Synthesis, Characterization, Antimicrobial and Anticarcinogenic Activity of Quinazoline Derivatives

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Summary: The reaction of β-dicarbonyl compounds as ethyl acetoacetate with hydrazines is a well-established route for the creation of pyrazole derivatives. In this research, 4-hydrazinoquinazoline (4) reacted with ethyl acetoacetate in boiling ethanol it gives ethyl 3-oxobutanoate quinazolin-4-yl-hydrazone (5). Cyclization of the compound (5) in the presence of glacial acetic acid is passed to form 4-(5-hydroxy-3-methyl-1H-pyrazol-1-yl)quinazoline(6). Furthermore, the reaction of phenylisothiocyanate compounds with hydrazines is a well-established way for the synthesis of tetrazinoquinazoline (8). The new compounds are confirmed by elemental analysis, nuclear magnetic resonance, infrared, and mass spectra. In-vitro anti-tumor evaluation of the new compounds across three cell lines HepG2 (liver carcinoma), HCT-116 (colon carcinoma), and MCF-7 (breast carcinoma) indicats high anti-tumor activity. In addition, antimicrobial activity of specific synthesized compounds are screened against four bacterial species. Amongst four, two Gram-negative are *Escherichia coli*, *Pseudomonas aeuroginosa* and gram-positive as *Staphylococcus aureus* and *Bacillus subtilis*. The antibacterial activity of Compounds (5) and (6) are greater comparing to standard drug (Amphicillin).

Keywords: Characterizations, Antimicrobial, Anticarcinogenic, Activity.

Introduction

At least half of all organic chemistry research worldwide involve heterocyclic chemistry. Derivatives of quinazolines are groups of fused heterocycles of a significant interest because they exhibit anticonvulsant, anti-inflammatory, antidiabetic, antibacterial properties and antihypertensive. On the other hand, the introduction of one or more fluorine atoms into organic molecules has been observed to improve their biological strength, bioavailability, and metabolic stability. Numerous derivatives of quinazoline is characterized as one of the utmost active classes of compounds [1-4]. Quinazolines derivatives have a widespread multiplicity of biological activities, as well as antibacterial [5], cytotoxic [6], antioxidant [7], anti-tumor [8], anti-inflammatory [9], analgesic [10], anti-hypertensive [11], anti-HIV [12], anticonvulsant [13, 14], anti-tubercular [15], anti-malarial [16] and antibacterial [17]. The aim of the current research is to investigate a novel compound of quinazolone derivatives, which shows the biological activity against cell lines.

Experimental

Melting points are calculated and uncorrected using a Kofler block. The IR spectra is passed out in the faculty of science, Alexandria University, Egypt. At 70 ev, HRESI-MS are renowned by 5980 series II GC, coupled with 5989 B mass spectrometer from the University of Cairo , Egypt, Faculty of Science.. The 1H NMR spectra is recorded in the Faculty of Science, Jordan University, Jordan. Thin-layer chromatography (TLC) has typically been followed by reactions with Merck Kiesel gel; precoated plastic sheets 60-F254. The Lines are noticed by UV-lamp. Reagent and solvent are obtained from the central lab. faculty of science, Alexandria University, Egypt and Al-Azhar University's Regional Center for Biotechnology & Mycology, Egypt

Ethyl 3-oxobutanoate quinazolin-4-yl-hydrazone **(5)**

Ethyl acetoacetate (3 mL) is added to 4-hydrazinoquinazoline[18]. (4, 3 g, 0.0187 mol) in ethanol (100 mL). The mixture is stirring overnight for

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24 hours at room temperature. The obtained product is filtered, washed with ethanol and crystallized from ethanol-chloroform to produce compound (5) as golden yellow needles (4 g, 78% yield); m.p. 141-142°; IR(KBr) v / cm-1: 3291, 1748, 1620, 1475,1429 ; HRESI-MS *m/z*, calcd. for $C_{14}H_{16}N_4O_2$ [M+1]: 272.30 ,Found: 272.17.

