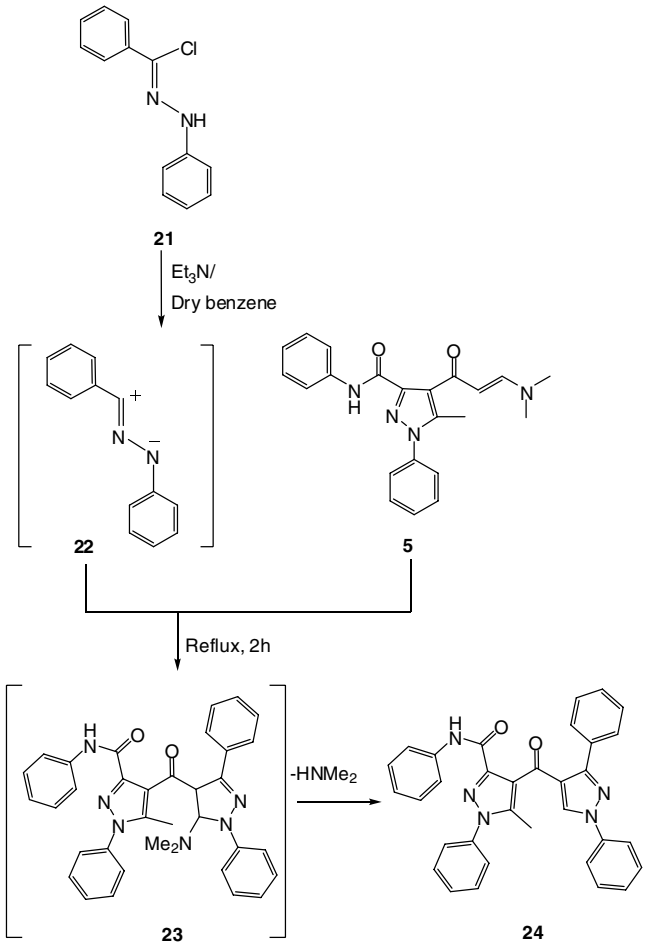
2.2.1. Antitumor screening test. The cytotoxic effects of the newly synthesized compounds and doxorubicin (Adriablastina)®, as a reference drug, in three deferent concentrations, were evaluated in the National Institute of Cancer, Cairo, Egypt.

IC_{50} was calculated with regard to saline control group and potency was calculated with regard to the percentage of the change of the doxorubicin and tested compounds, as depicted in Table 1.

3. Conclusions

The results of antitumor activity study of the newly synthesized compounds indicated that all compounds have no or low antitumor activity except compound **24** which was found to have moderate activity toward *Ehrlich Ascites* Carcinoma tumor cells (in vitro) in relation to the reference drug *Doxorubicin* (Table 1).

Compounds carrying two carbamoyl moieties are more active than those carrying one moiety. Moreover, compounds carrying carboxamide moiety are more active than those carrying acetyl or ester moieties. Also halogenated derivatives are more active than non-halogenated ones. *m*-alkyl substitution showed double the activity of *p*-alkyl as in compounds **9b**,**c**. Finally substitution with



Scheme 5.

electron withdrawing group such as NO_2 abolished the activity as in compound **15c**.

4. Experimental

4.1. Chemistry

4.1.1. General. All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H (300 MHz) and ^{13}C NMR (75.46 MHz) were run in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl chlorides **1**,[30] **6a–d**,[31] **12a–c**,[32] and **21**[33] were prepared following the procedures reported in the literature.

