Novel quinazolinone derivatives: Synthesis and Antimicrobial Activity

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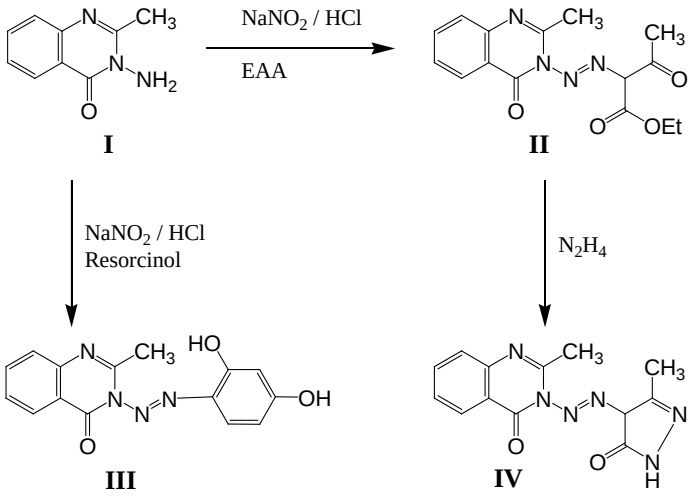
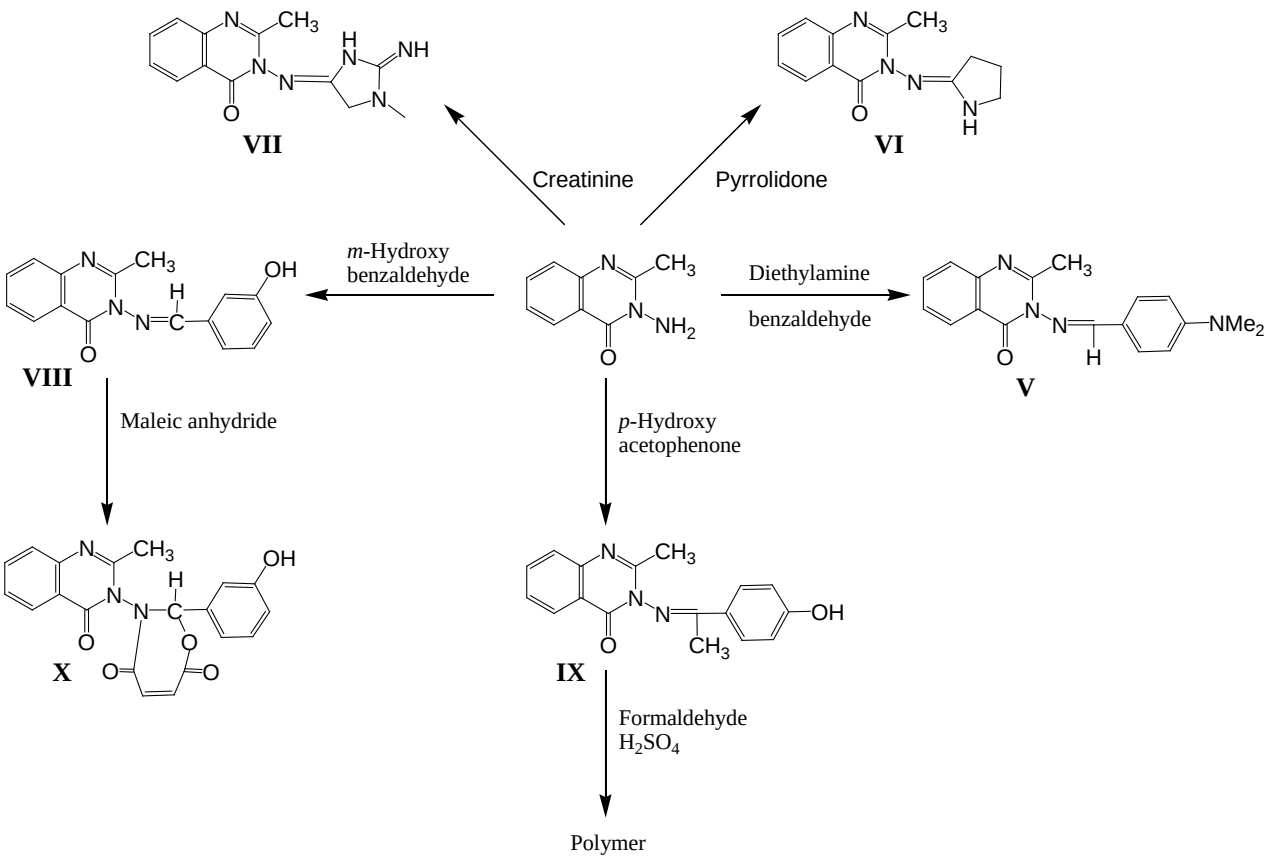
Abstract: Problem statement: This work involves to synthesis novel organic compounds and studies their pharmacological**. Approach:** The title compound quinazolinone derivatives were prepared by reacting 3 amino of 3-amino-2-methylquinazolin-4(3H)-one (first) with various aldehydes and ketones, and Second coupling through diazonium salts with resorcinol or with ethylacetoacetate then cyclized with hydrazine. The starting material 3-amino-2-methylquinazolin-4(3H)-one was synthesized by reacting hydrazine with 2-methyl-4H-benzo[d][1,3]oxazin-4-one, which in turn was prepared from anthranilic acid. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data (FT-IR, UV/visible spectra, ^{1}HNMR, and CHN analyses). All synthesized compounds were tested *in vitro* against a number of microorganisms (*Staphylococcus aurous, E.coli, Proteus vulgaris, Pseudomonas, and Klebsiella*) and two fungal *Aspergillus niger* and *Candida albicans* in order to assess their antimicrobial properties. **Results:** The study indicates that these compunds have high activity against tested bacteria. **Aim:** We aim to synthesize quinazolinone derivatives having active moieties to evaluate their antimicrobial activities. **Conclusion/Recommendations:** Based on the reported results, it may be concluded that 3-amino-2-methyl- quinazolin-3(4*H*)-one act as synthones for Schiff bases and for diazonium coupling.

