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Synthesis and In-vitro Biological Analyses of New quinazolin-2,4-dioneDerivatives

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Abstract

A series of quinazolin-2,4-dione analogues (1-17a,b) were prepared and their chemical structures were confirmed using spectral and microanalytical data. Analysis of the in vitro cytotoxic effect of the synthesized compounds using RBCs toxicity assay was achieved. Also, biological screening on their toxicity, their potential anticancer effect on hepatocellular carcinoma cell line (HepG2), and their antimicrobial activity against three microbes (bacteria, yeast, and fungus) were studied. Results of RBCs toxicity showed that synthesized compounds 1, 2b, 2d, 2e, 4, 6, 9, and 14 were non-toxic at a concentration of 100 µg/ml. In addition, only two compounds 5 and 17b had a moderate anti-HCC activity with calculated IC50 equals 382.9±6.9 µg/ml and 415.8±3.8 µg/ml, respectively in comparison to DOX anticancer drug which had IC50 equals 6.14±0.32 µg/ml. Compound 9 had the highest anti-*Aspergillus niger* effect, compound 5 had the highest anti-Candida albicans effect, compound 6 had the highest anti-Staphylococcus aureus effect, and compound 1 had the highest anti-Pseudomonas aeruginosa effect.

Keywords: Quinazolin-2,4-diones, HCC, RBCs toxicity, antimicrobial.

Introduction

Previous studies on Quinazolines and their derivatives proved their antitumor effect via different examined pathways as its inhibitory effect on receptor tyrosine kinases (e.g. epidermal growth factor receptor; EGFR), tubulins, and phosphatidylinositol-3-kinases; PI3K [1-3].

We focused on studying the anticancer effect on Hepatocellular carcinoma (HCC) because it is a worldwide problem, with epidemiological data varying from place to place. 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 [4-6].

Herein we studied the cytotoxic effect of a series of newly synthesized Quinazoline derivatives by measuring cellular damage as red blood cells (RBCs) appear to be an excellent model for evaluating the toxicity of natural or synthetic molecules. A compound's hemolytic activity is an indicator of its overall cytotoxicity to normal cells. RBCs are an excellent research model because they lack internal organelles, making them an ideal cell system for studying fundamental drug–membrane interactions. As a result, erythrocytes perform the basic functions assigned to the cell membrane while also having a simpler structure than other cell membranes [7].

Another biological activity of the synthesized Quinazolin-2,4-dione in this study was tested on different microbes. In recent years, there has been a massive increase in interest in researching and developing new antimicrobial agents derived from a variety of sources to combat microbial resistance. As a result, more emphasis has been placed on antimicrobial activity screening and evaluation methods [8].

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Materials and methods

Chemistry

The reagents used in this work were used without purification. Melting points of the prepared compounds were determined using Griffin apparatus and are uncorrected. The completion of the reactions was mentioned by Thin Layer Chromatography TLC technique on aluminum plates coated with silica gel. IR spectra were recorded on Shimadzu 408 and Bruker Vect. 22. NMR spectra were run at 400 MHz using TMS as the internal standard. The chemical shifts were measured in ppm (δ) related to TMS (0.00 ppm). MS were recorded on an HP model, Mass 5988 Mass spectrometer at 70 eV. All new compounds were analyzed for C, H, and N at Cairo University, Egypt.

Synthesis

Compounds **1-17a** and **17b** were synthesized as previously mentioned [9]

Spectral analyses of the synthesized compounds 1-17a,b

4.1.2. 4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzoic acid hydrazide **1**

FT-IR (KBr, υ, cm^{-1}) 3322 (NH), 3207 (NH_2), 1723, 1651 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.94 (d, 2H, Ar-H), 7.64 (d, 2H, Ar-H), 7.20-7.24 (m, 4H, Ar-H), 5.4 (s, 2H, NH_2). ^{13}C-NMR ($CDCl_3$): δ 113.6, 113.8, 115.1, 115.3, 125.0, 127.5, 128.6, 128.8, 131.5, 133.8, 135.5, 139.3, 150.0, 161.2, 164.9. MS (EI): m/z= 296.21 $[M]^+$. Anal. Calcd for $C_{15}H_{12}N_4O_3$: C, 60.81; H, 4.08; N, 18.91%. Found C, 60.86; H, 4.18; N, 19.08 %.

4.1.3. Schiff bases **2a-e**

2a: FT-IR (KBr, υ, cm^{-1}) 3197 (NH), 1724, 1662 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.68 (d, 2H, Ar-H), 7.57-8.74 (m, 10H, Ar-H+CH), 7.24 (d, 2H, Ar-H). ^{13}C-NMR (DMSO d_6, 100 MHz): δ 113.6, 113.8, 115.1, 115.3, 125.0, 127.5, 128.1, 128.3, 128.6, 128.7, 128.8, 128.9, 129.0, 131.7, 133.8, 135.5, 136.6, 139.3, 142.5, 150.0, 161.2, 161.8. MS (EI): m/z= 383.15 $[M]^+$. Anal. Calcd for $C_{22}H_{16}N_4O_3$: C, 68.74; H, 4.20; N, 14.58%. Found C, 68.86; H, 4.48; N, 14.77 %.

2b: FT-IR (KBr, υ, cm^{-1}) 3290 (NH), 1719, 1598 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 8.08 (d, 2H, Ar-H), 8.06 (d, 2H, Ar-H), 7.51 (d, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.22-7.98 (m, 5H, Ar-H+CH), 2.38 (s, 3H, CH_3). ^{13}C-NMR (DMSO d_6, 100 MHz): δ 22.6, 113.6, 113.8, 115.1, 115.3, 125.0, 127.5, 128.1, 128.3, 128.6, 128.7, 128.8, 128.9, 129.0, 131.7, 136.6, 139.3, 142.5, 149.2, 160.0, 172.8, 192.1. MS (EI): m/z= 398.42 $[M]^+$. Anal. Calcd for $C_{23}H_{18}N_4O_3$: C, 69.09; H, 4.11; N, 13.38 %. Found C, 69.36; H, 4.55; N, 14.06 %.

