Synthesis and anti-tumor activities of some new pyridazinones containing the 2-phenyl-1H-indolyl moiety

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ABSTRACT

β-aroylacrylic acid 2 condensed with hydrazines and hydroxylamine hydrochloride. Simultaneous cyclization of the condensed products yields pyridazinones and Oxazinone. The behaviour of the resulting pyridazinone toward formaldehyde/ piperidine, ethyl chloroacetate, chloroacetic acid, benzene sulfonyl chloride, bromine /acetic acid and aromatic aldehydes has also been studied. However, the reactions of the chloro derivative resulting from the reaction of pyridazinone with POCl₃ toward hydrazines, thiourea, sodium azide, aromatic amines and sulfa compounds have also been taken into consideration. The antitumor activity of some of the synthesized compounds were tested.

Keywords: Pyridazinone, Oxazinone and antitumor activity.

INTRODUCTION

In the last several decades, pyridazinone and indole derivatives have received considerable due to their wide range of applications. Pyridazinones are reported to antihypertensive[1],vasodilalory[2],analgesic[3],anti-inflammatory[3,4], antibacterial, antifungal[5], vasorelaxant [6], platelet aggregation inhibition [7] ‘antinoniccpleve[8] antagonist[9],cyclooxegenase inhibitory[10], herbicidal[11]and also have blood gluc-ose lowering effect[12].

On the other hand, indole derivatives exhibit antioxidant[13],anti-hepatitis C-virus [14]antimicrobial[15], antimalarial[16], anti-proliferative[17],antitumor[18] and also can be used as antitryponosomal[19]and antivascular agents[20]encouraged by these reports, we thought of
synthesizing a new series of pyridazinones containing the 2-phenylindole at the 4-position hoping to improve the antitumor activity of the new compounds [21].

**EXPERIMENTAL SECTION**

**General**

Melting points were determined in open capillary tubes on a gollen bamp melting point apparatus and are uncorrected. The elemental analyses were carried out at the Micro Analytical Unit, Faculty of Science, Cairo University by using Perkm – Elmer 2400CHN elemental analyses. The IR spectra recorded on Perkm Elmer spectrum RXIFT – IR systems as KBr discs the $^1$HNMR spectra were measured on Varion gemimi 200 MHz instrument with chemical shift (δ) expressed in ppm down field from TMS as internal standard in DMSO-d$_6$. Mass spectra were recorded on Shimadzu GC-MS, QP 1000 EX instrument operating at 70 ev.

**Reaction of aroylacrylic acid 1 with 2-phenylindole:**

To a solution of 1 (0.01 mole) in dry benzene (20 ml) and 2-phenylindole (0.01 mole) was added and the reaction mixture was refluxed for 10 hours. The solid that separated during reflux was filtered off and recrystallized from petroleum ether (40–60°C) to give compound 2. 4-(3,4-Dimethylphenyl)-4-oxo-2-(2-phenyl-1H-indole-3-yl) butanoic acid (2): yield 80%, white crystals; m.p. 210°C; IR(KBr) δ: 3437 (NH), 1700 (C=O) cm$^{-1}$; $^1$HNMR δ: 7.87-6.89 (12H, m, Ar-H), 3.19 (2H, d, CH$_2$-CH), 3.71 (1H, t, CH$_2$-CH), 2.23 (3H, s, CH$_3$), 11.51 (1H, s, NH). A mole calculated for C$_{26}$H$_{23}$N$_3$O (397.45); C 78.33; H 5.74; N 3.78.

**Reaction of the adduct 2 and the chloro derivative 12 with hydrazines, hydroxylamine hydrochloride, aldehydes, thiourea and amino compounds**

**General procedure:**

To a solution of 2 and/or 12 (0.01 mol) in absolute ethanol (50 ml) and equmolar amount of hydrazines, hydroxylamine hydrochloride, aromatic aldehydes, thiourea and amines was added and the reaction mixture was refluxed for 4-10 hrs. (TLC). A solution of KOH (2 gm) in 2 ml water was added in case of reaction with aldehydes. The crude material obtained after concentration and cooling was filtered off and recrystallized from the suitable solvent to give compounds 3a, b, 4, 11 a-c, 13a, b, 14, 16 and 19 respectively.

