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Synthesis and Evaluation of Some New Pyrazolopyrimidine and Thiazolidin-4-one Derivatives as Antimacrobial and Anticancer

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Abstract: In this work, it was of interest to synthesize new series of some novel pyrazolo [3, 4-d] pyrimidine and series of thiazolidinone derivatives. The antimicrobial and antitumor activity of some of these compounds was studied. Results revealed that extract no. **8, 10** then **5a** gives the highest antibacterial activity against all tested strains with a mean zone of inhibition equal 19.7, 19.3 and 18.2 respectively. Also, revealed that all tested extracts were highly effective against salmonella species which showed the widest zone of inhibition. The antitumor activity results indicated that all the eight tested compounds **(4b, 5b, 5c, 6a, 6b, 7b, 10** and **11)** showed antitumor activity against the tested liver cancer (HEPG2) cell line but with varying intensities in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. Moreover compound **10** showed the highest cytotoxic activity (IC_{50} equals 4.91 μg/ml) which was more effective than 5-Flurouracil.

Keywords: Pyrazolopyrimidine · thiazolidin-4-ones · antimicrobial and antitumor

INTRODUCTION

Certain pyrazolo [3, 4-d] pyrimidine exhibit phosphodiesterase inhibitory action [1] and others have shown herbicidal activity [2]. It well known that uric acid is breakdown product of purine in foods, so that allopurrinol is used to lower blood uric acid levels, prevent uric acid kidney stones and to prevent attacks arthritis recurrent gouty [3]. These finding encouraged us to synthesize some new pyrazolo [3, 4-d] pyrimidine ring systems as analogy to allopurrinol.

MATERIALS AND METHODS

Melting points were determined on a Gallenkamp melting point apparatus. IR spectra (KBr, ν, cm^{-1}) were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. ^{1}H-NMR spectra (DMSO-d_6, δ, ppm) were recorded on a Jeol EX-270 MHz spectrometer and TMS as the internal standard. Mass spectra were recorded on a Finnigan mat. SSQ-7000 GC-MS spectrometer. Microanalyses were performed at the Microanalytical Center of Cairo University.

Synthesis of 5-amino-3-(methylthio)-1-(2, 4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (3): A mixture of compound **1 (**1.71 g, 0.01 mol), compound **2** (1.98 g, 0.01 mol) and triethylamine (1 mL) in 15 mL ethanol was heated under reflux for 8-10 h. The product obtained after cooling was crystallized from acetic acid to give compound **3.** Yield 85% crystallized from acetic acid with m.p. 189-91°C, analysis for $C_{11}H_8N_6O_4S$, Calcd: C, 41.25, H, 2.53, N, 26.24. Found, C, 41.55, H, 2.73, N, 26.30. IR: 3407, 3322 (NH_2), 2215 (CN), 1539 (C=C) and 1283 (C-S-C). ^{1}H-NMR: 2.35 (3H, s, CH_3), 7.65-8.25 (3H, dd and s, Ar-H), 11.30 (2H, s, NH_2). MS (ESI+): *m/z* = 320 (M^+), (100%).

General method for preparation of Schiff's bases 4a-c.: A solution of compound **3** (3.2 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in 20 mL absolute ethanol containing few drops of glacial acetic acid was refluxed for 7-10 h. The formed solid, after cooling was filtered, washed with water, air dried and crystallized from the proper solvent.

(E)-5-(4-Chlorobenzylideneamino)-3-(methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazole-4-carbo-nitrile (4a): Yield 73%, crystallized from methanol, orange crystals, m.p.

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241-3°C, analysis: for $C_{18}H_{11}ClN_6O_4S$, M.wt. 442.84, Calcd: C, 48.82, H, 2.50, N, 18.98. Found: C, 48.95, H, 2.55, N, 19.01. IR: 3281 (C-H Ar), 2216 (CN), 1609 (C=N) and at 1545(C=C). ^{1}H-NMR: 2.35 (3H, s, CH_3), 7.25-8.35 (7H, m, Ar-H), 8.50 (1H, s, CH=N). MS (ESI+): *m/z* = 442 (M^+), (3.1%).