2c: FT-IR (KBr, υ, cm^{-1}) 3299 (NH), 1722, 1600 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.25-8.77 (m, 5H, Ar-H+CH), 8.06 (d, 2H, Ar-H), 7.95 (d, 2H, Ar-H), 7.71 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H). MS (EI): m/z= 418.60 $[M]^+$. Anal. Calcd for $C_{22}H_{15}N_4O_3$: C, 63.09; H, 3.61; N, 13.38%. Found C, 63.76; H, 3.68; N, 13.58 %. **2d:** FT-IR (KBr, υ, cm^{-1}) 3310 (NH), 1723, 1615 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.25-8.40 (m, 5H, Ar-H+CH), 8.06 (d, 2H, Ar-H), 7.95 (d, 2H, Ar-H), 7.71 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H). MS (EI): m/z= 431.12 $[M]^+$. Anal. Calcd for $C_{22}H_{15}N_5O_5$: C, 61.54; H, 3.52; N, 16.31%. Found C, 61.96; H, 3.72; N, 16.68 %.

2e: FT-IR (KBr, υ, cm^{-1})= 3312 (NH), 1720, 1610 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.03-8.61 (m, 5H, Ar-H+CH), 8.00 (d, 2H, Ar-H), 7.93 (d, 2H, Ar-H), 7.69 (d, 2H, Ar-H), 7.34 (d, 2H, Ar-H), 2.38-2.51 (s, 3H, CH_3). MS (EI): m/z= 398.42 $[M]^+$. Anal. Calcd for $C_{23}H_{18}N_4O_4$: C, 69.34; H, 4.55; N, 14.06%. Found C, 60.56; H, 4.78; N, 14.18 %.

2f: FT-IR (KBr, υ, cm^{-1})= 3322 (NH), 1718, 1615 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.99 (d, 2H, Ar-H), 7.61-7.67 (dd, 1H, HA), 7.46-8.84 (m, 5H, Ar-H+CH), 7.39 (d, 2H, Ar-H), 7.24-7.26 (dd, 1H, H_M), 7.11-7.16 (dd, 1H, H_X).MS (EI): m/z= 390.69 $[M]^+$. Anal. Calcd for $C_{20}H_{14}N_4O_3S$: C, 67.34; H, 4.55; N, 14.06%. Found C, 67.67; H, 4.78; N, 14.18 %.

4.1.4. Imides **3a-c**

3a: FT-IR (KBr, υ, cm^{-1}) 3322 (NH), 1715, 1602 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.96 (d, 2H, Ar-H), 7.71 (d, 2H, Ar-H),7.25-8.06 (m, 8H, Ar-H). MS (EI): m/z= 426.20 $[M]^+$. Anal. Calcd for $C_{23}H_{14}N_4O_5$: C, 64.79; H, 3.31; N, 13.14%. Found C, 64.86; H, 3.48; N, 13.14 %. **3b:** FT-IR (KBr, υ, cm^{-1}) 3325 (NH), 1715, 1605 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.36-8.06 (m, 4H, Ar-H), 7.56 (d, 2H, Ar-H), 7.26 (d, 2H, Ar-H). MS (EI): m/z= 562.21 $[M]^+$. Anal. Calcd for $C_{23}H_{10}Cl_4N_4O_5$: C, 48.79; H, 1.79; N, 9.93%. Found C, 49.86; H, 2.18; N, 10.08 %. **3c:** FT-IR (KBr, υ, cm^{-1}) 3312 (NH), 1715, 1625 (C=O's). MS (EI): m/z= 296.21 $[M]^+$. Anal. Calcd for $C_{27}H_{16}N_4O_5$: C, 48.79; H, 1.79; N, 9.93%. Found C, 49.86; H, 2.18; N, 10.08 %.

4.1.5. N-(1,4-Dioxo-3,4-dihydro-1H-phthalazin-2-yl)-4-(2,4-dioxo-1,4-dihydro-2H-quina zolin-3-yl)-benzamide **4**

FT-IR (KBr, υ, cm^{-1}) 3330 (NH), 1700, 1615 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 10.5 (s, 1H, NH), 10.1 (s, 1H, NH), 7.3-8.06 (m, 12H, Ar-H). MS (EI): m/z= 296.21 $[M]^+$. Anal. Calcd for $C_{23}H_{15}N_5O_5$: C, 60.81; H, 4.08; N, 18.91%. Found C, 60.86; H, 4.18; N, 19.08 %.

4.1.6. 4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzoic acid ethoxymethylene -hydrazide **5**

FT-IR (KBr, υ, cm^{-1}) 3300 (NH), 1620, 1590 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.4 (s, 1H, NH), 10.4 (s, 1H, NH), 7.94 (d, 2H, Ar-H), 7.65 (d, 2H, Ar-H), 7.23-7.94 (m, 4H, Ar-H), 5.4 (q, 2H, CH_2), 2.0 (s, 1H, CH), 1.8 (t, 3H, CH_3). MS (EI): m/z= 352.35 $[M]^+$. Anal. Calcd for $C_{18}H_{16}N_4O_4$: C, 61.36; H, 4.58; N, 15.91%. Found C, 61.50; H, 4.68; N, 16.08 %.

4.1.7. 3-[4-(3,5-Dimethyl-pyrazole-1-carbonyl)-phenyl]-1H-quinazolin-2,4-dione **6**

FT-IR (KBr, υ, cm^{-1}) 3327 (NH), 1725, 1619 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.51 (s, 1H, NH), 8.07 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H), 7.25-7.96 (m, 5H, Ar-H+pyrazole CH), 1.36 (s,6H,2CH_3). ^{13}C-NMR (DMSO d_6, 100 MHz): δ 22.6, 33.3, 103.2, 103.4, 113.6, 113.8, 115.1, 115.3, 125.0, 127.5, 128.6, 128.8, 131.5, 133.8, 135.5, 139.3, 150.0, 161.2, 164.9. MS (EI): m/z= 352.33 $[M]^+$. Anal. Calcd for $C_{20}H_{16}N_4O_3$: C, 63.3 5 H, 4.38; N, 17.38%. Found C, 63.46; H, 4.57; N, 17.68 %.