4-(5-Hydroxy-3-methylpyrazol-1-yl)quinazoline **(6)**

Ethyl 3-oxobutanoate quinazolin-4-yl-hydrazone (5, 0.7 g, 0.0025 mol) and glacial acetic acid (1mL) are heated at 100°C for 2 hours. Vaporization of the acetic acid gives a solid mass which crystallized with dioxane give compound (6) as colorless needles (0.4 g, 68% yield); m.p. 245-246° ; IR(KBr) v / cm-1: 3430 , 1614 , 1469 , 1425 ; 1H NMR (400 MHz,$CDCl_3$): δ 3.76 (s, 3H, CH_3), 7.78 (t, 1H, Ph-H), 7.96 (t, 1H, Ph-H), 8.18 (d, 1H, Ph-H), 8.51 (d, 1H, Ph-H), 8.86 (s, 1H, pz-H), 9.29 (s, 1H, pm-H) and 12.69 (s, 1H, OH).; HRESI-MS *m/z*, calcd. for $C_{12}H_{10}N_4O$ [M]: 226.23,Found: 226.10.

4-Phenylthiosemicarbazonoquinazoline **(7)**

A mix of 4-hydrazinoquinazoline (4, 2 g, 0.025 mol) and phenyl- isothiocyanate (3 miL) is refluxed in methanol (25 miL) for 84 hours. The reaction mixture is cooled to room temperature. Also, the product is filtered, washed with methanol and is crystallized with methanol to form compound (7) as colorless needles (2.4 g, 66% yield); m.p. 280° ; IR(KBr) v / cm-1:3454, 3352 ,1494 ,1436, 1240;1H NMR (400 MHz,$CDCl_3$):δ 1.88 (s, broad,1H, NH), 3.57 (s, broad,1H,exchangeable NH), 7.00-7.65 (m, 5H, Ph-H), 8.03 (s, 1H, pm-H), 7.82 (t, 1H, Ph-H), 8.18 (t, 1H, Ph-H), 8.31 (d, 1H, Ph-H) and 8.99 (d, 1H, Ph-H); HRESI-MS *m/z*, calcd. for $C_{15}H_{13}N_5S$ [M] :295.36, Found: 295.21.

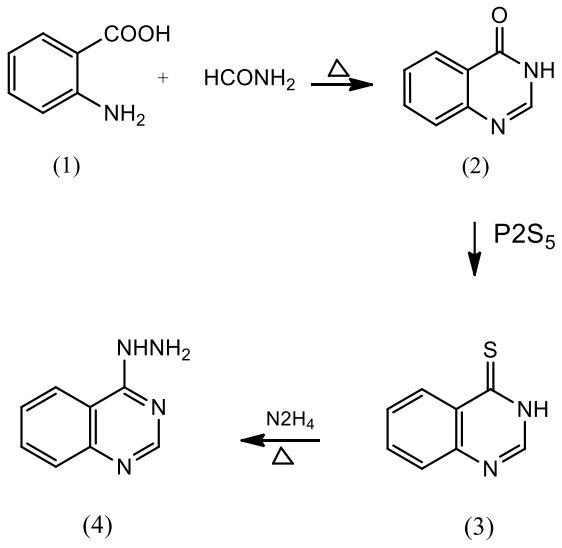
4-Phenyl-3-thioxo-2H-1,2,4,5 tetrazino[1,6-c]quinazoline **(8)**

A bromine solution (0.7 ml , 0.05 mol) in CCl4 (10 mL) is slowly added to 4-phenylthiosemicarbazonoquinazoline (7, 3 g, 0.0101 mol) in CCl4 (10 mL) by stirring for half-hour at room temperature. The mixture is poured onto crushed ice by stirring. Also, the product is filtered, washed with cold H_2O, dehydrated and crystallized with ethanol to form compound (8) as colorless needles (1.8 g, 60 % yield); m.p. 250° ; IR(KBr) v / cm-1:3466,1462,1414,1217;NMR: (DMSO-d6): δ 7.25 (s, broad,1H,exchangeable NH), 7.35 (t, 1H, Ph-H), 7.45 (t, 2H, Ph-H), 7.53 (t, 2H, Ph-H), 8.04 (d, 2H, Ph-H) 8.12 (d, 2H, Ph-H) and 8.40 (s, 1H, pm- H); HRESI-MS *m/z*, calcd. for $C_{15}H_{11}N_5S$ [M] : 293.35, Found: 293.30.

