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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 3-AMINO-6-(2'-AMINOPHENYL)-1,2,4-TRIAZIN-5(4H) ONE

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ABSTRACT

3-(Acyl/aroyl/ alkyl) amino-6- (2'-Acyl/aroyl/ alkyl) amino phenyl) - 1,2,4-triazin-5(4H) ones (3-10) have been synthesized derived from 3-amino-6-(2'-amino phenyl)- 1,2,4-triazin-5(4H) one (2). Structure of the new products have been established upon their elemental and spectral date. The newly prepared compounds showed good anti-microbial activity for more pathogenic microorganisms, prevalence of *Staph. aureus* growth and non-growth, that the growth had decreased almost at 9h and had increased number of non-growth bacteria by compound number 10. *E. coli* were inclined but less than *Staph. aureus*. *Candida albicans* growth had decreased almost at 9h. The most influential was the compound number (4, 8, 11 and 12). That concluded a promising template for anti-bacterial activities replacers.

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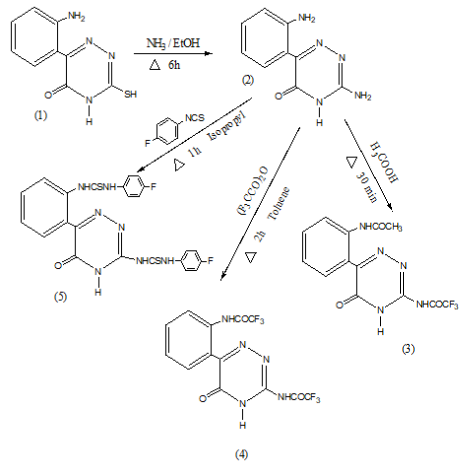
INTRODUCTION

Outstanding for affirmative pharmacological and biological properties of heterocyclic compounds and their derivatives had developed a reliable in medicinal research (Rajeesh, 2018). Temporarily their structural had fixed in many usual products as antibiotics, they had gained fixed position and were growing in nature as triazine (Zhou *et al.,* 2018). Recently, 3-amino -1,2,4- triazines and their derivatives attracted organic chemists very much attention, due to their biological and chemotherapeutic importance (Abdel-Rahman, 2001; Abdel-Rahman, 2001; Abdel-Rahman, 2001) , such as molluscicidal agents, against the snail responsible for Bilharzias (El-gendy, 2003), Potential inhibitors towards HIV-1 activity (Al-Romaizan, 2014; Abdel-Rahman, 2014) , CDK_2 inhibitors (Makki, 2015), antimicrobial (Abdel-Rahman, 2016) as well as use to removal a various ionic metals from wastewater (Baghlaf, 2013; Ramadan, 1993). Triazine-derivatives showed depart applications, present in natural sources had anti-bacterial properties, and it had a growing interest because in appropriated use of antibiotics resistant bacteria (Sojib, 2017). They had appeared with rise of antibiotic resistance bacteria; they acted as substructures and later addition importance (Julian, 2010).

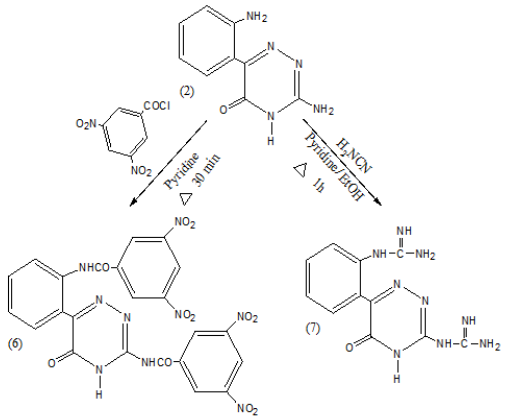
They had important role in medicinal chemistry due to biological activities as anti-bacterial. Triazine incorporated heterocyclic compounds were synthesized and screened for anti-bacterial activity against Gram-positive and negative bacteria. They had showed amazing *in-vitro* anti-bacterial activities against *Staph. aureus* and *E. coli* as major pathogenic bacteria (Rajeesh, 2018). 1, 2, 4-Triazines were had antifungal on different strains of fungi (Ashraf).