4.1.8. 3-(4-(3-Methyl-5-oxo-4,5-dihydro-pyrazole-1-carbonyl)-phenyl]-1H-quinazolin-2,4-dione **7**

FT-IR (KBr, υ, cm^{-1}) 3318 (NH), 1725, 1619 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.60-8.06 (m, 4H, Ar-H), 7.39 (d, 2H, Ar-H), 7.31 (d, 2H, Ar-H), 4.2 (s, 2H, CH_2), 1.3 (s, 1H, CH_3). ^{13}C-NMR (DMSO d_6, 100 MHz): δ 22.6, 103.4, 113.6, 113.8, 115.1, 115.3, 125.0, 127.5, 128.6, 128.8, 131.5, 133.8, 135.5, 139.3, 150.0, 156.3, 161.2, 164.9. MS (EI): m/z= 362.11 $[M]^+$. Anal. Calcd for $C_{19}H_{14}N_4O_4$: C, 63.49; H, 4.79; N, 14.81%. Found C, 6356; H, 4.82; N, 14.90 %.

4.1.9. 4-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N'-formylbenzohydrazide **8**

FT-IR (KBr, υ, cm^{-1}) 3315 (NH), 1720, 1615 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.60 (s, 1H, NH), 10.61 (s, 1H, CHO), 10.0 (s, 1H, NH), 8.31 (s, 1H, NH), 7.97 (d, 2H, Ar-H), 7.72 (d, 2H, Ar-H),7.25-8.31 (m, 4H, Ar-H).MS (EI): m/z= 324.1 $[M]^+$. Anal. Calcd for $C_{16}H_{12}N_4O_4$: C, 66.23; H, 3.92; N, 9.091%. Found C, 66.56; H, 4.10; N, 9.28 %.

4.1.10. 2-(4-(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)benzoyl)-N-phenylhydrazine-1-carbothioamide **9**

FT-IR (KBr, υ, cm^{-1}) 3298 (NH), 1774, 1619 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)= 11.5 (s, 1H, NH), 11.4 (s, 1H, NH), 9.8 (s, 1H, NH), 9.6 (s, 1H, NH), 7.95 (d, 2H, Ar-H), 7.66 (d, 2H, Ar-H), 7.15-8.08 (m, 9H, Ar-H). MS (EI): m/z= 431.41 $[M]^+$. Anal. Calcd for $C_{22}H_{17}N_5O_3S$: C, 69.17; H, 4.29; N, 10.52%. Found C, 69.19; H, 4.36; N, 10.68 %.

4.1.11. 3-(4-[1,3,4] Thiadiazol-2-yl-phenyl)-1H-quinazolin-2,4-dione **10**

FT-IR (KBr, υ, cm^{-1}) 3320 (NH), 1770, 1625 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.62 (s, 1H, NH), 7.94 (d, 2H, Ar-H), 7.92 (d, 2H, Ar-H), 7.19-8.57 (m, 5H, Ar-H+CH). MS (EI): m/z= 322.12 $[M]^+$. Anal. Calcd for $C_{16}H_{10}N_4O_2S$: C, 65.81; H, 4.22; N, 16.91%. Found C, 65.96; H, 4.38; N, 17.08 %.

4.1.12. 3-[4-(5-Phenylamino-[1,3,4]thiadiazol-2-yl)-phenyl]-1H-quinazolin-2,4-dione **11**

FT-IR (KBr, υ, cm^{-1}) 3299 (NH), 1760, 1628 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 7.94 (d, 2H, Ar-H), 7.92 (d, 2H, Ar-H), 7.3-8.06 (m, 10H, Ar-H+NH). MS (EI): m/z= 413.31 $[M]^+$. Anal. Calcd for $C_{22}H_{15}N_5O_2S$: C, 63.14; H, 5.30; N, 14.72%. Found C, 63.26; H, 5.42; N, 14.80 %.

4.1.13. 3-[4-(5-Mercapto-4-phenyl-4H-[1,2,4]triazol-3-yl)-phenyl]-1H-quinazolin-2,4-dione **12**

FT-IR (KBr, υ, cm^{-1}) 3325 (NH), 1774, 1618 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 10.7(s, 1H, SH), 7.92 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H), 7.3-8.61 (m, 9H, Ar-H). MS (EI): m/z= 413.41 $[M]^+$. Anal. Calcd for $C_{22}H_{15}N_5O_2S$: C, 64.11; H, 5.18; N, 14.51%. Found C, 64.26; H, 5.48; N, 14.78 %.

4.1.14. 4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-N-(4-oxo-2-p-tolyl-thiazolidin-3-yl)-benzamide **13**

FT-IR (KBr, υ, cm^{-1}) 3324 (NH), 1760, 1623 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 8.07 (d, 2H, Ar-H), 7.96 (d, 2H, Ar-H), 7.71 (d, 2H, Ar-H), 7.51 (d, 2H, Ar-H), 7.25-7.95 (m, 4H, Ar-H), 4.38 (s, 2H, CH_2), 2.3 (s, 1H, CH), 1.36 (s, 3H, CH_3). ^{13}C-NMR (DMSO d_6, 100 MHz): δ 21.2, 33.8, 66.2, 113.6, 113.8, 115.1, 115.3, 125.0, 127.5, 127.6, 127.8, 128.6, 128.8, 129.3, 129.5, 131.5, 133.8, 135.5, 136.9, 139.3, 139.7, 150.0, 161.2, 162.2, 167.2. MS (EI): m/z= 472.01 $[M]^+$. Anal. Calcd for $C_{25}H_{20}N_4O_4S$: C, 63.55; H, 4.27; N, 11.86%. Found C, 63.66; H, 4.38; N, 11.90 %.

4.1.15. N-(5-Benzylidene-4-oxo-2-p-tolyl-thiazolidin-3-yl)-4-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzamide **14**

FT-IR (KBr, υ, cm^{-1}) 3318 (NH), 1724, 1630 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.5 (s, 1H, NH), 8.07 (d, 2H, Ar-H), 7.96 (d, 2H, Ar-H), 7.71 (d, 2H, Ar-H), 7.47 (d, 2H, Ar-H), 7.19-8.27 (m, 10H, Ar-H+ olefinic CH), 2.28 (s, 1H, *sp3*CH), 1.36 (s, 3H, CH_3). MS (EI): m/z= 560.01 $[M]^+$. Anal. Calcd for $C_{32}H_{24}N_4O_4S$: C, 68.56; H, 4.31; N, 9.99%. Found C, 68.76; H, 4.41; N, 10.08 %.