Results and Discussion

Hydrazinoquinazoline derivatives are multipurpose nitrogen heterocyclic compounds which have long been known as a hopeful class of biologically active compound. A huge number of hydrazinoquinazoline derivatives have been synthesized to provide more effective medicines and to design synthetic drugs. On the other hand, the quinazoline nucleus is readily and cheaply on the industrial scale, it is useful for dyeing, pigments , in addition to use some of the foregoing quinazolines in drug manufacture, in coatings and in photography, for example 4-hydroxyquinazoline and 4-chloroquinazoline. Hence, this research includes the synthesis and characterization of certain new quinazoline derivatives. The prepared compounds are grouped according to their types and are arranged in order of numerous complexity of the newly formed heterocyclic ring irrespective of whether they are linked to quinazoline nucleus. Thus, numerous analogues of it have been produced and subjected to screen for biological activity using various animal models.

The hydrazinoquinazoline (4) is prepared from anthranilic acid (1) by cyclization with formamide to yield the quinazolin-4(3H)-one (2) it reacts with phosphorus pentasulfide in dry pyridine to give quinazolin-4(3H)-thione (3), it followed by reaction with hydrazine hydrate to yield the desired 4-hydrazinoquinazoline (4) in excellent yield (Scheme 1) [18].



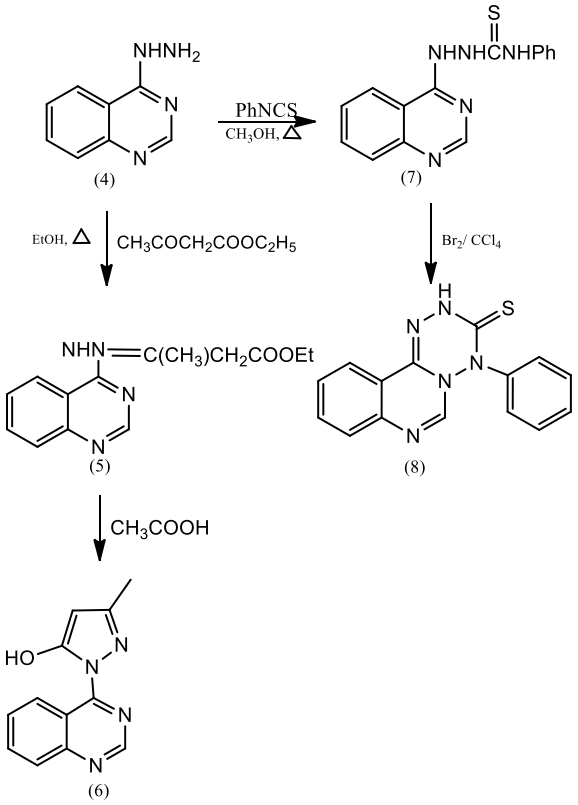
Scheme 1: Synthesis of hydrazinoquinazoline (4)

For good yield of the compound (4), it is reacted with ethyl acetoacetate in boiling ethanol to form ethyl 3-oxobutanoate quinazolin-4-yl-hydrazone (5). The structure of the quinazoline derivatives (5) is confirmed from its spectral analysis. The infrared spectrum of the compound (5) shows a broad NH band at 3291 cm-1, COOEt absorption at 1748 cm-1, also, the characteristic absorption bands of the quinazoline nucleus at 1475 cm-1.

Cyclization of compound 2 with glacial acetic acid under reflux affords the compound (6). Compound (6) shows a broad OH absorption at 3430 cm-1. Besides, the characteristic absorption bands of the quinazoline and pyrazole rings at 1469 and 1614, respectively by the infrared spectrum analysis. Hnmr spectrum of the compound (6), in $CDCl_3$, exhibits a broad singlet signals at δ 12.69 for exchange OH proton, a singlet at δ 8.86 for pyrazole proton, appear a singlet at δ 3.76 (3H) for methyl protons, in addition to two triplets at δ 7.78 and 7.96 (2H) and two doublets at δ 8.18 and 8.51(2H) for phenyl protons. Moreover, it showed a singlet at δ 9.29 for H-5 pyrimidine proton.

Also, the reaction of the compound (4) with phenylisothiocyanate affords 4-phenylthio-semicarbazonoquinazoline (7). The infrared spectrum of the compound (7) shows a broad NH absorption at 3454 and 3352 cm-1, in addition to the characteristic absorption bands of the quinazoline nucleus at 1464 cm-1. The 1Hnmr spectrum of 4-phenylthiosemicarbazonoquinazoline (7), in CDCl3, exhibits two broad singlets signals at δ 1.88 and 3.57 for an exchangeable NH protons, as well as a multiplet at δ 7.00–7.65 (5H) for phenyl protons, in addition to two triplets at δ 7.82 and 8.18(2H). The two doublets at δ 8.31 and 8.99 (2H) for phenyl protons, shows a singlet at δ 8.03 for H-5 pyrimidine proton.