(E)-5-(4-Hydroxy-3-methoxybenzylideneamino)-3-(methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (4b): Yield 68% crystallized from methanol, orange crystals, m.p. 261-3°C, analysis: for $C_{19}H_{14}N_6O_6S$, M.wt. 454, Calcd: C, 50.22, H, 3.11, N, 18.49. Found, C, 50.22, H, 3.11, N, 18.49. IR: 3551 (OH), 3227 (C-H Ar), 2210 (CN), 1623 (C=N) and at 1087 (C-S-C). ^{1}H-NMR: 2.43 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 7.0-8.10 (6H, m, Ar-H), 8.60 (1H,s, CH=N), 11.10 (1H,s, OH).

(E)-5-(3,4-Dimethoxybenzylideneamino)-3-(methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (4c): Yield 68% crystallized from methanol, red powered, m.p. 236-8°C, analysis: for $C_{20}H_{16}N_6O_6S$, M.wt. 468, Calcd: C, 51.28, H, 3.44, N, 17.94. Found, C, 51.35, H, 3.56, N, 17.99. IR: 3230 (C-H Ar), 2219 (CN), 1613 (C=N) and at 1080 (C-S-C). ^{1}H-NMR: 2.43 (3H, s, CH_3), 3.70 (6H, s, $2OCH_3$), 7.00-8.20 (6H, m, Ar-H), 8.60 (1H, s, CH=N).

General method for preparation of thiazolidinone (5a-c): To well stirred solution of compounds **4a, 4b** or **4c** (0.01 mol) in 50 mL dry benzene, thioglycolic acid (0.01 mol) was added and the mixture was refluxed in a water bath for 5 h. The excess solvent was evaporated under vacuum and the precipitated solid was filtered, washed with water, air dried and crystallized from the proper solvent.

5-(2-(4-Chlorophenyl)-4-oxothiazolidin-3-yl)-3-(methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (5a): Yield 63% Crystallized from methanol, brown crystals, m.p.142-5°C, analysis: for $C_{20}H_{13}ClN_6O_5S_2$, M.wt. 516.01, Calcd: C, 46.47, H, 2.53, N, 16.26.Found, C, 46.73, H, 2.55, N, 16.43. IR: 1710 (C=O), 1613 (C=N), 1540 (C=C) and 1305 (C-S-C). ^{1}H-NMR: 2.30 (3H, s, CH_3), 2.55 (2H, s, CH_2 of thiazolidinone), 3.65 (1H, s, CH of thiazolidinone), 7.10-8.60 (7H, m, Ar-H).

5-(2-(3-Hydroxy-4-methoxyphenyl)-4-oxothiazolidin-3-yl)-3-(methyl-thio)-1-(2, 4-dinitrophenyl)-1H-pyrazole-4-carbonitrile (5b): Yield 67%, crystallized from ethanol, red crystals, m.p. 200-3°C, analysis: for $C_{21}H_{16}N_6O_7S_2$, M.wt. 528.52, Calcd: C, 47.72, H, 3.05, N, 15.90. Found: C, 47.87, H, 3.52, N, 15.99. IR: 3425 (OH), 1708 (C=O), 1635 (C=N), 1568 (C=C) and 1109 (C-S-C). ^{1}H-NMR: 2.35 (3H, s, CH_3), 2.50 (2H, s, CH_2 of thiazolidinone), 3.60 (1H, s, CH of thiazolidinone), 3.80 (3H,s, OCH_3) and 7.15-8.25 (6H, m, Ar-H) and at 10.55 (1H,s,OH).

5-(2-(3, 4-Dimethoxyphenyl)-4-oxothiazolidin-3-yl)-3-(methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (5c): Yield 58% crystallized from methanol, red crystals, m.p. 187-9°C, analysis: for $C_{22}H_{18}N_6O_7S_2$, M.wt. 542.50, Calcd.: C, 48.70, H, 3.34, N, 15.49 Found, C, 48.86, H, 3.52, N, 15.96. IR: 3185 (C-H Ar), 1705 (C=O), 2220 (CN), 1610(C=N) and at 1570 (C=C). ^{1}H-NMR: 2.30 (3H, s, CH_3), 2.55 (2H, s, CH_2 of thiazolidinone), 2.65 (1H, s, CH of thiazolidinone), 3.85 (6H, s, 2 OCH_3) 7.35-8.10 (6H, m, Ar-H).