6-(3,4-dimethylphenyl phenyl-1H-indol-3-yl)pyridazin-3(2H)-one)-4-(2- (3a) :

Yield 72 %, yellowish orange crystals; m.p. 250°C (ethanol); IR(KBr) δ: 3261 (NH), 1666 (C=O) cm$^{-1}$, 1609(C=N) cm$^{-1}$; $^1$HNMR δ: 7.87-6.89 (12H, m, Ar-H), 3.71 (1H, t, CH$_2$-CH), 3.19 (2H, d, CH$_2$-CH), 2.19 (3H, s, CH$_3$), 2.16 (3H, s, CH$_3$), 11.51 (1H, s, NH), 11.03 (1H, s, NH); MS m/z (%) 393 (M$^+$ 9.81); A mole calculated for C$_{26}$H$_{23}$N$_3$O (393.47); C 79.36; H 5.89; N 10.68. Found: C 79.57; H 6.10; N 10.90%.

4,5-Dihydro-6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3(2H)-one (3b):

Yield 70 %, dark yellow crystals; m.p. 235°C (ethanol); IR(KBr) δ: 3405 (NH), 1657 (C=O) cm$^{-1}$, 1591(C=N) cm$^{-1}$; $^1$HNMR δ: 7.87-6.70 (17H, m, Ar-H), 4.01 (1H, t, CH$_2$-CH), 3.40 (2H, d, CH$_2$-CH), 2.23 (3H, s, CH$_3$), 2.17 (3H, s, CH$_3$), 11.51 (1H, s, NH); MS m/z (%) 469 (M$^+$ 1.22); A mole calculated for C$_{32}$H$_{27}$N$_3$O (469.50); C 81.86; H 5.80; N 9.12. Found: C 81.93; H 5.96; N 9.12 %.

![Image of page 249](image-url)
4-(3,4-dimethylphenyl)-4-oxime-2-(2-phenyl-1H-indol-3-yl)butanoic acid (4):
Yield 56%, dark green crystals; m.p. 140°C (ethanol); IR(KBr) \( \nu \): 3435 (NH), 1694 (C=O) cm\(^{-1}\), 1611 (C=N) cm\(^{-1}\); \(^1\)HNMR \( \delta \): 7.92-6.88 (12H, m, Ar-H), 3.87 (1H, t, CH\(_2\)-CH), 2.98 (2H, d, CH\(_2\)-CH), 2.17 (3H, s, CH\(_3\)), 2.14 (3H, s, CH\(_3\)), 11.86 (1H, s, NH), 11.59 (2H, s, 2xOH); A mole calculated for C\(_{26}\)H\(_{24}\)N\(_2\)O\(_3\) (412.47); C 75.71; H 5.87; N 6.80. Found: C 75.92; H 6.10; N 6.96%.

5-(4-hydroxybenzyl)-6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3(2H)-one (11a):
Yield 85%, brown crystals; m.p. 170°C (ethanol); IR(KBr) \( \nu \): 3394 (NH), 1658 (C=O) cm\(^{-1}\), 1596 (C=N) cm\(^{-1}\); MS m/z (%) 496 (M\(^+\), 1.15, 1.93); A mole calculated for C\(_{33}\)H\(_{27}\)N\(_3\)O\(_2\) (497.57); C, 79.65; H, 5.47; N, 8.45. Found: C, 79.82; H, 5.79; N, 8.53%.

5-(3,4,5-trimethoxybenzyl)-6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3(2H)-one (11b):
Yield 87%, orange crystals; m.p. 275°C (ethanol); IR(KBr) \( \nu \): 3438 (NH), 1665 (C=O) cm\(^{-1}\), 1617 (C=N) cm\(^{-1}\); \(^1\)HNMR \( \delta \): 7.79-6.84 (14H, m, Ar-H), 3.91 (3H, s, OCH\(_3\)), 3.80 (3H, s, OCH\(_3\)), 3.71 (3H, s, OCH\(_3\)), 2.49 (2H, s, CH\(_2\)), 2.17 (3H, s, CH\(_3\)), 2.10 (3H, s, CH\(_3\)), 8.58 (2H, s, NH); MS m/z (%) 571 (M\(^+\), 0.46); A mole calculated for C\(_{36}\)H\(_{33}\)N\(_3\)O\(_4\) (571.65); C, 75.63; H, 5.82; N, 7.35. Found: C, 75.82; H, 6.05; N, 7.57%.