4.1.2. 4-Acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (3). A mixture of 2-(2-phenylhydrazono)-2-chloro-*N*-phenylacetamide (**1**) (5.18 g, 20 mmol) and pentan-2,4-dione (**2**) (2 ml, 20 mmol) was added to 20 ml freshly prepared sodium ethoxide solution [prepared by adding 460 mg sodium metal into absolute ethanol (20 mmol)] and the mixture was stirred for 1 h at room temperature. The yellow precipitated product was filtered off, washed with distilled water to get rid of NaCl salt, and dried. Recrystallization from ethanol afforded 5.1 g of 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) (80% yield), mp 141–142 °C. IR (KBr) υ_{max}/cm^{-1}: 3259 (NH), 1751 (C=O), 1666 (C=O), 1596 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H), 2.76 (s, 3H), 7.12–7.7 (m, 10H), 9.01 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 7.167, 26.744 (2 CH_3, aliphatic), 114.82, 119.25, 120.65, 123.87, 124.28, 124.32, 132.54, 132.99, 138.99, 139.85 (11 CH, aromatic carbons), 154.17 (C=O, amide), 192 (C=O, ketonic carbon); MS (*m*/*z*, %): 319 (M^+, 100), 227 (99.5), 157 (26.5), 118 (22.9), 77 (54); analysis for $C_{19}H_{17}N_3O_2$ (319.36), Calcd: C, 71.46; H, 5.37; N, 13.16%, found C, 71.61; H, 5.79; N, 13.23%.

4.1.3. 4-[(E)-3-(Dimethylamino)acryloyl]-5-methyl-1-phenyl-3- phenylcarbamoyl-1H-pyrazole (5). A mixture of 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) (6.38 g, 20 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (**4**) (2.38 ml, 20 mmol) was taken in dry xylene (20 ml) and the mixture was refluxed for 4 h, then left to cool to room temperature. The reddish-yellow precipitated product was filtered off, washed with petroleum ether (60/80 °C), and dried. Recrystallization from benzene afforded 6.73 g of 4-((*E*)-3-(dimethylamino)acryloyl)-5-methyl-*N*-1-diphenyl-1*H*-pyrazole-3-carboxamide (90% yield), mp 169–170 °C. IR (KBr) υ_{max}/cm^{-1}: 3421 (NH), 1681 (C=O), 1627 (C=O), 1591 (C=N); ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3 H), 2.88, 3.11 (s, 6H, $N(CH_3)_2$), 5.55 (d, 1H, J = 12.5 Hz), 7.03–7.79 (m, 10H), 7.3 (d, 1H, J = 12.5 Hz), 11 (s, br., D_2O exchangeable NH); ^{13}C NMR (DMSO-d_6): δ 12.95 (CH_3, aliphatic), 37.4, 43.6 ($N(CH_3)_2$), 97.74 (CH, aliphatic), 119.87, 122.73, 123.5, 125.66, 128.65, 128.7, 129.03, 138.64, 138.7, 140.48, 145.31 (11 CH, aromatic carbons), 154.55 (–*C*H=CH–$N(CH_3)_2$), 159.57 (amide), 187.5 (ketonic carbon); MS (*m*/*z*, %): 374 (M^+, 22.6), 330 (15.5), 304 (15.1), 282 (32.3), 118 (26.8), 98 (100), 77 (57), 70 (68.2); analysis for $C_{22}H_{22}N_4O_2$ (374.44), Calcd: C, 70.57; H, 5.92; N, 14.96%, found C, 70.61; H, 5.79; N, 14.93%.

4.1.4. 3-Acetyl-1-aryl-4-[(5-methyl-1-phenyl-3-phenylcarbamoyl)- *1H*-pyrazol-4-yl]carbonylpyrazoles (9a–d): General procedure. To a mixture of enaminone **5** (0.748 g, 2 mmol) and the appropriate 2-oxo-*N*-arylpropanehydrazonyl chloride **6** (2 mmol), in benzene (10 ml), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 2 h and the solvent was distilled off under reduced pressure. The residual brown viscous liquid was taken in ethanol and the resulting solid was collected by filtration, washed thoroughly with ethanol, dried, and finally recrystallized from ethanol/DMF to afford corresponding pyrazole derivatives **9a–d** in 70–80% yield. The phys-

ical and spectral data of compounds **9a**–**d** are listed below.