Keywords: 3-amino-2-methyl- quinazolin-3(4*H*)-one, antibacterial activity.

Introduction: Pharmacologically, quinazolin-4-ones are among the most important classes of heterocyclic compounds. These compounds possess versatile type of biological activities; some of these are well known for their anticancer[1-2], antitubercular[3], antibacterial[4], antifungal[5], anti-HIV[6], anthelmintic[7], anti-inflammatory[8], antihypertensive activities[9], antiulcer[10], analgesic[11], and antiproliferative [12] activities as well as inhibitory effects for thymidylate synthase[13]. Some reports have suggested that 2-styrylquinazolin-4-ones (SQZ) [14,15] could be effective inhibitors of tubulin polymerization. The 2,3-disubstituted quinazolones have been predicted to possess antiviral and antihypertensive activities [16]. The present reports the senthesis of novel quinazolinone derivatives. The main pbjective of this work is th prepare aseries of derivatives of quinazolin. The basic ring was desined to be a 3-amino-2-methylquinazolin-4(3H)-one with additional derivatives as diazo, Schiff bases and cyclized Schiff bases (Sheme 1 and 2).



Scheme 1



Scheme 2

Exparimentals:

All chemical used were of reagent grade (supplied by either Merck or Fluka) and used as supplied. The FTIR spectra in the range (4000–400) cm^{-1} were recorded as KBr disc on FTIR 8300 Shimadzu Spectrophotometer. Proton NMR spectrum was recorded on Bruker-DPX 300 MHz spectrometer with TMS as internal standard in Jordan University. The UV-Visible spectra were measured in ethanol using Shimadzu UV-Vis. 160 A spectrophotometer in the range (200-1000) nm. Elemental microanalysis was carried out using CHNOS elemental analyzer model 5500 Carlo-Erba instruments(Italy made). Gallen Kamp M.F.B.600.010 F melting point apparatus was used to measure the melting point of all the prepared compounds.

Synthesis of 3-amino-2-methylquinazolin-4(3H)-one (I):

Method A: The 3-amino-2-methylquinazolin-4(3H)-one was prepared by stirring equimolar quantities of 2-methyl-4H-benzo[d][1,3]oxazin-4-one and hydrazine in ethanol for 10 h, then refluxed for 24 h, The reaction mixture was cooled and stirred into cold water (50 ml). Crude product was filtered, washed with cold water and dried it at 100^{o}C. Crude product was recrystallised from ethanol.