Prompted by these observations, the present work approach toward the synthesis of new acyl/aroyl/alkyl amino-1,2,4-triazinones in view of their anti-bacterial activity. That were done by using standard bacterial strains were considered as a major pathogenic bacterium.

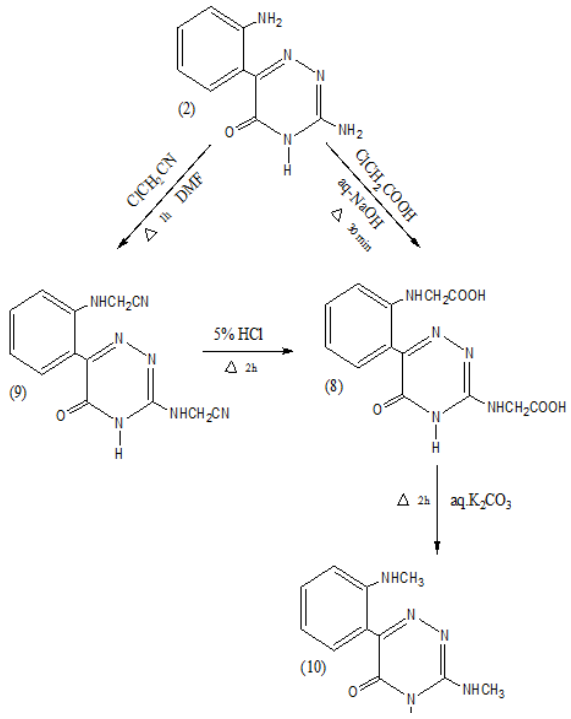
Nowadays bacteria had acquired antibiotics resistance genes. Heterocyclic compounds had a power for very effective anti-bacterial agents. The decisions had enlarged the investigation by using this heterocyclic compound as example of antibiotics for more pathogenic microorganisms' substituent with confirmation.



Scheme 1.



Scheme 2



Scheme 3.

MATERIALS AND METHODS

Melting points determined on an electro thermal Bibby Stuart SMP3 (UK) Scientific melting point apparatus and are uncorrected. The infrared (IR) spectra recorded on Perkin–Elmer RXI FT-IR infrared spectrophotometer using the KBr pellet technique. Electronic absorption spectra were recorded in DMF on Shimadzu UV- Visible 3101 PC spectrophotometer. 1H NMR spectra recorded on a Bruker DPX-400FT-NMR spectrometer at Cairo-University**,** Egypt. Using tetramethylsilane as the internal standard DMSO-d6 as a solvent (Chemical shifts in δ, ppm)**.** ^{19}F-NMR Spectra determined at 84.25 MHz using hexafluorobenzene as a solvent. Splitting patterns were designated as follows: s: single; m: multiple; Mass spectra measured on a GCMS-Q 1000 Ex spectrometer**.** Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values within ± 0.4% of the theoretical values. Follow up of the reaction and checking the homogeneity of the compounds made by TLC on silica gel- protected aluminum sheets (Type 60 F254, Merck). Compound **1** was prepared according to reported method.

3-Amino -6-(2'-aminophenyl)-1,2,4-triazin-5(4H) one (2): A mixture of 1 (0.01mol) and liquid NH_3 (20 ml) in abs. EtOH (20 ml) refluxed for 6h, cooled. The solid obtained filtered off and crystalized form EtOH to give 2. Yield 70 % . M.p 263-265 °C . IR (γ) cm^{-1}: 3500-3200 (b, band), NH_2, 3100 (NH), 1670 (C=O), 1600(C=N). ^{1}HNMR (DMSO-d_6) δ (ppm): 13.4 (s,1H, NH of 1,2,4-triazine), 5.5 & 3.4 (each s, two NH_2), 8.6, 7.6, 7.0, 6.9 (4H, aromatic protons). ^{13}CNMR (DMSO-d_6) δ (ppm): 172.95 (C=O), 152.2(C=N), 147.82 (C=N), 130.54, 130.32, 115.46, 114.87, 114.75 (aromatic and hetero carbons). Analy. Calcd., C: 53.20; H, 4.43; N, 34.48 % for $C_9H_9N_5O$ (203). Found: C, 55.84; H, 4.01; N, 34.20 %.