Also, cyclization of the compound (7) with bromine in carbon tetrachloride leds the formation of 4-phenyl-3-thioxo-2H-1,2,4,5 tetrazino[1,6-c]quinazoline (8). The infrared spectrum of the tetrazinoquinazoline (8) shows a broad NH absorption at 3466 cm-1, thiocarbonyl C=S band at 1217 cm-1. Furthermore, the 1Hnmr spectrum of compound (8), in DMSO-*d6*, exhibits a singlet signals at δ 7.25 for exchange NH proton, in addition to three triplets at δ 7.35,7.45 and 7.53 (5H). The two doublets at δ 8.03 and 8.12 (4H) for phenyl protons, shows a singlet at δ 8.40 for H-5 pyrimidine proton (Scheme.2).



Scheme 2: Reactions with hydrazinoquinazoline (4)

The newly synthesized compounds are estimated for its growth inhibition potential versus cancer cell lines, specifically HCT-116 colon cancer, HepG2 hepatocellular carcinoma, and MCF-7 breast cancer cells by the SRB colorimetric test [19]. Doxorubicin (Dox) is utilized as positive control which is a most effective anticarcinogenic agent that known for wide-ranging spectrum against different types of solid tumors. It is also known to have potent pro-apoptic effects [20]. In addition, the antimicrobial actions of some new synthesized compounds are covered against four species of bacteria for their antibacterial activity. Amongst four two are Gram-negative as *Escherichia coli and Pseudomonas aeuroginosa* and gram-positive as *Staphylococcus aureus* and *Bacillus subtilis* [21].

Biological Screening

In-vitro anticancer activity

The recently synthesized compounds is assessed by colorimetric assay of sulforhodamine B (SRB) [20] for its growth inhibition against cancer cells, namely human colon tumor HCT, hepatocellular carcinoma HepG2, and MCF-7 breast cancer, tested in the Regional Center for Biotechnology &, Mycology Al-Azhar University, Egypt. For in vitro anticancer activity different concentration of new compounds is

evaluated by SRB cells. SRB cells are coated with compounds 24 hours before the treatment in a multiwall plate to enable the cell to be attached to the plate wall.

The cell monolayer with different concentrations of the new compounds is detected and incubated at 37 °C for 48 h with 5% CO_2. The cells are then continuously washed and spotted with SRB strain after 48 h. it is recovered with tris EDTA buffer eliminating the extra stain by washing with acetic acid and linked stain. The level of the color is measured by using ELISA reader. Finally, the tumor cell lines survival curve are plotted to show the relationship between surviving segment and drug concentration. Anticancer activities are expressed as the median growth inhibitory concentration (IC_{50}).

Evaluation of cytotoxicity against colon carcinoma cell lines

In case of HCT-116 cells as demonstrated that at 50 µg (Fig 1, 2), it exhibited low viability cells about 26.50 and 33.08 % for compounds (6) and (8) respectively.

The inhibitory concentration that inhibits the viable cells for human colon tumor HCT-116 by 50% (IC_{50}) of the compound (6), are exhibited a strong growth inhibition (IC_{50}= 31.32 or 40.52 µg) for compound (6) and (8) respectively.

Evaluation of cytotoxicity against hepatocellular carcinoma cell lines

In case of HepG2 cells, it indicats low viability cells about 36.12 and 20.68 % for compound (6) and (8) respectively, at 50 µg as shown in Fig (1). Also, when the tumor cells is treated with 25 µg of each compound shows 42.33 % inhibition against HepG2 for compound (6) but 51.30 % inhibition against HepG2 for compound (8).