4.1.4.1. 3-Acetyl-1-phenyl-4-[(5-methyl-1-phenyl-3-phenylcarbamoyl)-1H-pyrazol-4-yl]carbonylpyrazole (9a). Yield (74%), mp 240–242 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3413 (NH), 1701 (C=O), 1670 (C=O), 1635 (C=O), 1600 (C=N);^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 2.52 (s, 3H, CH_3CO), 6.97–7.83 (m, 15H, ArH's), 8.84 (s, 1H, pyrazole-5-CH), 10.31 (s, br., D_2O exchangeable, NH);^{13}C NMR (DMSO-d_6): δ 11.99, 27.35 (2 CH_3, aliphatic), 119.59, 119.70, 120.35, 123.78, 125.18, 125.50, 127.94, 128.51, 129.13, 129.54, 129.60, 131.82, 137.94, 138.23, 138.49, 143.39, 148.24, (17 aromatic carbons), 149.58 (pyrazole-5-*C*H), 160.34 (amide), 183.69, 192.96 (2 ketonic carbons); MS (*m*/*z*, %): 489 (M^+, 16.8), 446 (40.1), 355 (100), 327 (12.3), 118 (20.9), 77 (18.1). Analysis for $C_{29}H_{23}N_5O_3$ (489.52): Calcd: C, 71.15; H, 4.74; N, 14.31%; found C, 71.39; H, 4.91; N, 14.36%.

4.1.4.2. 3-Acetyl-1-(4-methylphenyl)-4-[(5-methyl-1-phenyl-3-phenylcarbamoyl)pyrazol-4-yl]carbonyl-pyrazole (9b). Yield (76%), mp 205–207 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3417 (NH), 1797 (C=O), 1670 (2 C=O), 1596 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.51 (s, 3H, CH_3CO), 6.99-7.63 (m, 10H, ArH's), 7.670 (d, 2H, J = 2.7 Hz), 7.677 (d, 2H, J = 2.7 Hz), 8.79 (s, 1H, pyrazole-5-CH), 10.32 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 11.96, 20.55, 27.39 (3 CH_3, aliphatic), 119.51, 119.76, 120.49, 123.78, 125.13, 125.50, 128.52, 129.10, 129.52, 129.96, 131.75, 136.32, 137.52, 137.98, 138.30, 143.36, 148.20, (17 CH, aromatic carbons), 149.49 (pyrazole-5-*C*H), 160.35 (amide), 183.74, 192.99 (2 ketonic carbons); MS (*m*/*z*, %): 503 (M^+, 14.3), 460 (32.2), 369 (100), 118 (18.1), 77 (16.2); analysis for $C_{30}H_{25}N_5O_3$ (503.55), Calcd: C, 71.56; H, 5.00; N, 13.91%, found C, 71.59; H, 5.01; N, 14.00%.

4.1.4.3. 3-Acetyl-1-(3-methylphenyl)-4-[(5-methyl-1-phenyl-3-phenylcarbamoyl)pyrazol-4-yl]carbonyl-pyrazole (9c). Yield (71%), mp 208–209 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3433 (NH), 1790 (C=O), 1680 (C=O), 1616 (C=O), 1523 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.52 (s, 3H, CH_3CO), 6.96–7.68 (m, 14H, ArH's), 8.78 (s, 1H, pyrazole-5-CH), 10.33 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 11.97, 20.97, 27.41 (3 CH_3, aliphatic), 116.79, 119.68, 120.12, 120.38, 123.75, 125.15, 125.50, 128.34, 128.5, 129.1, 129.38, 129.53, 131.84, 137.97, 138.3, 138.49, 139.31, 143.39, 148.32 (19 CH, aromatic carbons), 149.57 (pyrazole-5-*C*H), 160.38 (amide), 183.69, 192.96 (2 ketonic carbons); MS (*m*/*z*, %): 503 (M^+, 13.9), 460 (30.4), 369 (100), 118 (16.1), 77 (23.2); analysis for $C_{30}H_{25}N_5O_3$ (503.55), Calcd: C, 71.56; H, 5.00; N, 13.91%, found C, 71.60; H, 5.08; N, 13.99%.