Method B: A mixture of orth-acetamido(ethyl)benzoate (0.01 mole) and (0.01 mole) of hydrazine hydrate in 150mL. of ethanol were refluxed for 12h. then left to cool at room temp. The reaction mixture was washed with water, the recrystalized from ethanol.

Synthesis of diazocompounds (II and III): A solution of the 3-amino-2-methylquinazolin-4(3H)-one (0.05 mole) in a mixture of (25ml) of hydrochloric acid and (50ml) of glacial acetic acid was diazotized at 0 ^{o}C with sodium nitrite solution (0,055 mole) dissolved in 10 ml of water. After Keeping at this temperature for 3 hours the resulting solution of diazonium salt was filtered and added to a mixture of 75ml. of glacial acetic acid, (0.05mole) of ethyl acetoacetate EAA (or resorcinol) and (0.37mole) of sodium acetate at 0 ^{o}C to 5 ^{o}C and pH=5.5. The mixture was left for 10hr , then an equal volume of water was added and the crude product was collected, washed with ethanol, then water, and recrystallized from ethanol.

Synthesis of (E)-2-methyl-3-((3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)quinazolin-4(3H)-one (IV): To a solution of (E)-ethyl 2-((2-methyl-4-oxoquinazolin-3(4H)-yl)diazenyl)-3-oxobutanoate (0.01 mole) in acetic acid (30mL.) was added hydrazine (0.012mole) and anhydrous

sodium acetate(0.82g, 0.01mole). The reaction mixture was heated under reflux for 4 hours then poured into ice-cold water and stored in refrigerator. The crude product was separated, washed with water, dried and recrystallized from ethanol.

Synthesis of Schiff bases (V-IX): A mixture of 0.0lmole of 3-amino-2-methylquinazolin-4(3H)-one and 0.0l mole of aldehyde or ketone in (10 ml) absolute ethanol was refluxed in water bath for (30min) then left to cool in ice -water. The solid was filtered, washed with 2%HCl then water and recrystallized twice from ethanol.

Cyclization of Schiff base (Synthesis of (Z)-2-(3-hydroxyphenyl)-3-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2,3-dihydro-1,3-oxazepine-4,7-dione) (X): Mixture of 0.01 mole of Schiff base ((E)-3-(3-hydroxybenzylideneamino)-2-methylquinazolin-4(3H)-one) with 0.01 mole of maleic anhydride in 10mL. of dry benzene was refluxed in water bath for 2 hours. The solvent was removed and the precipitate was recrystallized from tetrahydrofuran(THF).

Polymerization: The resin was synthesized by methods reported earlier[17-19]. The IX-formaldehyde resins were synthesized in molar ratio by adding IX and formaldehyde (37% aqueous solution). The pH (9.0) was adjusted by using of sufuric acid. The reaction mixture was refluxed for 30 minutes at 90.

Biological activities

Antibacterial activity: The Test Organisms used were: *Staphylococcus aureus* as gram positive bacteria, and *Escherichia coli, Proteus vulgaris, Klebsiella and Pseudomonas aeruginosa* as gram negative bacteria. Hole diffusion method was used to measure the inhibitory activity as indicated by the diameter of the inhibition zone. Concentration of 1mg/mL of test compounds were prepared by dissolving the compounds in dimethyl sulfoxide (DMSO), for each concentration, 0.2 ml of synthesized compounds II-X (1 mg/ml) was added to each hole. The plates were allowed to stand at room temperature for two hours and then incubated. The organisms were grown in nutrient agar at 37°C for 24 hours. After incubation period, the growth inhibition zones diameters were carefully measured in mm. The clear zone around the wells was measured as inhibition zones. The absence of a clear zone around the well was taken as inactivity.

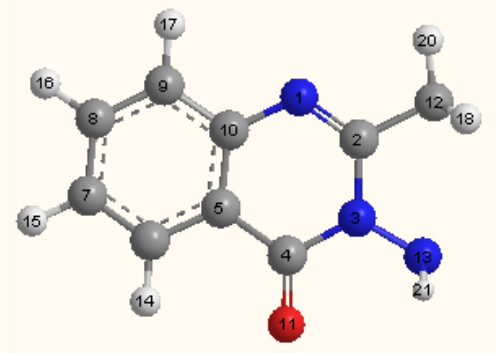
Antifungal activity: The test organisms used were *Aspergillus niger* and *Candida albicans*. Samples II-X were dissolved in DMSO then 0.5 ml sample of each compound (1 mg/ml) plus 0.1 ml of the tested fungal suspension were mixed thorougly with 20 ml of agar medium, which was maintained at 45°C. The inoculated medium was poured into sterile Petri-dishes, allowed to solidify, and incubated at 25°C for seven days. The plates were examined for evidence of inhibition of growth.