General method for preparation of compounds 6a, b: A solution of compound **5a** or **5b** (0.01 mol) and p-hydroxy benzaldehyde (0.01 mol) in 20 mL absolute ethanol was refluxed for 7-10 h. The formed solid, after cooling was filtered, washed with water, air dried and crystallized from the proper solvent.

5-((Z)-5-(4-Hydroxybenzylidene)-2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-3-(methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (6a): Yield 64% crystallized from acetic acid to give yellowish brown crystals, m.p. 212-5 °C, Analysis: for $C_{27}H_{17}ClN_6O_6S_2$, M.wt. 621.04, Calcd.: C, 52.22, H, 2.76, N, 13.53, Found, C, 52.19, H, 2.70 N, 13.55. IR: 3425 (OH), 3262 (C-H Ar), 2216 (CN), 1709 (C=O), 1610 (C=N), 1549 (C=C) and at 1091 (C-S-C). ^{1}H-NMR: 2.40 (3H, s, CH_3), 3.65 (1H, s, CH of thiazolidinone), 7.10-8.75 (12H, m, Ar-H + C=CH) and at 10.45 (1H, s, OH).

5-((Z)-5-(4-Hydroxybenzylidene)-2-(4-hydroxy-3-methoxyphenyl)-4-oxo-thiazolidin-3-yl)-3-(methylthio)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbonitrile(6b): Yield 71% crystallized from ethanol/DMF, to give red crystals, m.p. 270-3°C, Analysis: for $C_{28}H_{20}N_6O_8S_2$, M.wt. 632.62, Calcd.: C, 53.16, H, 3.19, N, 13.28. Found, C, 53.20, H, 3.22, N, 13.25. ^{1}H-NMR: 2.45 (3H, s, CH_3), 3.69 (1H, s, CH of thiazolidinone), 3.78 (3H, s, OCH_3) 7.20-8.85 (11H, m, Ar-H + C=CH) and at 10.15, 11.50 (2H, 2s, 2OH). MS (ESI+): *m/z* = 632.62, (45.3%).

General method for preparation of compounds 7a, b: A solution of compound **3** (3.20 g, 0.01 mol) and sulfonyl chloride derivatives (0.01 mol), namely, benzene sulfonyl chloride and toluene sulfonyl chloride in 20 mL methanol containing 1mL pyridine was refluxed for 4-6 h. The formed solid, after cooling was filtered, washed with water, air dried and crystallized from the proper solvent.

5-Benzensulfonamido-3-(methylthio)-1-(2, 4-dinitrophenyl)-*1H*-pyrazole-4-carbonitrile (7a): Yield 69%, crystallized from ethanol to give brown crystals, m.p. 135-7°C, Analysis: for $C_{17}H_{12}N_6O_6S_2$, M.wt. 460.44, Calcd.: C, 44.34, H, 2.63, N, 18.25. Found: C, 44.37, H, 2.65, N, 18.30. IR: 3390 (NH), 3234 (C-H Ar), 2222 (CN), 1615 (C=N), 1510 (C=C) and at 1280 (C-S-C). ^{1}H-NMR: 2.50 (3H, s, CH_3), 7.15-8.10 (8H, m, Ar-H) and at 9.75 (1H, s, NH).

3-(Methylthio)-1-(2,4-dinitrophenyl)-5-(tosylamino)-*1H*-pyrazole-4-carbonitrile(7b): Crystallized from acetic acid to give red crystals, m.p. 160-163°C, yield 65%. Analysis: for $C_{18}H_{14}N_6O_6S_2$, M.wt.474.47, Calcd.: C, 45.57, H, 2.97, N, 17.71. Found: C, 45.51, H, 2.85, N, 17.75. IR: 3405 (NH), 3240 (C-H Ar), 2218 (CN), 1610 (C=N), 1525 (C=C) and at 1285 (C-S-C). ^{1}H-NMR: 2.35 (3H, s, CH_3), 2.55 (3H, s, CH_3-ph), 7.25-8.15 (7H, m, Ar-H) and at 10.15 (1H, s, NH).