4.1.16. N-(5-Amino-6-cyano-7-phenyl-2-p-tolyl-7H-pyrano[2,3-d]thiazol-3-yl)-4-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzamide **15**

FT-IR (KBr, υ, cm^{-1}) 3405 (NH), 3331 (NH_2), 1650, 1573 (C=O's). ^{1}H-NMR (DMSO d_6, 400 MHz): δ (ppm)=11.95 (s, 1H, NH), 7.98 (d, 2H, Ar-H), 7.94 (d, 2H, Ar-H), 7.64 (d, 2H, Ar-H), 7.25 (d, 2H, Ar-H), 7.23-8.77 (m, 11H, Ar-H+NH_2), 2.39-2.42 (d, 2H, 2CH), 1.3 (s, 3H, CH_3). MS (EI): m/z= 626.01 $[M]^+$. Anal. Calcd for $C_{35}H_{26}N_6O_4S$: C, 67.08; H, 4.18; N, 13.41%. Found C, 67.16; H, 4.28; N, 13.58 %.

4.1.17. 4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-N-[4-oxo-5-(phenyl-hydrazono)-2-p-tolyl-thiazolidin-3-yl]-benzamide **16**

FT-IR (KBr, υ, cm^{-1}) 3324 (NH), 1744, 1618 (C=O's). MS (EI): m/z= 544.64 $[M]^{+2}$. Anal. Calcd for $C_{31}H_{24}N_6O_4S$: C, 65.47; H, 4.36; N, 10.75%. Found C, 65.50; H, 4.48; N, 11.02 %.

4.1.18. N-[5-(4-Chloro-benzylidene)-4-oxo-2-p-tolyl-thiazolidin-3-yl]-4-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzamide **17a**

FT-IR (KBr, υ, cm^{-1}) 3354 (NH), 1651, 1600 (C=O's). MS (EI): m/z= 574.64 $[M]^+$ and 576.64 $[M^++2]$ due to the presence of chlorine atom. Anal. Calcd for $C_{32}H_{23}ClN_4O_4S$: C, 68.97; H, 4.56; N, 9.75%. Found C, 69.00; H, 4.68; N, 10.02 %.

4.1.19. 4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-N-[5-(4-methoxy-benzylidene)-4-oxo-2-p-tolyl-thiazolidin-3-yl]-benzamide **17b**

FT-IR (KBr, υ, cm^{-1}) 3322 (NH), 1750, 1620 (C=O's). MS (EI): m/z= 590.01 $[M]^+$. Anal. Calcd for $C_{33}H_{26}N_4O_5S$: C, 67.11; H, 4.44; N, 9.49%. Found C, 67.26; H, 4.58; N, 9.61 %.

Pharmacology

Cytotoxicity assay

One of the various cytotoxicity assays that assess the possible toxicity of red blood cells is hemolysis. The key cells in circulation are red blood cells, which are responsible for carrying oxygen; indeed, any modifications to this mechanism may be lethal. One toxicity evaluation technique is based on calculating the release of hemoglobin, called hemolysis, from suspended red blood cells. Therefore, the signal stabilization of the erythrocyte cell membrane is the loss of hemoglobin [10].

The blood was collected from a healthy human volunteer in a Heparin tube. The tube was centrifuged at 1000 rpm, 4 ºC for 5 min, and washed three times with an equal volume of PBS (pH 7.4). The volume of blood was measured and reconstituted as a 10% v/v suspension with PBS (pH 7.4). Samples at different concentrations were added to erythrocytes suspension at a ratio of 4:1. Sterile phosphate buffer saline received the negative control, while 0.1 percent Triton X-100 received positive control because it induces swelling, followed by erythrocyte hemolysis. After incubation for 30 min at 37 ºC, centrifugation was carried out at 1000 rpm, 4 ºC for 3 min. Spectrophotometric measurement detected the hemoglobin release at 540 nm [11].

The hemolysis percentage was calculated using the following equation:

% hemolysis= (Abs of sample-Abs of (-) control)/Abs of (+) control x 100

All experiments were performed in triplicate and mean values were used for the calculation

The degree of *in vitro* cytotoxicity to hemolytic activity is evaluated using the mortality rate observed: 0% to 9%= non-toxic; 10% to 49% = slightly toxic; 50% to 89% = toxic; and 90% to 100% = highly toxic. The LC0-9 is referred to as a non-toxic concentration [12].

Anticancer activity

The anti-hepatocellular carcinoma (HCC) effect of the samples was analyzed using the cell line HepG2 (ATCC® CCL-75™, Vacsera, Egypt). The cells were inoculated without any samples on 96-well plates ($1x10^4$ cells/100 µl medium/well). The RPMI 1640 medium (Biowest, USA), was supplemented with 10% inactivated fetal bovine serum (FBS) filter-sterilized (LONZA), 1% antibiotics (penicillin/streptomycin) fungizone solution (LONZA), 1% of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer 1M (Biowest, USA. After 24 h incubation at 37 ºC and 5% CO_2, the medium was aspirated after and the samples were diluted in an RPMI medium maintenance medium containing 2% FBS, 1% antibiotic solution, and 1% HEPES) at varying concentrations (500, 250, 100, and 50 µg/ml). Doxorubicin (Adriamycin) was used as the standard drug. Samples were applied to the cells and incubated for 24 h. The samples were collected from each well, and then cells were dyed with 20 µl of 0.5% crystal violet and incubated at room temperature for 10 min. Each well was washed 3

times with 200 µl distilled H_2O. After washing, the plate was inverted on filter paper to remove any remaining liquid. Two hundred microliters of methanol were added to each well, and the plate was incubated with its lid on for 20 min at room temperature. The absorbance was measured at 570 nm (OD570) using ELISA reader [13].

Percent of cell viability= (absorbance of treated cells/absorbance of control cells) x 100

Percent of cell cytotoxicity= 100 – percent of cell viability. All experiments were performed in triplicate and the mean values were used for calculation.