Results and Discussion

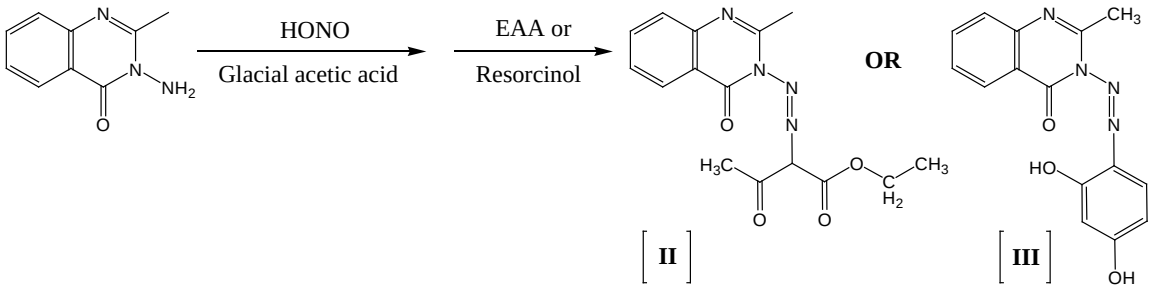
3-amino-2-methylquinazolin-4(3H)-one (I): Compound (I) is the key intermediates for the compounds synthesized later in this work. It has been prepared by the tow methods, first by the reaction of 2-methyl-4H-benzo[d][1,3]oxazin-4-one with hydrazine by using of ethanol as asolvent. Yield 50% and m.p. 153^{o}C. Proton NMR **(** S. 1.2(3H) for CH_3, 6.3 for NH_2, m. 7.3 for H aromatic, m. 8.2 for H aromatic**).** FT-IR spectrum (1665.9 cm^{-1} for carbonyl, 3305cm^{-1} for N-H, and 1259.3 cm^{-1} for C-N), CHN analysis: C, 61.12(61.70); H, 4.87(5.18); N, 24.01(23.99).

Second by reaction of orth-acetamido(ethyl)benzoate with hydrazine hydrate using ethanol as asolvent. Yield 60%, and m.p. 151^{o}C. The second method for preparation of compound (I) has better yield than first method.

The difference in the reactivity of the carbon atoms in the compound (I) molecule toward electrophilic substitution was observed. This is indicated by the (-ve) charge on the different carbons of compound (I). The data obtained for the minimized geometry i.e. charge, bond length, bond angle, twist angle, heat of formation and steric energy of the reactants, intermediate and the products were calculated using simiempirical AMI module in the CS chemoffice molecular modeling package. The data obtained show that the heat of formation is about (37.41423Kcal), and the highest atomic charge in compound (I) molecule is at [(O-11) (-0. 954)] the next charge value is at [(N-1) (-0.367)], [(C-12) (-0.20)], [(N-13) (-0.132)], [(C-7) (-0.085)], [(C-9) (-0.085)], [(C-8) (-0.05)] and [(C-6) (-0.038)]. These data show clearly that these tow atoms are the most reactive toward the electrophilic substitution reaction on benzene ring, in compound (I). The determined bond angle and twist angle and 3D-geometrical structure, indicate that this molecule is planar.