3-(Methylthio)-1-(2,4-dinitrophenyl)-*1H*-pyrazolo[3,4-d]pyrimidin-4(5H)-one (8): A solution of compound **3** (3.2 g, 0.01 mol) and formic acid 15 mL was refluxed for 5-8 h. The formed solid, after cooling was filtered, washed with water, air dried and crystallized from acetic acid to give **8**, m.p. 169-171°C, yield 73%. Analysis: for $C_{12}H_8N_6O_5S$, M.wt. 348.29, Calcd.: C, 41.38, H, 2.32, N, 24.13. Found: C, 41.41, H, 2.35, N, 24.17. IR: 3320 (NH), 2217 (CN), 1725 (C=O), 1617 (C=N) and 1520 (C=C). ^{1}H-NMR: 2.40 (3H, s, CH_3), 7.20-7.75 (3H, dd and s, Ar-H), 8.90 (1H, s, CH of pyrimidinone), 10.35 (1H, s, NH). MS (ESI+): *m/z* = 348.29 (21.89%).

4-Chloro-3-(methylthio)-1-(2,4-dinitrophenyl)-1H-pyrazolo[3,4-d] pyrimidine (9): A mixture of compound **8** (3.48 g, 0.01 mol), phosphorus penta chloride (0.01 mol) and phosphorus oxychloride (5 mL) was heated under reflux on a water bath for 3 h and the mixture was poured slowly on crushed ice. The formed solid, after cooling was filtered, washed with water, air dried and crystallized from methanol to give yellowish brown crystals **9**. Yield 59%, crystallized from methanol, m.p. 130-3°C, Analysis: for $C_{12}H_9ClN_6O_4S$, M.wt. 368.76, Calcd: C, 39.09, H, 2.46 N, 22.79 Found, C, 39.12, H, 2.43, N, 22.75. IR: 3330 (NH), 1725 (C=O), 1622 (C=N), 1510 (C=C) and 1300 (C-S-C). ^{1}H-NMR: 2.35 (3H,s, CH_3), 7.55-8.20 (3H, dd and s, Ar-H), 8.75 (1H,s, CH of pyrimidin).

Synthesis of fused pyrazolo [3, 4-d] pyrimidine derivatives (10-12): A solution of compound **9** (3.66 g, 0.01 mol), anthranilic acid (0.015 mol) in 30 mL butanol was heated under reflux for 12 h. The product obtained was crystallized from ethanol to give compound **10**.

Compound 10: Yield 71% crystallized from methanol to give brown crystals, m.p. 143-145°C, Analysis: for $C_{19}H_{11}N_7O_5S$, M.wt. 494.40, Calcd: C, 50.78, H, 2.47, N, 21.82. Found: C, 50.90, H, 2.61, N, 21.90. IR: 3210 (C-H Ar), 1710 (C=O), 1617 (C=N), 1520 (C=C) and 1300 (C-S-C). ^{1}H-NMR: 2.45 (3H, s, CH_3), 7.20-8.65 (7H, m, Ar-H) and 9.10 (1H, s, CH of pyrimidin).

Compound 11: A solution of compound **9** (3.66 g, 0.01mol) and sodium azide (0.05 mol) in 30 mL glacial acetic acid was refluxed for 3 h, The product obtained was crystallized from chloroform/ petroleum ether to give orange crystals**,** yield 59% crystallized from chloroform/petroleum ether, m.p. 186-8°C, Analysis: for $C_{12}H_7N_9O_4S$, M.wt. 373.30, Calcd.: C, 38.65, H, 1.89, N, 33.77. Found: C, 38.70, H, 1.59, N, 33.83. ^{1}H-NMR: 2.40 (3H, s, CH_3), 7.25-8.30 (3H, dd and s, Ar-H) and 8.95 (1H, s, CH of pyrimidin). MS (ESI+): *m/z* = 373.03 (9.75%).

Compound 12: A solution of compound **9** (3.66 g, 0.01mol) and glycine (0.01 mol) in 30 mL n-butanol was heated under refluxed for 3 h, the solid separated was refluxed with acetic anhydride for 2h,The product obtained was filtered off crystallized from methanol to give **12,** yield 69%. Crystallized from ethanol/DMF to give brown crystals, m.p. 280-3°C, Analysis: for $C_{14}H_9N_7O_5S$, M.wt. 387, Calcd: C, 43.40, H, 2.34, N, 25.31. Found:C, 43.45, H, 2.51, N, 25.42. IR: 3122 (C-H Ar), 1718 (C=O), 1609 (C=N), 1535 (C=C) and 1285(C-S-C). ^{1}H-NMR: 2.40 (3H, s, CH_3), 2.65 (2H, s, CH_2 of imidazolone) 7.35-8.10 (3H, dd and s, Ar-H) and at 8.90 (1H, s, CH-pyrimidin).