4.1.4.4. 3-Acetyl-1-(4-bromophenyl)-4-[(5-methyl-1-phenyl-3-phenylcarbamoyl)pyrazol-4-yl]carbonyl-pyrazole (9d). Yield (80%), mp 258–259 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3431 (NH), 1670 (2C=O), 1596 (C=O), 1529 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.97–7.66 (m, 14H, ArH's), 8.87 (s, 1H, pyrazole-5-CH), 10.31 (s, br., D_2O exchangeable, NH); MS (*m*/*z*, %): 569 (M^++2, 12.0), 567 (M^+, 12.3), 526 (21.1), 524 (22.0), 477 (1.5), 475 (1.5), 435 (97.5), 433 (100), 407 (0.3), 405 (0.3), 304 (2.5), 276 (0.7), 118 (14.0), 77 (27.9); analysis for $C_{29}H_{22}BrN_5O_3$ (568.42), Calcd: C, 61.28; H, 3.90; N, 12.32%, found C, 61.60; H, 3.92; N, 12.40%.

4.1.5. Ethyl 1-aryl-4-(4[(5-methyl-1-phenyl-3-phenylcarbamoyl)-1H-pyrazolyl]carbon-yl)pyrazole-3-carboxylates (15a–c): General procedure. To a mixture of enaminone **5** (0.748 g, 2 mmol) and the appropriate chloro(arylhydrazono)ethyl acetate **12a**–**c** (2 mmol), in benzene (10 ml), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 2 h and the solvent was distilled off at reduced pressure. The residual viscous liquid was taken in ethanol then the resulting solid was collected by filtration, washed thoroughly with ethanol, dried, and finally recrystallized from ethanol/DMF to afford corresponding pyrazole derivatives **15a**–**c** in 70–80% yield. The physical and spectral data of compounds **15a**–**c** are listed below.

4.1.5.1. Ethyl 4-(4[(5-methyl-1-phenyl-3-phenylcarbamoyl)-1H-pyrazolyl]carbonyl)-1-phenylpyrazole-3-carboxylate (15a). Yield (80%), mp 120–122 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3413 (NH), 1732 (C=O), 1639 (C=O), 1600 (C=O), 1531 (C=N); ^{1}H NMR (DMSO-d_6): δ 1.23 (t, 3H, CH_3, J = 6.9), 2.49 (s, 3H, CH_3), 4.259 (q, 2H, CH_2, J = 6.9), 6.97–7.74 (m, 15H, ArH's), 8.79 (s, 1H, pyrazole-5-CH), 11.2 (s, br., D_2O exchangeable, NH); MS (*m*/*z*, %): 519 (M^+, 15.8), 427 (40.1), 355 (100), 118 (10.1), 77 (20.5); analysis for $C_{30}H_{25}N_5O_4$ (519.6), Calcd: C, 69.35; H, 4.85; N, 13.48%, found C, 69.70; H, 4.93; N, 13.52%.