Antimicrobial activity

The samples were prepared by dissolving 2 mg in 2 ml of DMSO and 100µl (containing 100 µg) was used in this test. The antimicrobial activity of different synthesized compounds was investigated by the agar cup plate method. Four different test microbes namely: *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Candida albicans* (yeast), and *Aspergillus niger* (fungus) were used. Nutrient agar plates were heavily seeded uniformly with 1ml of 10^5-10^6 cells/ml in the case of bacteria and yeast. A Czapek-Dox agar plate seeded by the fungus was used to evaluate the antifungal activities. Then a hole was made in media by gel cutter (Cork borer no.4) in a sterile condition. Then one drop of melted agar was poured into the hole and allowed to solidify to make a base layer. After that specific amount of culture filtrate (0.1 ml) was poured into the hole. Then plates were kept at low temperature (4 °C) for 2-4 h to allow maximum diffusion. The plates were then incubated at 37 °C for 24 hours for bacteria and at 30°C for 48 h in an upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of the zone of inhibition expressed in millimeters (mm). The experiment was carried out more than once and the mean of reading was recorded [1].

Statistical Analysis

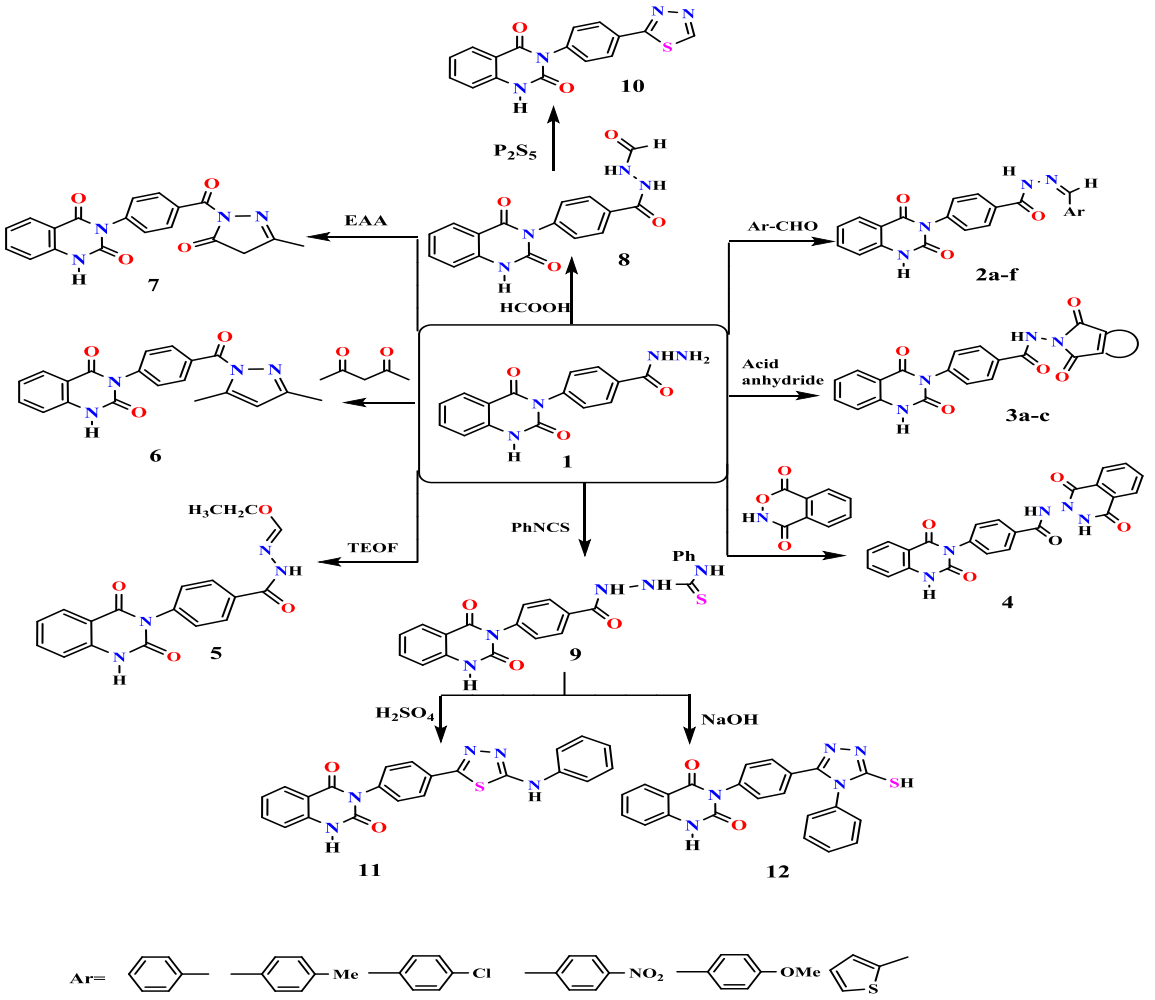
The test of significance was performed using the GraphPad Prism 8 (San Diego, California, USA). The experimental results were expressed as the mean ± Standard Deviation (SD). Group comparisons were performed using One way ANOVA test and two-way ANOVA of repeated measures (RM) were performed to determine the significant difference among the mean values of different groups. Multiple T-test was used to detect the relative significance between each sample and control at different concentrations. Statistical significance was determined using the Holm-Sidak method. The p-value $\leq$ 0.05 was considered statistically significant (^{a}), p-value $\leq$ 0.01 was considered highly significant (^{b}), and p-value $\leq$ 0.0001 was considered very highly significant (^{c}).

Results and discussion

In an extension of our ongoing efforts towards an investigation of novel bioactive heterocyclic compounds [6, 14-22], the present work is a follow to our previous study in which we synthesized a series of quinazolin-2,4-diones **1-17a** and **17b** [9]. Herein we decided to study their potential anticancer effect on hepatocellular carcinoma cell line (HepG2) and their antimicrobial activity against three microbes (bacteria, yeast, and fungus).