3-(Methylthio)-1-(2, 4-dinitrophenyl)-N-phenyl-*1H*-pyrazolo[3,4-d]pyrimidin-4-amine (13): A solution of compound **9** (3.66 g, 0.01 mol) and aniline (0.02 mol) in 30 mL butanol was heated under reflux for 6 h. The formed

solid, after cooling was filtered, washed with water, air dried and crystallized from acetic acid to give **11** as dark red crystals, yield 71%, m.p. 146-8°C, Analysis: for $C_{18}H_{13}N_7O_4S$, M.wt. 423.41, Calcd.: C, 51.06, H, 3.09, N, 23.16. Found: C, 51.16, H, 3.13, N, 23.22. IR: 3345 (NH), 3122 (CH-Ar), 1618 (C=N), 1545 (C=C) and. ^{1}H-NMR: 2.35 (3H, s, 3H, CH_3), 7.35-8.20 (8H, m, Ar-H), 8.85 (1H, s, H-pyrimidin), 10.70 (1H, s, NH).

Antitumor Screening: Chemotherapy is a major therapeutic approach for the treatment of both localized and metastasized cancers. In the present work selected compounds related to pyrazolopyrimidine derivatives were evaluated as inhibitors of the growth of liver cancer (HEPG2) cell line in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin as a trial to get more effective and less toxic agent.

Preliminary experiments were done using the human tumor cell line to identify the potential toxicity of eight selected newly synthesized compounds (**4b, 5b, 5c, 6a, 6b, 7b, 10** and **11**) in comparison to the known anticancer drugs 5-Flurouracil and Doxorubicin by SRB using the method Skehan *et al.* [4].

- Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hrs before treatment with compounds to allow attachment of cell to the wall of the plate.
- Different concentration of the compound under test (0, 1, 2.5, 5 and 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 hrs at 37°C and in atmosphere of 5% CO_2.
- After 48 hrs, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer.
- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