4.1.5.2. Ethyl 4-(4[(5-methyl-1-phenyl-3-phenylcarbamoyl)-1H-pyrazolyl]carbonyl)-1-(4-nitrophenyl)-pyrazole-3-carboxylate(15b). Yield (79%), mp 121–122 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3413 (NH), 1739 (C=O), 1631 (2 C=O), 1596 (C=N); ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 3H, CH_3, J = 6.9), 2.45 (s, 3H, CH_3), 4.21 (q, 2H, CH_2,J = 6.9), 6.97–7.64 (m, 10H, ArH's), 7.693 (d, 2H, J = 9), 7.723 (d, 2H, J = 9), 8.96 (s, 1H, pyrazole-5-CH), 10.26 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 11.96, 13.72 (2 CH_3, aliphatic), 61.34 (CH_2), 119.89, 120.5, 123.76, 125.25, 125.48, 126.66, 128.45, 129.2, 129.54, 132.55, 137.89, 138.13, 142.69, 143.41, 144.28, 146.05 (17 CH, aromatic carbons), 147.64 (pyrazole-5-*C*H), 159.95 (amide), 160.96, 182.59 (2 ketonic carbons); MS (*m*/*z*, %): 564 (M^+, 35.4), 472 (75.8), 400 (100), 354 (37.6), 185 (16.2), 118 (49.5), 77 (62.3); analysis for $C_{30}H_{24}N_6O_6$ (519.6), Calcd: C, 62.82; H, 4.28; N, 14.89%, found C, 62.89; H, 4.33; N, 14.92%.

4.1.5.3. Ethyl 1-(4-bromophenyl)-4-{4[(5-methyl-1-phenyl-3-phenylcarbamo-yl)-1H- pyrazolyl]carbonyl} pyrazole-3-carboxylate (15c). Yield (71%), mp 178–180 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3367 (NH), 1732 (C=O), 1681 (2 C=O), 1596 (C=N); ^{1}H NMR

(DMSO-d_6): δ 1.17 (t, 3H, CH_3, $J = 7.2$), 2.48 (s, 3H, CH_3), 4.235 (q, 2H, CH_2, $J = 7.2$), 6.93–7.704 (m, 10H, ArH's), 8.098 (d, 2H, $J = 9$), 8.352 (d, 2H, $J = 9$), 9.15 (s, 1H, pyrazole-5-C*H*), 10.28 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 11.89, 13.79 (2 CH_3, aliphatic), 61.18 (CH_2), 120.01, 120.54, 120.72, 121.45, 123.77, 125.53, 126.11, 128.5, 129.19, 129.55, 132.00, 132.48, 137.68, 137.99, 138.26, 143.19, 143.53 (17 CH, ArC's), 147.56 (pyrazole-5-*C*H), 159.95 (C=O, amide), 161.25 (C=O, ketonic carbon), 182.68 (C=O, ester carbonyl carbon); MS (*m*/*z*, %): 599 (M^++2, 8.8), 597 (M^+, 7.9), 507 (22.0), 505 (23.5), 479 (0.7), 477 (0.7), 435 (97.4), 433 (100), 354 (37.6), 157 (2.5), 185 (16.2), 118 (50.9), 77 (44.7); analysis for $C_{30}H_{24}N_6O_6$ (598.4), Calcd: C, 60.21; H, 4.04; N, 11.70%, found C, 60.45; H, 4.14; N, 11.72%.

4.1.6. 4-[(5-Methyl-1-phenyl-3-phenylcarbamoyl-pyrazol-4-yl)carbonyl]-1-phenyl-3-phenylcarbamoyl-1H-pyrazole (20). To a mixture of **5** (0.748 g, 2 mmol) and 2-(2-phenylhydrazono)-2-chloro-*N*-phenylacetamide (**1**) (0.518 g, 2 mmol) in benzene (10 ml), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 2 h. The solvent was distilled off at reduced pressure and the residual viscous liquid was taken in ethanol. The resulting solid was collected by filtration, washed thoroughly with ethanol, dried, and finally recrystallized from ethanol/DMF to afford the corresponding pyrazole derivative **20** in 95% yield, mp 183–184 °C. IR (KBr) υ_{max} /cm^{-1}: 3386 (NH), 3228 (NH), 1662 (2 C=O), 1604 (C=O), 1527 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 7.02–7.93 (m, 20H, ArH's), 8.06 (s, 1H, pyrazole-5-CH), 10.23 (s, br., D_2O exchangeable, NH), 10.76 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 11.76 (CH_3, aliphatic), 119.55, 119.83, 120.24, 121.27, 123.79, 123.90, 124.32, 125.61, 127.92, 128.54, 128.85, 129.10, 129.39, 129.66, 132.95, 138.10, 138.38, 138.51, 138.66, 142.33, 146.63 (21 ArC's), 147.93 (pyrazole-5-*C*H), 159.39, 159.83 (2 C=O, amide), 184.05 (C=O, ketonic carbon); MS (*m*/*z*): 566 (M^+, 23.9), 446 (18.6), 355 (100), 118 (14.5), 77 (29.5); analysis for $C_{34}H_{26}N_6O_3$ (566.6), Calcd: C, 72.07; H, 4.64; N, 14.83%, found C, 72.45; H, 4.70; N, 14.92%.