3-Acetamido-6-(2'-acetamidophenyl)-1,2,4-triazin-5(4H) one (3): Amixture of 2 (0.5 gm) and glacial acetic acid (10 ml) refluxed 1h, cooled then poured into ice. The solid obtained filtered off and crystalized form EtOH to give 3. Yield 73 %. M.p 272-274 °C. IR (γ) cm^{-1}: 3100, 3080 (NH), 2900, 1480 (CH_3), 1670, 1650 (C=O, CONH), 1580(C=N). ^{1}HNMR (DMSO-d_6) δ (ppm): 13.66 (NH of 1,2,4-triazinone), 10.01 & 9.90 (each s, 2H, 2NHCO), 7.52, 7.21, 7.1, 7.01, 6.95 (aromatic protons). Analy. Calcd., C: 54.35; H, 4.52; N, 24.39 % for $C_{13}H_{13}N_5O_3$ (287). Found: C, 54.13; H, 4.21; N, 24.59 %.

3-(Trifluoroacetamido)-6-(2'-trifluoroacetamidophenyl)-1,2,4-triazin-5(4H) one (4): A mixture of **2** (0.5 gm) and trifluoro acetic anhydride (5 ml) in dry toluene (20 ml) refluxed for 2h, cooled then poured into petroleum ether 60-80 . The result ant solid filtered off and crystalized form THF to give 4. Yield 75%. M.p.: 279-280 °C. IR cm^{-1}: 3180, 3200 (2NH), 3070 (NH), 1700, 1680 (2C=O), 1570 (C=N), 1250 (C-F), 820 (aryl group). ^{1}HNMR (DMSO-d_6) (ppm): 13.55, 10.78, 9.22 (each s, 3NH), 8.98,8.52, 7.9, 7.4 (aromatic protons). ^{13}CNMR (DMSO-d_6) (ppm): 163.9 (C=C), 148.27, 148.12 (2CONH), 142.94 (C-F), 137.11, 133.96 (2C=N), 130.83, 130.12, 128.87, 126.8 (aromatic carbons) Analy. Calcd., C: 39.49; H, 1.77; N, 17.72 % for $C_{13}H_7N_5F_6O_3$ (395). Found: C, 39.19; H, 1.55; N, 17.45 %.

N-(4'-Fluorophenyl-N-(4'-fluorophenylthiouriamdophenyl-5'-hydroxy-1,2,4-triazin-3'-yl) thiourea (5): Equimolar amounts of **2** and 4-fluorophenyl iso thio cyanate in isopropyl alcohol (20 ml) refluxed for 1 h, cooled. The solid produced filtered off and crystalized form EtOH to give 5. Yield 70%. M.p.: 275-277 °C. IR cm^{-1}: 3200, 3100 (b, NH, NH), 1660 (C=O), 1200, 1190 (C=S), 1250 (C-F), 820 (p-substituted phenyl). ^{1}HNMR (DMSO-d_6) (ppm): 13.8, 11.5, 9.9 (NH), 8.32_7.37 (m, 12H, aromatic protons), 4.43 (s, C-SH). Analy. Calcd., C: 73.20; H, 4.52; N, 25.99, S, 28.23 % for $C_{23}H_{17}N_7F_2S_2O$ (377). Found: C, 72.92; H, 4.11; N, 25.59; S, 27.89 %. M/S (Int.%): 380 (M+3, 1:11 %), 95 (100).