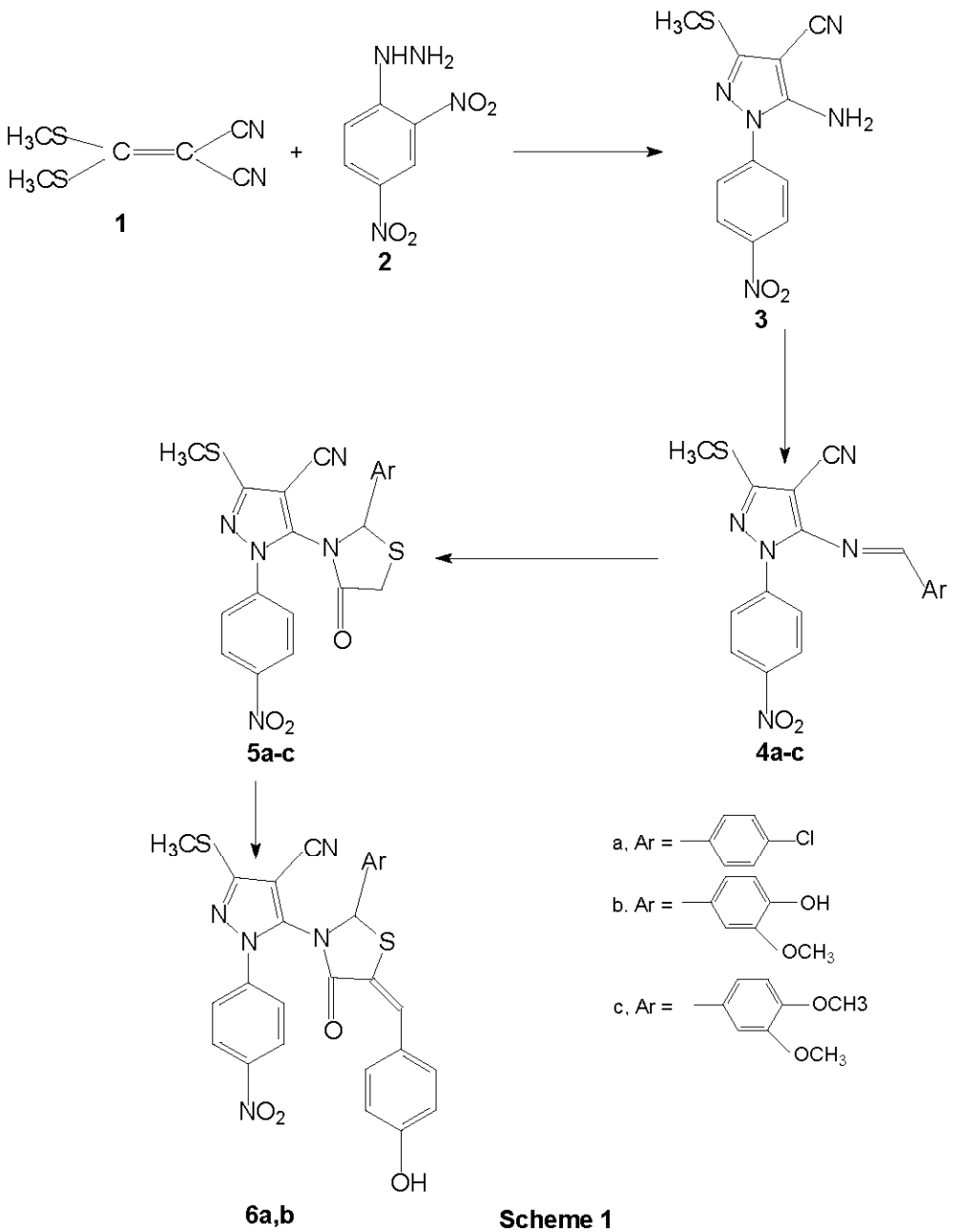
RESULTS AND DISCUSSION

The starting compound, namely, 5-amino-3-(methylthio)-1-(2,4-dinitro-phenyl)-1*H*-pyrazolo-4-carbonitril (**3**) was prepared in a good yield (85%) by the heating of ketene **(1)** with 2,4-dinitrophenyl hydrazine **(2)** in basic medium [5-7] according to Afred Kreutzberger *et al.* [8]. It has been reported that Schiff's bases have gained promise pharmacological activity [9, 10]. So, it was of interest to synthesize some Schiff's bases incorporated to pyrazole moiety. So compound **3** condensed with different aromatic aldehydes, namely, 4-chlorobenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and 3, 4-dimethoxybenzaldehyde in the presence of few drops of acetic acid to give compounds **4a-c,** respectively. Also cyclocondensation [11] of **4a-c** with thioglycolic acid in dry benzene gave the corresponding thiazolidinone derivatives **5a-c** (Scheme 1). Which in turn could be condensed with 4-hydroxybenzaldehyde to give arylidine derivatives [12].

On the other hand, compound **3** react with benzene sulfonyl chloride and/or toluene sulfonyl chloride to give the corresponding sulphamides **7a, b**. Also, our goal is to develop new general and convenient procedures for the preparation of fused pyrazolo [3, 4-d] pyrimidine derivatives [13]. Thus, the interaction of **3** with formic acid lead to formation of 3-(methylthio)-1-(2, 4-dinitrophenyl)-1*H*-pyrazolo [3,4-d] pyrimidin-4(5H)-one (**8**). The chloronation of the latter compound using phosphorus oxychloride and phosphorus pentachloride gave 4-chloro-3-(methylthio)-1-(2, 4-dinitrophenyl)-1*H*-pyrazolo [3,4-d] pyrimidine (**9**) (Scheme 2). In recent series of publication [14, 15], it has been reported that, 4-chloropyrazolo [3,4-d] pyrimidine is a useful precursor in synthesis of fused nitrogen bridged benzopyrimidine pyrazolopyrimidine. Similarly, we found that, the reaction of compound **9** with anthranilic acid in boiling butanol gave **10**. Furthermore, El-Hashash *et al.* [16] and Deeb *et al.* [17], when compound **9** was treated with sodium azide in boiling acetic acid we got compound **11**. Also, on treatment of compound **9** with glycine in boiling butanol and the ring closure by AC_2O gave compound **12**). Finally, compound **9** was reacted with aniline in boiling butanol to give the corresponding 4-anilinopyrazolo [3, 4-d] pyrimidine **13** (Scheme 2).