4.1.7. 1,3-Diphenyl-4-[(5-methyl-1-phenyl-3-phenylcarbamoylpyrazol-4-yl)carbonyl]-1H-pyrazole (24). To a mixture of the enaminone **5** (0.748 g, 2 mmol) and *N*-phenylbenzenecarbohydrazonoyl chloride (**21**) (0.46 g, 2 mmol) in benzene (10 ml), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 2 h and the solvent was removed under reduced pressure. The residual viscous liquid was taken in ethanol and the resulting solid was collected by filtration, washed thoroughly with ethanol, dried, and finally recrystallized from ethanol/DMF to afford compound **24**. Yield (92%), mp 241–242 °C (ethanol/DMF). IR (KBr) υ_{max}/cm^{-1}: 3382 (NH), 1678 (2 C=O), 1596 (C=O), 1527 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 7.01–7.89 (m, 20H, ArH's), 8.91 (s, 1H, pyrazole-5-CH), 10.23 (s, br., D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6): δ 11.53 (CH_3, aliphatic), 119.14, 120.11, 121.72, 123.47, 123.78, 125.57, 127.33, 127.88, 128.51, 128.56, 128.80, 129.05, 129.44, 129.56, 132.14, 133.38, 138.18, 138.36, 138.76, 141.82, 147.04, (21 CH, ArC's), 152.48 (pyrazole-5-*C*H), 160.13 (C=O, amide), 183.76 (C=O, ketonic carbon); MS (*m*/*z*): 523 (M^+, 25.6), 431 (100%), 247 (24.5%), 118 (11%), 77 (35.1%); analysis for $C_{33}H_{25}N_5O_2$ (523.6), Calcd: C, 75.70; H, 4.81; N, 13.38%, found C, 75.75; H, 4.84; N; 13.52%.

4.2. Pharmacology

4.2.1. Materials. RPMI 1640 medium (Sigma), Ehrlich Ascites Carcinoma cells (EAC) suspension (2.5.105/ml), and Trypan blue dye. A stock solution was prepared by dissolving one gram of the dye in distilled water (100 ml). The working solution was then prepared by diluting (1 ml) the stock solution with 9 ml of distilled water. The stain was used then for staining the dead EAC cells.

4.2.2. Antitumor activity of the E.A.C.[34–36] A set of sterile test tubes was used, where 2.5×10^5 tumor cells/ ml were suspended in phosphate-buffered saline. Then 25, 50, 100 μg/ml from tested compound were added to the suspension, kept at 37 °C for 2 h. Trypan blue dye exclusion test was then carried out to calculate the percentage of nonviable cells.

Doxorubicin (Adriablastina)® is taken as a positive control. The percentage of the non-viable cells is calculated by the following equation; % of non-viable cells = $N/N_t \times 100$, where N is the number of non-viable cells counted, N_t is the total number of cells. The test was repeated four times for each compound.

4.2.3. Statistical analysis. Data are expressed as means ± SE. Differences between control and treated tubes were tested using one-way ANOVA followed by multiple comparisons by the Duncan's multiple rang test. A probability value less than 0.05 was considered statistically significant.

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