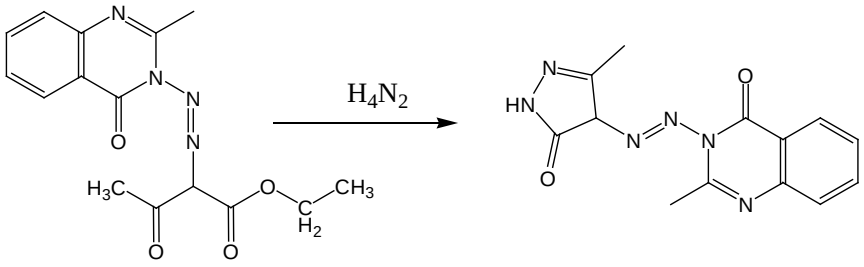


Synthesis of diazocompounds ((E)-ethyl 2-((2-methyl-4-oxoquinazolin-3(4H)-yl)diazenyl)-3-oxobutanoate(II) and (E)-3-((2,4-dihydroxyphenyl)diazenyl)-2-methylquinazolin-4(3H)-one (III)): Diazotisation is carried out in concentrated acid is used for the diazo component, since hydrolysis of the diazonium salt occurs in dilute acid. Here, the acid of choice was concentrated hydrochloric acid. used in amixture with glacial acetic acid. A particularly important reagent combination is the nitrosyl hydrochloric acid which is used extensively as a nitrosating agent for compound (I). Coupling is usually accompanied by some evidence of decomposition however, by careful addition of diazonium salt solution at 0-5 ^{o}C to a solution of coupling component in acetic acid, 30, 45% yield of azoquinazolin was usually obtained. To complete the coupling, particularly for reactions using nitrosyl hydrochloric acid in the diazotisation, the pH of the reaction mixture was adjusted to approximately 4-5. Thus, an appropriate amount of 10 % sodium acetate solution was slowly added below 5 ^{o}C. The yield of compound (II) was 30% (and m.p.=135 ^{o}C), but the yield of compound (III) was 70% (and m.p. =125 ^{o}C). The IR spectra of (II and III) showed the disappearance of the NH group absorption bands and the presence of bands at 1495 cm^{-1} and 1470 cm^{-1} for (N=N), and the absorption band for C=O groups (II and III) were 1710 and 1700 cm^{-1} respectively. In addition of 3175 cm^{-1} for O-H group for the compound (III).

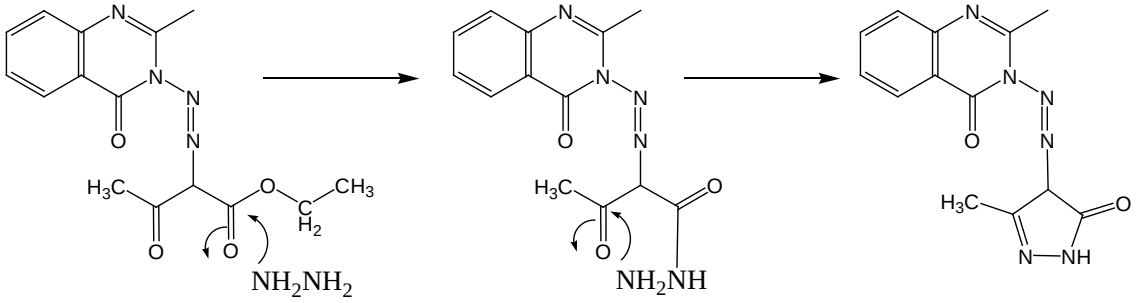


Synthesis of (E)-2-methyl-3-((3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)quinazolin-4(3H)-one (IV): Compound (IV) were obtained by thermal cyclization of (II) with hydrazine hydrate in precence of acetic acid. The yield of compound (IV) was 80% (and m.p. =171 ^{o}C). The IR spectra of (IV) showed absorption band for C=O group at 1675 cm^{-1}**.**

Reaction:



Mechanism:



Synthesis of Schiff Bases (V-IX): In general, Schiff ‘s bases are prepared by refluxing equi molar amounts of the primary amines (aromatic or aliphatic or related derivatives) with carbonyl compounds (aldehyde or ketones, aromatic or aliphatic derivatives) in appropriate solvent (cyclohexane, benzene and preferentially ethanol). Sometimes, the reaction is catalyzed by few drops of glacial acetic or piperidine and refluxing for 2 hours. The physical and spectral data are shown in table 1.

Table 1: The physical and spectral data of Schiff Bases (V-IX).