Biological Activity

Antibacterial Activity Test: Thirteen extract diluted in DMSO with conc. 300 μg/ml, 50μl of each diluted extract were added into wells of 6mm diameter formed into Muller Hinton agar plates (ref 300μg/). Tobramycin (10 μg/ml) was used as control positive broad spectrum antibiotic. DMSO was used as control negative. Strains selected for being tested are isolated from mastitic cow milk

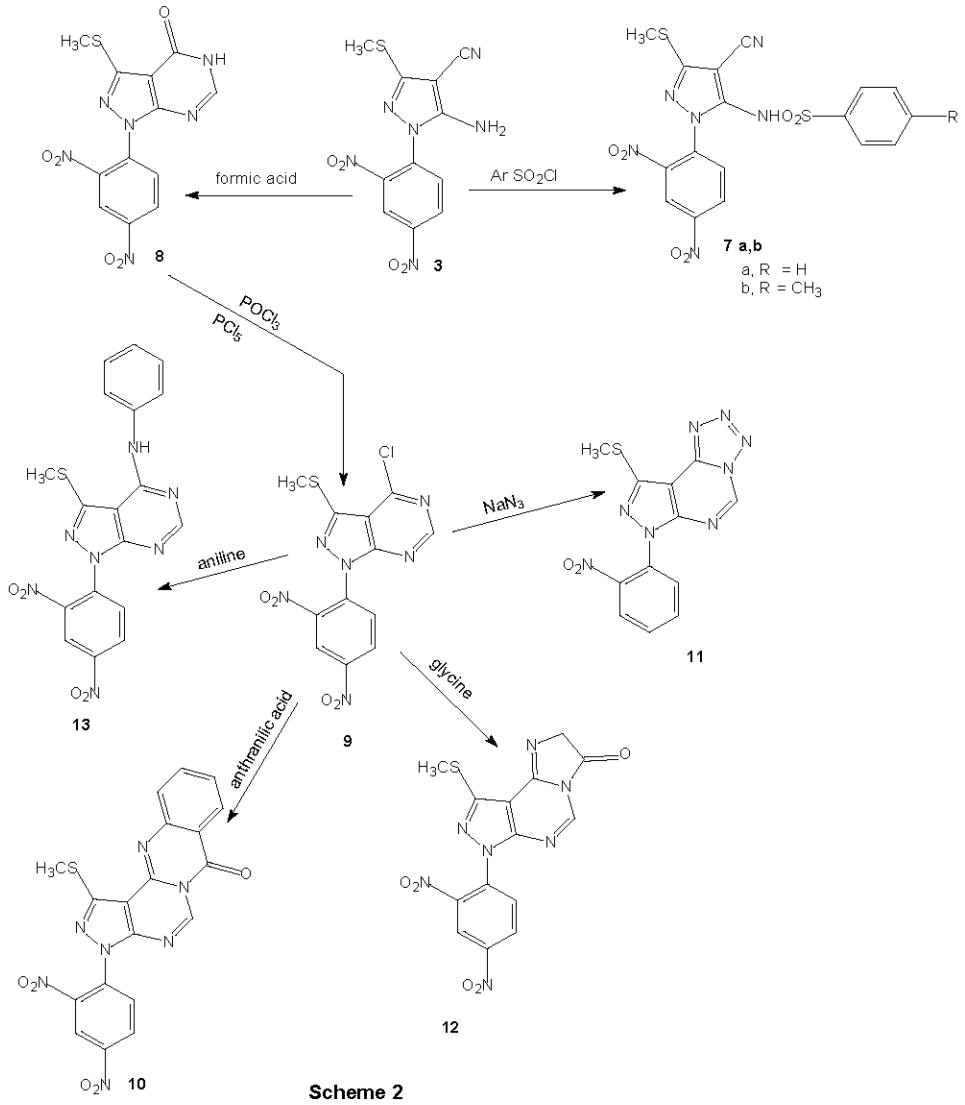


Scheme 1

Scheme 1:

including *E.coli*, *Salmonella*, *L.monocytogenes*, *S.aureus*, *Ps. aerigenosus* and *B. cereus*. These strains are commonly accused of being a cause of food poisoning and diarrhea in human. Muller Hinton agar plates were inoculated with strains prepared in conc. equivalent with 0.5 MacFerland and streaked onto the agar plates using sterile swabs, then 50µl of the prepared extract were added in each well previously formed into the agar using sterile pasture pipette. All plates were incubated at 37°C/24hrs (Well diffusion assay) [18]. Zone of inhibition were measured in mm using a ruler. The experiment was carried out in triplicate and the mean of the zone of inhibition was tabulated in Table 1.