Chemistry

Carbohydrazide **1** was fabricated via the hydrazinolysis of 4-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzoic acid ethyl ester with hydrazine hydrate. The starting compound **1** was submitted to react with a series of chemical reagents to produce novel bioactive derivatives. Condensation of compound **1** with a series of selected aldehydes " benzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, 4-methoxybenzaldehyde (anisaldehyde), 4-methylbenzaldehyde, and thiophene-2-carboxaldehyde " yielded a series of Schiff base derivatives **2a-f** respectively. Moreover, the hydrazide **1** was submitted to react with the appropriate of various acid anhydrides like phthalic and tetrachlorophthalic, 1,8-naphthalic anhydride through a dehydrative condensation reaction, to afford the corresponding imides **3a-c**, respectively. Compound **4** was prepared via the reaction of compound **1** with Isatoic anhydride. On the other hand, hydrazide **1** was treated with triethyl orthoformate to afford gave 4-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzoic acid ethoxymethylene-hydrazide **5**. In addition, compounds **6** and **7** were synthesized *via* Knorr pyrazole synthesis by cyclization of compound **1** with β-di-ketone like acetylacetone and ethyl acetoacetate respectively, (Scheme 2). Compound **1** was refluxed with formic acid for 6 h to give 4-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)-benzoic acid N'-formyl-hydrazide **8**. Cyclization of **8** with phosphorous pentasulfide yielded new heterocyclic moiety thiadiazole attached to quinazoline analogue **10**. The reaction of **1** with phenyl isothiocyanate afforded **9**. **11** and **12** were synthesized by intermolecular cyclization of phenyl-thiosemicarbazide **9** under the action of conc. H_2SO_4 and 5% NaOH. Cyclization of **2b** with thioglycolic acid yielded **13**. **14** was performed by treatment **13** with benzaldehyde. Cyclization of **13** and **14** was achieved by reactions with benzylidene malononitrile and malononitrile, respectively to give **15**. The structure of all yielded compounds was confirmed by their spectral and microanalytical data [9].



Scheme 1. The synthesis of compounds **2-12.**

Pharmacology

Cytotoxicity assay

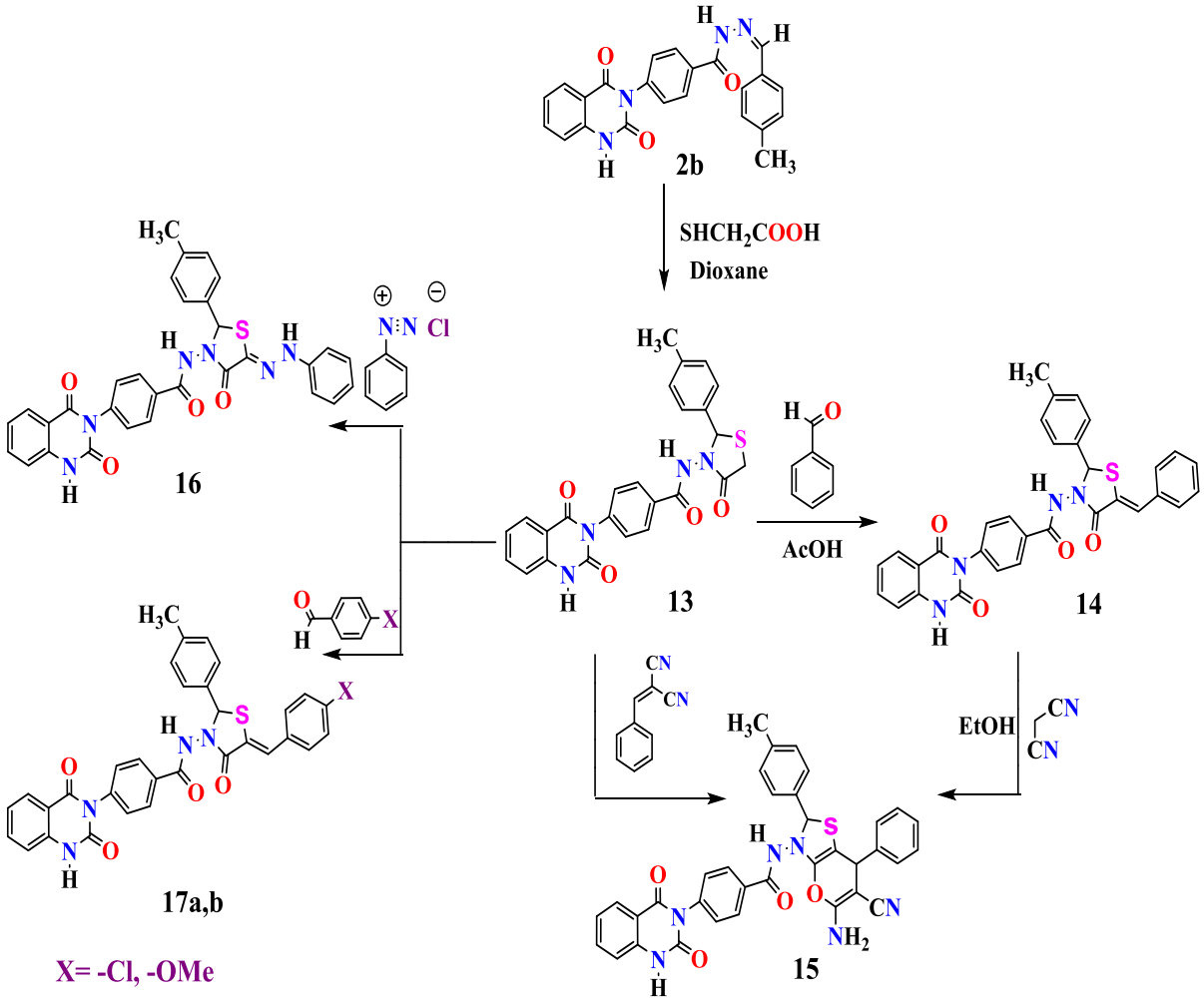
Comparing compounds and positive control that gave 100% hemolysis using RM two-way ANOVA showed that compounds were significant at different concentrations (p= 0.017). Results in **Table 1** showed that all synthesized compounds were toxic at a concentration of 500 µg/ml and the standard drug had the highest toxicity in all tested concentrations. Nevertheless; at concentrations 100 µg/ml, synthesized compounds **1, 2b, 2d, 2e, 4, 6, 9,** and **14** were non-toxic. Besides, the lowest cytotoxic effect was obtained at a concentration of 100 µg/ml of sample **2b**.