6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3(2H)-thione (14):
Yield 65%, yellow crystals; m.p. 180°C (ethanol); IR(KBr) \( \nu \): 3376 (NH), 1610 (C=N) cm\(^{-1}\), 1459 (N=C=S) cm\(^{-1}\), 1405 (C=S) cm\(^{-1}\); MS m/z (%) 407 (M\(^+\) 0.23 %); A mole calculated for C\(_{26}\)H\(_{21}\)N\(_3\)S (407.52); C, 76.62; H, 5.19; N, 10.31, S, 7.87. Found: C, 76.83; H, 5.30; N, 10.28; S, 8.10%.

1-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3-yl)hydrazine (13a):
Yield 90%, yellow crystals; m.p. 160°C (ethanol); IR(KBr) \( \nu \): 3437 (NH), 1615 (C=N) cm\(^{-1}\); \(^1\)HNMR \( \delta \): 7.88-6.88 (13H, m, Ar-H), 2.26 (3H, s, CH\(_3\)), 2.16 (3H, s, CH\(_3\)), 11.58 (4H, s, NH+NNH\(_2\)); A mole calculated for C\(_{36}\)H\(_{23}\)N\(_5\)S (481.58); C, 79.80; H, 5.65; N, 14.54. Found: C, 80.10; H, 5.73; N, 14.63%.

1-[6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3-yl]-2-phenylhydrazine (13b):
Yield 88%, brown crystals; m.p. 140°C (ethanol); IR(KBr) \( \nu \): 3294 (NH), 1598 (C=N) cm\(^{-1}\); MS m/z (%) 482 (M\(^+\), 0.37 %); A mole calculated for C\(_{32}\)H\(_{27}\)N\(_5\)S (482.56); C, 79.64; H, 5.43; N, 11.61. Found: C, 79.83; H, 5.52; N, 11.73%.
6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)-N-pyridin-2-yl]pyridazin-3-amine (17): yield 84%, brown crystals; m.p. 132°C (ethanol); IR (KBr) \( \nu \): 3534 (NH), 1618 (C=N) cm\(^{-1}\); MS m/z (%) 467 (M\(^+\), 0.67%); A mole calculated for C\textsubscript{31}H\textsubscript{25}N\textsubscript{5} (467.55); C, 79.63; H, 5.39; N, 14.98. Found: C, 79.57; H, 5.52; N, 15.12%.

6-(3,4-dimethylphenyl)-N-(benzenesulfonyl-2-aminothiazol)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3-amine (18): yield 86%, orange crystals; m.p. 249°C (ethanol); IR (KBr) \( \nu \): 3538 (broad NH, OH), 1584 (C=N) cm\(^{-1}\); \( ^1 \)HNMR \( \delta \): 8.48-6.54 (20H, m, Ar-H), 2.23 (3H, s, CH\textsubscript{3}), 2.16 (3H, s, CH\textsubscript{3}); A mole calculated for C\textsubscript{35}H\textsubscript{28}N\textsubscript{6}O\textsubscript{2}S\textsubscript{2} (628.75); C, 66.85; H, 4.49; N, 13.37; S, 10.20. Found: C, 67.10; H, 4.63; N, 13.54; S, 10.24%.

6-(3,4-dimethylphenyl)-N-(benzenesulfonyl-2-amino-pyrimidine)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3-amine (19): yield 85%, orange crystals; m.p. 256°C (ethanol); IR (KBr) \( \nu \): 3370 (broad NH, OH), 1586 (C=N) cm\(^{-1}\); \( ^1 \)HNMR \( \delta \): 8.07-6.89 (12H, m, Ar-H), 3.81 (1H, t, CH\textsubscript{2}-CH), 3.2 (2H, d, CH\textsubscript{2}-CH), 2.21 (3H, s, CH\textsubscript{3}), 2.18 (3H, s, CH\textsubscript{3}), 1.15-1.2 (1H, s, NH); A mole calculated for C\textsubscript{36}H\textsubscript{29}N\textsubscript{7}O\textsubscript{2}S (623.71); C, 69.32; H, 4.69; N, 15.72; S, 5.14. Found: C, 69.58; H, 4.73; N, 15.91; S, 5.29%.