Results revealed that extract no. **8, 10** then **5a** gives the highest antibacterial activity against all tested strains with a mean zone of inhibition equal 19.7, 19.3 and 18.2



Scheme 2

Scheme 2:

Table 1: The preliminary antimicrobial screening test for the prepared compounds using Tobramycin and DMSO as control

Tested strains	*E.coli*	*Salmonella*	*L. monocytogenes*	*S. aureus*	*Ps. areginosus*	*B. cereus*	Mean zone of inhibition
Tested chemicals							
3	13	15	18	14	15	13	14.7
4a	_	_					
4b	13	15	13	13	14	15	13.8
4c	11	15	12	10	11	12	10.3
5a	23	18	23	19	18	18	18.2
5b	14	19	13	10	12	13	13.5
6b	_	_					
7a	23	18	16	13	23	14	17.8
7b	11	15	13	15	14	16	14
8	21	17	27	18	17	18	19.7
9	23	16	13	11	18	13	15.7
10	13	19	23	18	18	25	19.3
11	13	19	14	13	16	18	15.5
Tobramycin (10µg/ml)	+++	++	++	+++	+++	++	
DMSO	_	_	_	_	_	_	

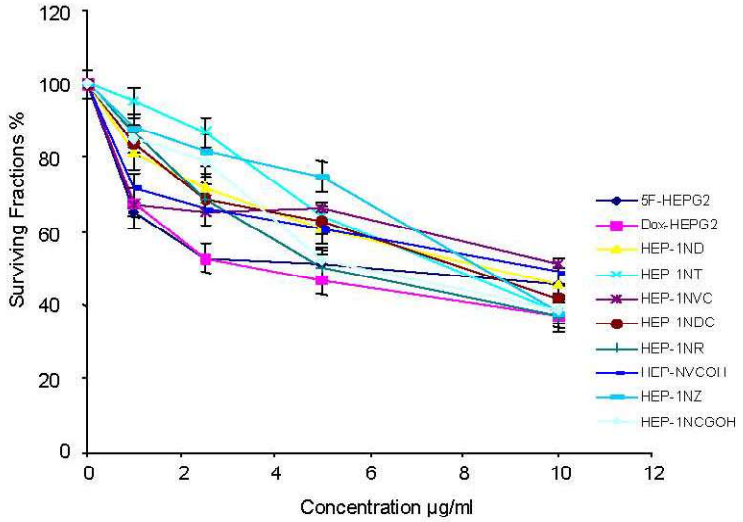


Fig. 1: Cytotoxic effect of (4b,5b,5c,6a,6b,7b,10 and 11) on liver cancer HEPG2 compared to 5-Flurouracil and Doxorubicin

Table 2: Effect of some selected newly synthesized compounds on liver carcinoma cell line (HEPG2)

Comp.	IC_{50}
5-Flurouracil	5 µg/ml
Doxorubicin	3.56 µg/ml
4b	8.57 µg/ml
5b	8.27 µg/ml
5c	10.00 µg/ml
6a	5.75 µg/ml
6b	9.62 µg/ml
7b	7.64 µg/ml
10	4.91 µg/ml
11	8.45 µg/ml

IC50: dose of the compound which reduces survival to 50%

respectively. Followed by extract no. **7a** (17.8), then no. **9** (15.7), then no.**11** and **3** (14.7) and finally no. **7b** (14). On the contrary extract no. **4a** and **6b** showed no antibacterial activity against all tested strains results revealed that all tested extracts were highly effective against salmonella species which showed the widest zone of inhibition.

CONCLUSION

The antitumor activity results indicated that all the eight derivatives showed antitumor activity against the tested liver cancer (HEPG2) cell line but with varying intensities in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. Moreover compound **10** showed the highest cytotoxic activity (IC_{50} equals 4.91 µg/ml) which was more effective than 5-Flurouracil (IC_{50} equal 5 µg/ml), while compound 6a was slightly higher than 5-Flurouracil (IC_{50} equal 5.75 µg/ml). Results were illustrated in Table 2 and Fig. 1 for the cytotoxic activities of the eight compounds (**4b, 5b, 5c, 6a, 6b, 7b, 10** and **11)** in comparison to 5-Flurouracil and Doxorubicin.

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