Anticancer activity

Results showed that only two synthesized compounds had a potential anti-HCC effect; **5** and **17b**. The IC_{50} of compound **5** was detected at 382.9±6.9 µg/ml and the IC_{50} of compound **17b** was detected at 415.8±3.8 µg/ml. Compared to DOX as an anticancer drug that had IC_{50} equal to 6.14±0.32 µg/ml, these two compounds; **5** and **17b**, were considered to have moderate anti-HCC activity.

Figure (2). Statistical significance analysis between each concentration of compound **5** on cancer cells compared to hemolysis showed that a significant interaction was found in each concentration 500, 250, 100, and 50 µg/ml with p-value= 0.01, 0.009, 0.05, and 0.004 respectively.

However, statistical significance analysis between each concentration of compound **17b** on cancer cells compared to hemolysis revealed that only concentrations 500 and 250 had a significance with p-value= 0.006 and 0.009, respectively. DOX exhibited the highest cytotoxic effect at a concentration of 50 µg/ml so, compounds **5** and **17b** considerably showed a moderate anti-HCC effect.



Scheme 2. The synthesis of compounds **13-17a,b.**

A recent study has been revealed that Quinazoline derivatives (BLU9931) had a suppressed effect on fibroblast growth factor 19-fibroblast growth factor receptor 4 (FGF19-FGFR4) signaling pathway that promotes HCC progression [11].

Antimicrobial activity

The repeated results of the clear zone (ϕmm) were analyzed using ANOVA statistical tool indicated that there is a significant difference in the sensitivity of the tested microorganisms to the various compounds (*p*-value= 0.0034). **Figure (3)**

Compound **9** had the highest anti-*Aspergillus niger* effect, compound **5** had the highest anti-*Candida albicans* effect, compound **6** had the highest anti-*Staphylococcus aureus* effect, and compound **1** had the highest anti-*Pseudomonas aeruginosa* effect.

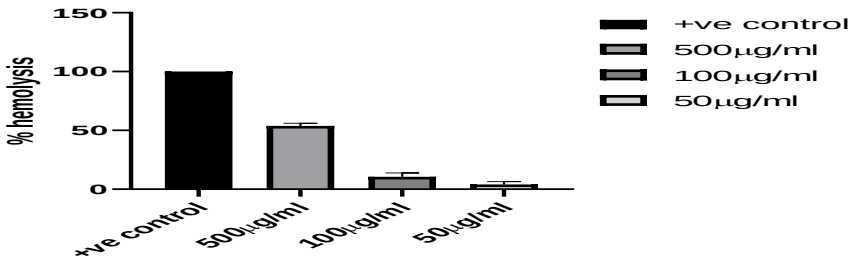
Conclusion

In the current study, we were able to successfully synthesize quinazolin-2,4-dione analogues as promising derivatives with possible antimicrobial and anti-HCC effects. The in vitro cytotoxic activity against HepG2 cell lines showed that the synthesized analogues **5** and **17b** had moderate anticancer effects. Nevertheless, compounds 1, 5, 6, and 9 had notable antimicrobial effects on *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, and *Aspergillus niger*, respectively.

Finally, we recommend further in vivo preclinical investigations for the compounds that had potential biological effects so that they can be developed as therapeutic drugs.

Table 1. Hemolytic activity of synthesized compound**s**

	500 µg/ml	**100µg/ml**	**50µg/ml**
Diclofenac	57.8±1.7	28.9±1.8	12±1.3
1	53.6±3	9.7±1.6	1.7±0.6
2a	52.1±5.1	12.8±1.2	6.1±1.8
2b	52.3±3.1	4.6±1.2	1±0.8
2c	52.1±3.3	7.8±1.9	3±0.4
2d	56.4±4.6	9.5±0.8	2.8±0.9
2e	55.4±4.2	8.7±0.8	7.9±2.4
2f	53.6±5.5	11.2±1.4	7.3±1.2
3a	53.8±5.4	11.2±1.9	3.6±2.2
3b	51.1±2.1	15.8±2.9	4.5±0.8
3c	54.7±1.6	11.3±2.3	3.9±1.8
4	56.4±3.2	8.3±1.1	3.4±1.2
5	57.1±6.6	14.1±3.5	4.6±1.5
6	52.3±4.1	7.9±1	4.4±1.2
8	50.6±2	10.5±1.4	2.7±0.6
9	49.6±2	8.3±0.6	2.4±1.5
13	51.8±1.8	9.6±1.6	2.7±1.1
14	55.1±3.3	4.9±2.6	1.4±1
15	57.4±4.1	10.8±1.9	7.9±1
17a	55.4±0.9	12.3±3.1	1.2±0.8
17b	55.6±3.4	11.1±0.7	8.6±1.1



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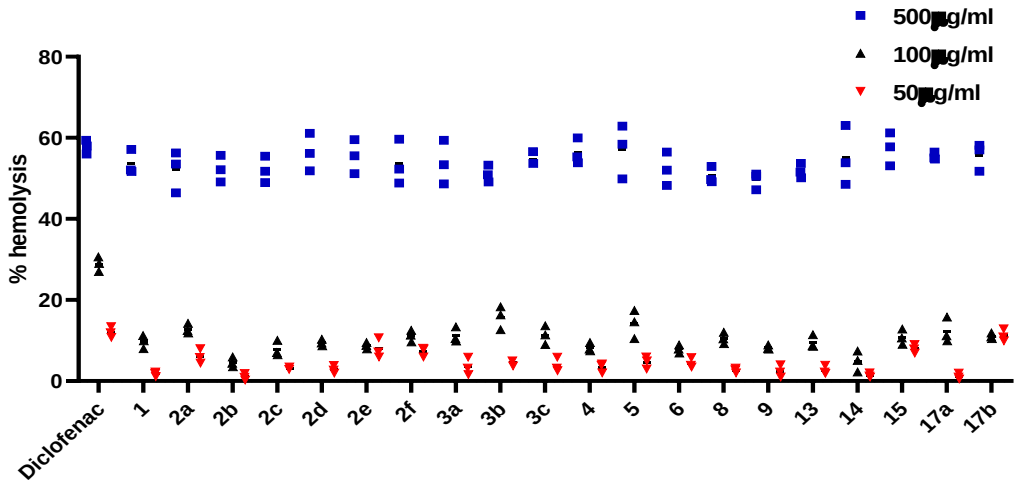

Figure (1): These graphs represent the percent of hemolysis of synthesized compounds and standard drug (Diclofenac) at different concentrations (500, 100, and 50 µg/ml).