4,5-Dihydro-3-(3,4-dimethylphenyl)-5-(2-phenyl-1H-indol-3-yl)oxazin-6-one (5): A mixture of 2 (0.01 mol), NH\textsubscript{2}OH.HCl (0.01 mol) and pyridine (20 ml) was gently refluxed for 10 hrs., cooled, poured onto cold dil. HCl and the solid obtained was crystallized from ethanol to give (5). Yield 62%, faint green crystals; m.p. 165°C (ethanol); IR (KBr) \( \nu \): 3408 (NH), 1670 (C=O) cm\(^{-1}\), 1618 (C=N) cm\(^{-1}\); \( ^1 \)HNMR \( \delta \): 8.07-6.89 (12H, m, Ar-H), 3.81 (1H, t, CH\textsubscript{2}-CH), 3.2 (2H, d, CH\textsubscript{2}-CH), 2.21 (3H, s, CH\textsubscript{3}), 2.18 (3H, s, CH\textsubscript{3}), 11.52 (1H, s, NH); A mole calculated for C\textsubscript{26}H\textsubscript{22}N\textsubscript{2}O\textsubscript{2} (394.45); C, 79.16; H, 5.62; N, 7.10. Found: C, 79.28; H, 5.81; N, 6.98%.

4,5-Dihydro-6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)-2-(pyrrolidin-1-yl)methyl)pyridazin-3(2H)-one (6): A mixture of 3a (0.01 mol), piperidine (0.02 mol), formaldehyde (2.5 ml) and methanol (50 ml) was refluxed for 5 hrs., then kept over night at room temperature, then treated with H\textsubscript{2}O and the precipitated solid filtered and crystallized from ethanol to give (6). Yield 78%, yellowish crystals; m.p. 230°C; IR (KBr) \( \nu \): 3436 (NH), 1666 (C=O), 1600 (C=N) cm\(^{-1}\); \( ^1 \)HNMR \( \delta \): 7.87-6.92 (12H, m, Ar-H), 3.82 (1H, t, CH\textsubscript{2}-CH), 3.19 (2H, d, CH\textsubscript{2}-CH), 2.23 (3H, s, CH\textsubscript{3}), 2.16 (3H, s, CH\textsubscript{3}), 2.51-1.13 (10H, m, (CH\textsubscript{2})\textsubscript{5}), 2.61 (2H, s, CH\textsubscript{2}), 11.43 (1H, s, NH); A mole calculated for C\textsubscript{32}H\textsubscript{34}N\textsubscript{4}O (490.62); C, 78.33; H, 6.99; N, 11.42. Found: C, 78.28; H, 7.20; N, 11.59%.

Reaction of 2 with ethylchloroacetate, chloroacetic acid and benzene - sulfonyl chloride;

**General procedure:**
A mixture of 3a (0.01 mol), anhydrous potassium carbonate (0.03 mol), ethyl chloroacetate, chloroacetic acid and benzenesulfonyl chloride (0.03 mol) was refluxed for 24 hrs. After filtration while hot and removing the excess solvent, the product was recrystallized from ethanol to give 7-9, respectively.

Ethyl-2-(5,6-dihydro-3-(3,4—dimethylphenyl)-6-oxo-5-(2-phenyl-1H-indol-3-yl)pyridazin-1(4H-yl)acetate (7):
Yield 89%, yellow crystals; m.p. 180°C (ethanol); IR (KBr) \( \nu \): 1739 (C=O, ester), 1668 (C=O), 1609 (C=N) cm\(^{-1}\); MS m/z (%) 479 (M\(^+\), 0.60); A mole calculated for C\textsubscript{30}H\textsubscript{29}N\textsubscript{3}O\textsubscript{3} (479.56); C, 75.13; H, 6.10; N, 8.76. Found: C, 74.92; H, 6.29; N, 8.93%.
6-(3,4-dimethylphenyl)-2-methyl-4-(2-phenyl-1H-indol-3-yl)4,5-dihydro-3(2H)-pyridazinone (8):
Yield 87%, yellow crystals; m.p. 190°C (ethanol); IR(KBr) \( \nu \): no C=O (acid), 3432(NH), 1666(C=O), 1600(C=N) cm\(^{-1}\); A mole calculated for \( \text{C}_{27}\text{H}_{25}\text{N}_{3}\text{O} \) (407.37); C, 79.60; H, 6.16; N, 10.32. Found: C, 79.78; H, 6.30; N, 10.38%.

6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)-2-(phenylsulfonyl)-4,5-1H-(3H)pyridine (9):
Yield 92%, yellowish crystals; m.p. 140°C (ethanol); IR(KBr) \( \nu \): 1666(C=O), 1607(C=N) cm\(^{-1}\); MS m/z (%), 534 (M\(^+\) 1.15, 0.78); A mole calculated for \( \text{C}_{32}\text{H}_{27}\text{N}_{3}\text{O}_{3}\text{S} \) (533.566); C, 72.03; H, 5.10; N, 7.88; S, 6.01. Found: C, 71.82; H, 5.26; N, 8.02; S, 5.87%.