3- (3',5'-Dinitrobenzamido)- 6-(2'-(3",5"-dinitrobenzamido) phenyl) -1,2,4-triazin-5 (4H)one (6): A mixture of **2** (0.01 mol) and 3,5-dinitrobenzoyl chloride (0.02 mol) in dry pyridine (20 ml) warmed for 30 min, cooled then poured into ice. The solid obtained filtered off and crystalized form dioxan to give **6**. Yield 70 **%**. M.p 279-281 °C IR cm^{-1}: 3200, 3100 (NH), 1680, 1660 (C=O), 1580 (C=N), 1530, 1350 (NO_2), 900,810 (substituted phenyl groups). ^{1}HNMR (DMSO-d_6) (ppm): 13.76, 12.62, 10.27 (3NH), 8.92, 7.68,

7.61, 7.51, 7.40, 7.34, 7.21, 7.19, 7.09, 6.98 (10H, aromatic protons). ^{13}CNMR (DMSO-d_6) (ppm): 206, 191, 178 (3C=O), 148.15, 147.67, 142.33 (C=N), 133.59, 132.80, 131.46, 130.69, 129.29, 127.13 (aromatic carbons), 119.94, 118.42, 114.71(carbons of 1,2,4-triazine). Analy. Calcd., C: 49.11; H, 2.31; N, 22.95 % for $C_{23}H_{13}N_9O_{11}$ (562). Found: C, 48.88; H, 2.11; N, 22.59 %. M/S (Int. %): 564 (M+2, 1.01 %): 167 (100).

3-Guanidyl-6-(2'-guanidylphenyl)-1,2,4-triazin-5(4H) one (7): A mixture of **2** (0.01 mol) and Cyanamid (0.02 mol) in dry pyridine (20 ml) refluxed for 1h, cooled then poured into ice-acetic acid. The yielded solid filtered off and crystalized form EtOH to give **7**. Yield 70 %. M.p 273-275 °C. IR cm^{-1}: 3400 - 3100 (b, NH_2NH), 1660 (CONH), 1620 (deformation NH_2), 1580 (C=N), 1330 (N=CNH). ^{1}HNMR (DMSO-d_6) (ppm): 14.59, 13.71, 12.48, 11.26 (4H, NH), 10.92 (s, 1H, OH of 1,2,4-triazin one), 8.69, 7.99, 7.55, 7.40, 7.2 (4H, aromatic protons), 3.7 & 3.4 (4H, two NH_2). ^{13}CNMR (DMSO-d_6) (ppm): 179.01, (C=O), 162, 155 (C=NH), 143.06, 132.0, 131.24 (C=N), 130.75, 123.01, 122.35, 121.35 (aromatic carbons), 119.93, 117.6 (carbons of 1,2,4-triazinone). Analy. Calcd., C: 45.99; H, 4.52; N, 43.90 % for $C_{11}H_{13}N_9O$ (287). Found: C, 45.66; H, 4.32; N, 43.59 %.

5-Carboxymethylamino-6-(2'-carboxymethylaminophenyl)-1,2,4-triazin-5(4H)-one (8): A mixture of **2** (0.01 mol) and monochloroacetic acide (0.02 mol) in aq.NaOH (5 %, 50 ml) refluxed for 30 min , cooled then poured into ice-HCl. The yielded solid filtered off and crystalized form EtOH to give **8**. Yield 75 %. M.p 265-267 °C. IR cm^{-1}: 3500 - 3400 (OH), 3100 (NH), 3020 (aromatic CH), 2920 (aliphatic CH) 1700, 1670 (2C=O), 1560 (C=N), 1440 (derformation CH_2), 880 (substituted phenyl). ^{1}HNMR (DMSO-d_6) (ppm): 14.60, 13.67, 13.29 (each s, 3H, 3NH), 10.95 (s, 1H, OH), 9.47 (s, 1H, OH of 1,2,4-triazinone), 8.0, 7.64, 7.55, 7.38 (4H, aromatic protons), 2.52 (2H, CH_2), 2.51 (2H, CH_2). ^{13}CNMR (DMSO-d_6) (ppm): 173.56, 173.02, 171,72 (3C=O), 146.45 (C=N), 130.95, 130.13, 129.66, 128.29 (aromatic carbons), 117.33, 116.61, 114.46 (carbons of 1,2,4-triazine), 40.02, 38.76 ($2CH_2$ protons). Analy. Calcd., C: 48.90; H, 4.07; N, 21.94 % for $C_{13}H_{13}N_5O_5$ (319). Found: C, 48.59; H, 3.88; N, 21.59 %.