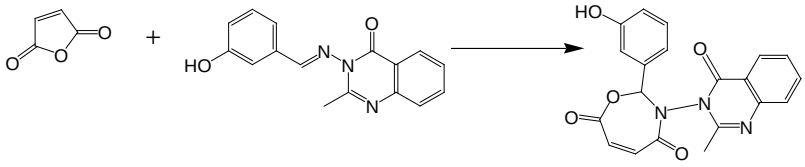
No.	Name	Melting point in ^{o}C	Color	Yield %	IR spectroscopy in cm-1		
					-NH	-OH	C=O
V	3-(4-(dimethylamino)benzylideneamino)-2-methylquinazolin-4(3H)-one	Oily	Red brown	80	-	-	1670

VI	(E)-2-methyl-3-(pyrrolidin-2-ylideneamino)quinazolin-4(3H)-one	Oily	Yellow	90	3300	-	1679
VII	(Z)-3-(2-imino-1-methylimidazolidin-4-ylideneamino)-2-methylquinazolin-4(3H)-one	141	Milky	50	3270	-	1680
VIII	3-(3-hydroxybenzylideneamino)-2-methylquinazolin-4(3H)-one	Oily	Dark brown	85	-	3155	1671
IX	(Z)-3-(1-(4-hydroxyphenyl)ethylideneamino)-2-methylquinazolin-4(3H)-one	Oily	Brown	70	-	1134	1675

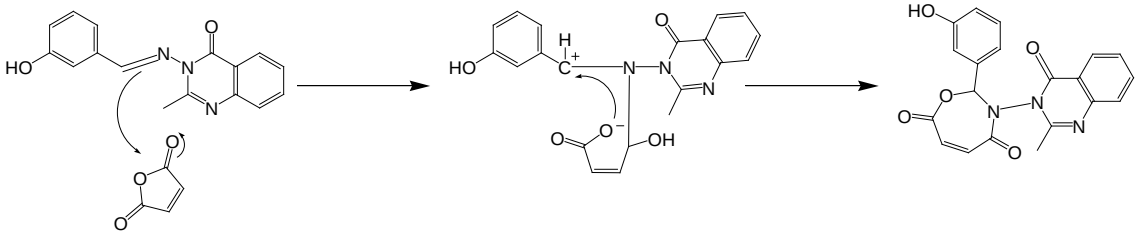
Proton NMR for the compound (VII): (S. 1.6(3H) for CH_3, S. 1.9(3H) for CH_3, S. 1.1(2H), 2.5 for N-H, 4.5 for NH_2, m. 7.5 for H aromatic**).** CHN analysis: C, 58.22(57.77); H, 4.70(5.22); N, 30.20(31.09).

Reaction of Schiff Base (VIII) with Maleic Anhydride: Treatment of Schiff bases with maleic,n anhydride results in the formation of (Z)-2-(3-hydroxyphenyl)-3-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2,3-dihydro-1,3-oxazepine-4,7-dione. The yield of compound (X) was 55% (and m.p.=166 ^{o}C). The IR spectra of (IV) showed absorption band for C=O group at 1723 cm^{-1}

Reaction:



Mechanism:



Biological Activity [20-22]: The antimicrobial screening data show that the compounds exhibit antimicrobial properties and it is important to note that the new derivatives exhibit more inhibitory effects than the orginal molecule (I). From table (2) it is clear that the zone of inhibition against the gram-negative bacteria and gram-positive bacteria. The increased activity of the new derivatives can be explained that act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the orginal molecule (I). The π-electron delocalization

over the new derivatives increases the lipophilic character and favours its permeation through the lipoid layer of the bacterial memberanes. It was reported that 3*H*-quinazolin-4-one derivatives have interesting antimicrobial activity against different species of Gram positive bacteria, Gram negative bacteria and pathogenic Fungi[23-30]. Schiff's bases have been widely reported to be biologically versatile compounds having antifungal , fungicidal, herbicidal and plant growth regulating properties. The presence of imino linkage (-N=C-) in these compounds has been regarded as being assential for the enhancement of antibacterial and antimicrobaial [31] activities.

Table 2. Antimicrobial activity of novel synthesized compounds.

No.	Bactria					Funji	
	Staphylococcus aurous	***E.coli***	***Proteus vulgaris***	***Pseudomonas***	***Klebsiella***	***Aspergillus niger***	***Candida albicans***
II	8	7	4	4	3	11	12
III	9	6	4	4	5	12	9
IV	9	7	4	3	5	13	13
V	19	19	8	9	8	12	14
VI	20	21	15	17	17	12	9
VII	19	14	11	13	16	12	10
VIII	16	12	14	12	12	11	11
IX	16	18	15	16	13	9	8
X	17	19	12	15	13	10	11

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