5-bromo-6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3(2H)one (10):
A solution of 3a (0.01 mol) in glacial acetic acid (10ml) and bromine (0.01 mol) was stored at room temperature for 3 hrs. The solid product obtained was filtered off, washed with petroleum ether (40-60°C) and recrystallized from ethanol.
Yield 72%, yellow crystals; m.p. 164°C (ethanol); MS m/z (%), 470 (M\(^+\) 1.15, 0.48); A mole calculated for \( \text{C}_{26}\text{H}_{20}\text{N}_{3}\text{OBr} \) (470.36); C, 66.39; H, 4.29; N, 8.93; Br, 16.99. Found: C, 66.61; H, 4.35; N, 9.12; Br, 16.97%.

3-(3-chloro-6-(3,4-dimethylphenyl)pyridazin-4-yl)-2-phenyl-1H-indol (12):
A mixture of 3a (0.01 mol) and POCl\(_3\) (10 ml) was gently refluxed for 3 hrs., cooled, treated with crushed ice and the precipitated solid filtered and crystallized from ethanol to give 12.
Yield 65%, yellow crystals; m.p. 170°C; IR(KBr) \( \nu \): no C=O; 3436(NH), 1588(C=N) cm\(^{-1}\); \(^{1}\)HNMR \( \delta \): 7.87 – 6.89(13H, m, Ar-H), 2.31(3H, s, CH\(_3\)), 2.28(3H, s, CH\(_3\)), 11.50(1H, s, NH); A mole calculated for \( \text{C}_{26}\text{H}_{20}\text{N}_{3}\text{Cl} \) (409.90); C, 76.18; H, 4.92; N, 10.25; Cl, 8.65. Found: C, 76.29; H, 5.21; N, 10.29; Cl, 8.87%.

6-(3,4-dimethylphenyl)-8-(2-phenyl-1H-indol-3-yl)tetrazolo[1,5-b]pyridazine (15):
A mixture of 12 (1gm), sodium azide (1gm), water (5 ml) and dimethyl formamide (20 ml) was boiled for 2 hrs. and cooled. The solid obtained upon dilution with water was filtered and crystallized from ethanol, greenish crystals; m.p. 224°C; IR(KBr) \( \nu \): 3433(NH), 1588(C=N) cm\(^{-1}\); \(^{1}\)HNMR 8: 7.87 – 6.89(13H, m, Ar-H), 2.36(3H, s, CH\(_3\)), 2.31(3H, s, CH\(_3\)), 11.50(1H, s, NH); A mole calculated for \( \text{C}_{26}\text{H}_{20}\text{N}_{6} \) (416.47); C, 74.98; H, 4.84; N, 20.16. Found: C, 75.24; H, 4.98; N, 20.32%.

Sulforhodamine-B(SRB) assay of cytotoxic activity:
MCF7 (breast carcinoma cell line), HEPG2 (hepatocellular carcinoma cell line), HCT116 (colon carcinoma cell line) were obtained frozen in liquid nitrogen (-180°C) from the American type culture collection. The tumor cell lines were maintained in the National cancer Institute, Cairo, Egypt, by serial sub-culturing. Potential cytotoxicity of 2-5, 7, 11b, 12, 13a, 16 and 19 were tested using the method of Skehan et al.

Principle:
The sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a brought pink aminoxanthrene dye with two sulfonic group. It a protein stain that binds to the amino group of intracellular proteins under mildly acidic conditions to proceed a sensitive index of cellular protein content.

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Procedure
1- Cells were used when 90% confluence was reached in T25 flasks. Adherent cell lines were harvested with 0.025 % trypsin. Viability was determined by trypan blue exclusion using the invested microscope (Olympus 1x70, Tokyo, Japan).
2- Cells were seeded in 96-well microtiter plates at a concentration of $5 \times 10^4 - 10^5$ cell / well in a fresh medium and left to attach to the plates for 24 hrs.
3- After 24 hrs., cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200µl volume / well using fresh medium and incubation was continued for 24, 48 and 72 hrs. cells were treated with vehicle alone. For each drug concentration, 4 wells were used.
4- Following 24, 48 and 72 hrs. treatment, the cells were fixed with 50 µl cold 50 % trichloroacetic acid for 1 hr. at 4ºC.
5- Wells were washed 5 times with distilled water and stained for 30 min. at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid.
6- The wells were then washed 4 times with 1 % acetic acid.
7- The plates were air-dried and the dye was solublized with 100µl / well or 10 mM tris base (Ph 10.5) for 5min on a shaken (orbital shaken 0520, Boeco, Germany) at 1600 rpm.
8- The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meller tech Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The relation between survival fraction and compound concentration was plotted to get the survival curve of each tumor all lines (fig.1). The IC50 values (the concentrations of thymoquinone required to produce 50% inhibition of cell growth (fig. 2).

RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in scheme 1 and 2. Treatment of 4-(3,4-dimethylphenyl)-4-oxo-2-(2-phenyl-1H-indol-3-yl)butanoic acid (2) with hydrazine hydrate, phenyl hydrazine in absolute ethanol and hydroxylamine hydrochloride in pyridine afforded the corresponding pyridazinones (3a, b) and oxazinone 5, respectively in a one pot reaction, through concentration and simultaneous cyclization. However, reaction of 2 with hydroxylamine hydrochloride in absolute ethanol afforded the corresponding oxime 4. Interestingly, oxime 4 could be converted to oxazinone 5 by boiling in acetic anhydride.

The reaction of 3a with formaldehyde piperidine under Mannich’s reaction conditions and/or ethylchloro acetate, benzenesulfonyl chloride in boiling ethanol in presence of potassium carbonate afforded the 2-substituted pyridazinone derivatives 6,7 and 9, respectively.

Interestingly, the reaction of 3a with monochloroacetic acid in dry acetone/K$_2$CO$_3$ yielded the 2-methyl pyridazinone derivative 8 through nucleophilic substitution and decarboxylation. Compound 8 can be prepared through an alternative route, through the reaction of 3a with methyl iodide in dry acetone / K$_2$CO$_3$ to give one and the same compound[8] identified by m.p., mixed m.p. and IR comparison. Treatment of 3a with bromine-acetic acid mixture afforded compound 10. The formation of this compound can be mechanistically explained on the basis that the first step is dehydrogenation followed by addition of bromine on the formed double bond and the elimination of hydrogen bromide in a similar manner to that observed in the bromination of pyrazoline[22].

Compound 3a was subjected to further studies. Thus, condensation of 3a with p-hydroxybenzaldehyde in absolute ethanol with a catalytic amount of ethanolic KOH took place at the 5-position to give 4,5,6-trisubstituted pyridazinone(11a-c).
The behaviour of pyridazinone derivative 3a towards electrophilic reagents like POCl₃ gave 3-chloro pyridazine derivative 12, by substitution of the enolic hydroxyl group with chlorine together with dehydrogenation.

The resulting chloro derivative has been used as starting material for the preparation of a series of new compounds.

Thus, reaction of 12 with hydrazine hydrate and/or phenylhydrazine gave the hydrazine derivatives 13a and 13b, respectively.

The reaction of 12 with thiourea in absolute ethanol gave the pyridazine thione 14, while the reaction of 12 with sodium azide in DMF gave tetrazolo pyridazine derivative 15.
Table (1a,b,c) : Effect of some new prepared compounds on different types of tumor cells as cytotoxic drug

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<td>Conc. (µg/ml)</td>
<td>HCT 116</td>
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Cytotoxicity against different human cancer cell lines in vitro

For evaluation of anti-tumor cytotoxicity of compounds 2-5, 7, 11b, 12, 13a, 16 and 19, three different human cancer cell lines were used: MCF7 (breast carcinoma cell line), HEPG2 (hepatocellular carcinoma cell line), HCT116 (colon carcinoma cell line). Cytotoxicity and IC50 values of the tested compounds are shown in Fig. 1 and 2. The survival fractions were gradually decreased as the concentration of the tested compounds were increased (Table 1). From figure 2, it has been shown that 3a, 4, 7, 16 and 19 are the compounds of lowest IC50 which means that they are the most effective cytotoxic drugs, accordingly compounds 4 and 19 can be
used as very potent cytotoxic drug for breast carcinoma cell, 3a for liver carcinoma cell and 7, 16 as colon carcinoma cell cytotoxic drug, while 2, 5 and 12 as moderate cytotoxic drug for breast and colon carcinoma cell respectively, while the remaining compounds are very weak cytotoxic drug.

![IC50(µg/ml)]

MCF7 HE PG2 HCT116

Fig. (2) : 0-10 Very potent cytotoxic drug, 10-20 Moderate cytotoxic drug, >20 Very weak cytotoxic drug

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