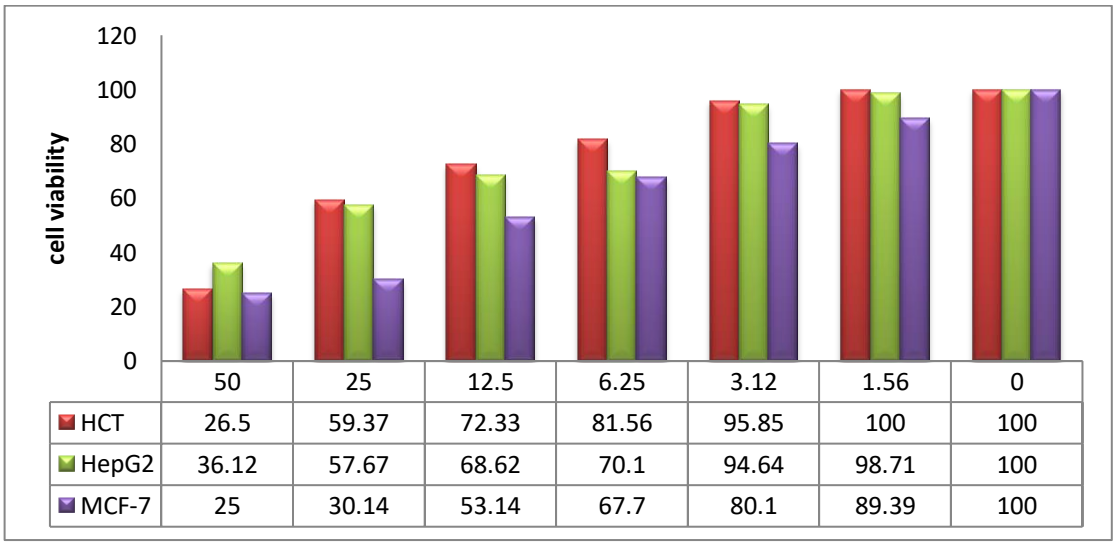
The inhibitory concentration that inhibits the viable cells for HepG2 by 50% (IC50) of the new compounds exhibited a growth inhibition (IC_{50}=28.9 and 23.1 µg) for compound (6) and (8) respectively.

Evaluation of cytotoxicity against breast carcinoma cell lines

In case of HCF-7 cells as in Fig (1), it shows 75.00 % very strong inhibition against HCF-7 for compound (6) at 50 µg. Moreover, when the breast tumor cells is treated with 25 µg from each compound showing low viability cells about 30.14 % for compound (6).

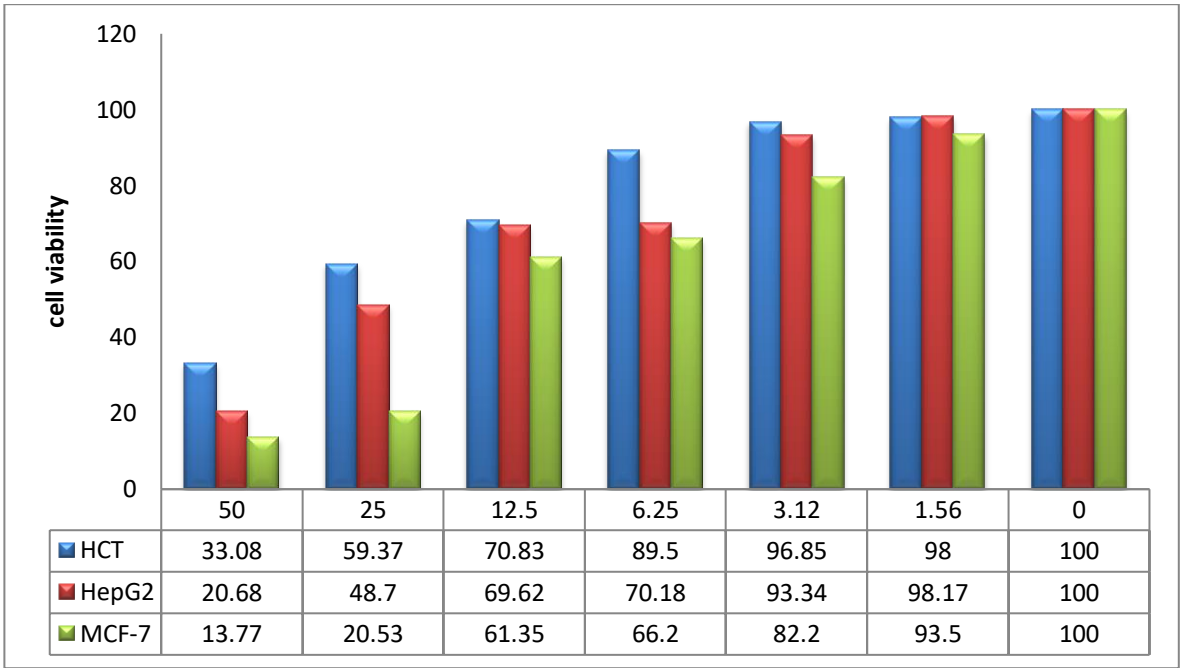
Furthermore, 86.23 % are very strong inhibition against HCF-7 for compound (8) at 50 µg. Also, when the breast tumor cells is treated with 25 µg from each compound showing low viability cells about 20.53 % for compound (8) in Fig (2).