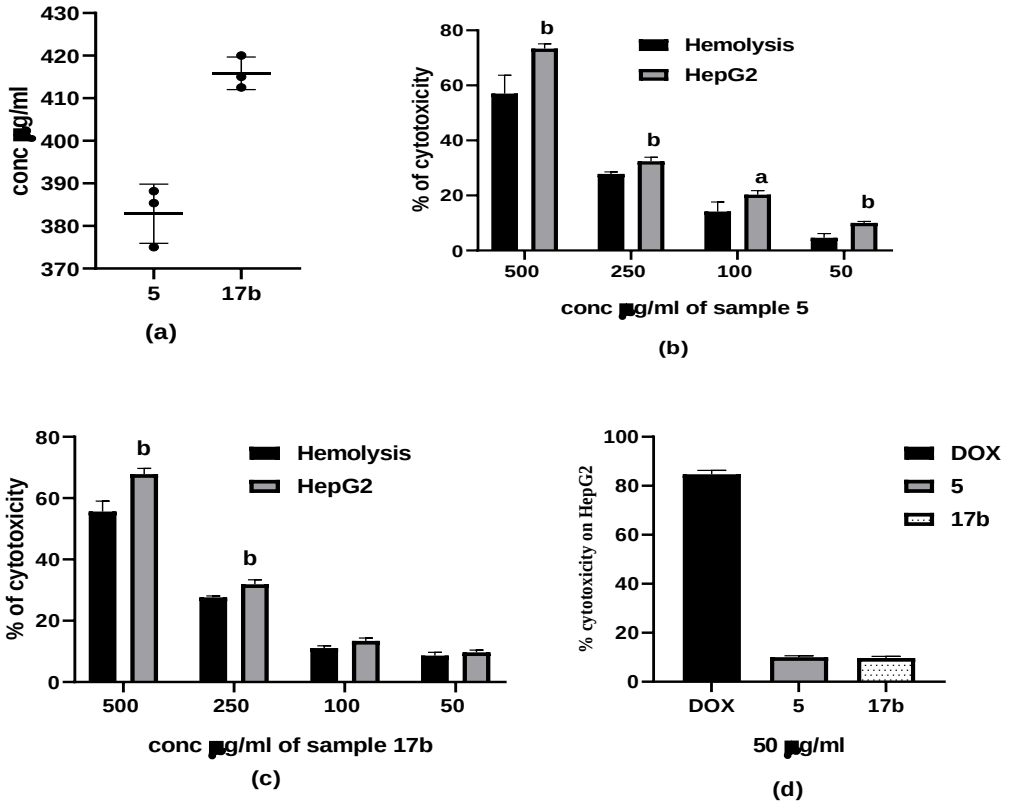

Figure (2) (a): This graph represents the IC_{50} of compounds 5 and 17b on HepG2 cells. **(b):** This graph represents the effect of different concentrations of compound 5 on normal cells (RBCs) and cancer cells (HepG2). [a] *p*-value ≤ 0.05 and [b] *p*-value ≤ 0.01. **(c):** This graph represents the effect of different concentrations

of compounds 17b on normal cells (RBCs) and cancer cells (HepG2). [b] *p*-value≤ 0.01. **(d):** Percent of cytotoxicity on HepG2 of DOX, compounds 5 and 17b.

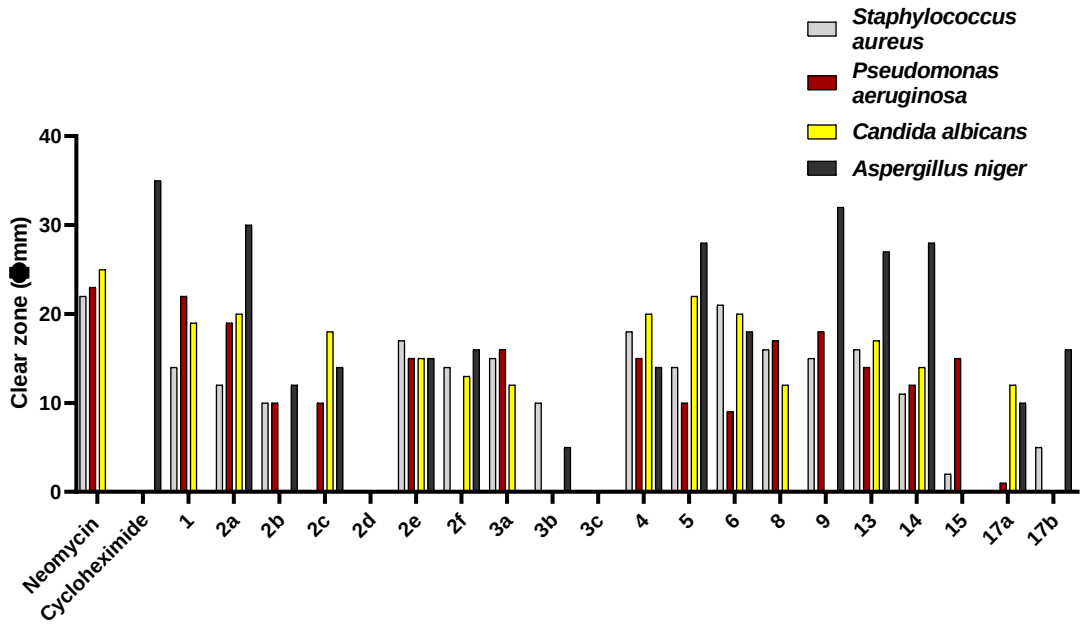

Figure (3): The antimicrobial activity of tested compounds was analyzed in comparison to neomycin and cycloheximide antibiotics against 4 different microbes: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*.

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