In addition, the inhibitory concentration that inhibits the viable cells for HCF-7 by 50% (IC_{50}) of the compound (6) and (8) are exhibited a very strong growth inhibition (IC_{50}= 16.4 and 20.5 µg), respectively.



	50	25	12.5	6.25	3.12	1.56	0
HCT	26.5	59.37	72.33	81.56	95.85	100	100
HepG2	36.12	57.67	68.62	70.1	94.64	98.71	100
MCF-7	25	30.14	53.14	67.7	80.1	89.39	100

Fig. 1: In vitro anticancer activities of the newly synthesized compound (6) against hepatocellular carcinoma (HepG2), colon cancer (HCT-116), and breast cancer (MCF-7) cell lines.



	50	25	12.5	6.25	3.12	1.56	0
HCT	33.08	59.37	70.83	89.5	96.85	98	100
HepG2	20.68	48.7	69.62	70.18	93.34	98.17	100
MCF-7	13.77	20.53	61.35	66.2	82.2	93.5	100

Fig. 2: In vitro anticancer activities of the newly synthesized compound (8) against hepatocellular carcinoma (HepG2), colon cancer (HCT-116), and breast cancer (MCF-7) cell lines.

Table-1: Antimicrobial activity of chemically synthesized compounds against bacteria.

Compound Code	Inhibition field (mm)			
	S. aureus	*B.subtili*	*E. coli*	*P.aeuroginosa*
COMP 5	**9**	**12**	**4**	**14**
COMP 6	**13**	**11**	**6**	**26**
COMP 7	**3**	**1**	**2**	**9**
COMP 8	**5**	**2**	**6**	**10**
Ampicillin	**7**	**6**	**2**	**23**

The result obtained is in the same line with a previous study conducted by Mohamed *et al.* [22] who develops novel antitumor molecules containing 4-substituted quinazoline pharmacophore. In addition, another study conducted by Chen *et al.* [23] states that 30 novel quinazolinyl-diaryl urea derivatives are designed, manufactured and screened for their biological data by measuring antitumor activity against three cancer cell lines A549 (denocarcinomic human alveolar basal epithelial cells), HepG2, and MGC-803 (human gastric).

Furthermore, a recent mini-review conducted by Solyanik [24] described the pharmacological effects of quinazolines basically depend on their structure. The restrictive factor is certainly the poor solubility of many quinazoline compounds. Also, high chemical reactivity can show the study of their specific antitumor or antimetastatic activities. Although quinazolines symbolize promising "chemical construction set" for creating anticancer drugs with an extensive arsenal of targets for therapeutic intervention.

Antimicrobial activity of chemically newly synthesized compound

The antimicrobial activity of specific synthesized compounds is covered against four species of bacteria. Amongst four two are Gram-negative as *Escherichia coli and Pseudomonas aeuroginosa* and gram-positive as *Staphylococcus aureus* and *Bacillus subtilis*, subsequent a beaker dish agar dispersion technique [21]. The new structures are melted in dimethyl sulfoxide to get 1 mg/µL. The inhibition zone is weighted in mm at the end of the incubation period for approximately 48 h at a temperature of 37^0 C. Ampicillin is used as a standard reference to evaluate the potency of the tested compounds. The newly compounds of quinazoline derivatives (5, 6, 7and 8) is established for their antibacterial activity in vitro against a typical strain panel of *S. aureus, B. subtilis, E. coli* and *P.*

aeuroginosa. Dimethyl sulfoxide doesn't present any inhibition zones. The newly compounds (5) and (6) appear greater antimicrobial activity than the ampicillin. Compound (8) presents moderate antimicrobial activity against Gram (-ve) bacteria and low activity against Gram (+ve) bacteria compared to ampicillin. Although, compound (7) shows lower activity against Gram (+ve) and Gram (- ve) bacteria as shown in Table-1.

Conclusion

The new compounds in the presence of pyrazole and tetrazine ring act as an anticancer agent and show antitumor activity against liver HepG2, colon HCT-116 and breast MCF-7 cancer cell lines. Antibacterial activity of compounds (5) and (6) show greater than the standard drug Amphillicin.

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