Formation of 8: Compound **9** (0,05gm) in dilute HCl (5 %, 20 Mml) refluxed for 1h ,cooled, then poured onto ice. The solid obtained filtered off and crystalized form to give **8.**M.p. and mixed M.p. gave no- depresses.

3-Cyanomethylamino-6-(2'-cyanomethylaminophenyl)-1,2,4-triazin-5(4H)-one (9): A mixture of **2** (0.01 mol) and chloroacetonitrile (0.02 mol) in DMF (20 ml) refluxed 1h, cooled then poured into ice. The resultant solid filtered off and crystalized form dioxin to give **9**. Yield 70 %**.** M.p 263-265 °C. IR cm^{-1}: 3100 - 3080 (NH), 3080 (aromatic CH), 2880 (aliphatic CH), 2230(C, 1670 (C=O), 1550 (C=N), 820 (substituted phenyl). ^{1}HNMR (DMSO-d_6) (ppm): 14.43, 11.21, 10.35 (3NH), 8.9, 8.63, 8.03, 7.7 (aromatic protons), 4.23, 4.21 ($2CH_2$). ^{13}CNMR (DMSO-d_6) (ppm): 167.79 (C=O), 155.54, 155.08 (C, 148 (C=N), 130.09, 127.74, 126.98 (aromatic carbons), 118.34 (carbons of 1,2,4-triazine), 40.06, 38.81 ($2CH_2$ carbons). Analy. Calcd., C: 58.86; H, 4.15; N, 36.98 % for $C_{13}H_{11}N_7$ (265). Found: C, 58.55; H, 4.01; N, 36.60 %.

3-Methylamino-6-(2'-methylaminophenyl)-1,2,4-triazin-5(4H)-one (10): Compound 8 (0.5gm) and aq K2CO3 (5 %, 20 ml) refluxed for 2h , cooled then poured into acetic acid. The solid thus obtained filtered off and crystalized form EtOH to give 10. Yield 70 %. m.p 269-270 °C. IR cm-1: 3100 - 3080 (NH), 3020 (aromatic CH), 2890 (aliphatic CH), 1670 (C=O), 1550 (C=N), 1480 (deformation CH3) 880 (substituted phenyl). 1HNMR (DMSO-d6) (ppm): 13.69 (NH, 1,2,4-triazine), 11.88, 11.55 (NHMe), 8.39, 8.20, 7.94 (aromatic protons), 2.88, 2.99 (2CH3 protons). 13CNMR (DMSO-d6) (ppm): 170.33 (C=O), 151, 144 (C=N), 128.01 - 122.5 (aromatic carbons), 40.04, 39.62 (2CH3). Analy. Calcd., C: 57.14; H, 5.62; N, 30% for C11H13N5O (231). Found: C, 56.89; H, 5.33; N, 30.01 %.

RESULTS AND DISCUSSION

3-Amino-6- (2'-aminophenyl)-1,2,4-triazin-5(4H) one (2) (19) as starting material was obtained from refluxed 3-thioxo-6-(2'-aminophenyl)-1,2,4-triazin-5(2H, 4H) one (1) (18) with NH_3 in abs. EtOH (Scheme 1). Acylation of an amino-groups of compounds 2 was deduced from refluxed with glacial acetic acid and /or trifluoro acetic anhydride in dry toluene to give the corresponding N-acyl derivatives 3 (19) and /or 4 respectively, while addition of 4-fluoro phenyl iso thiocyanate to compound 2 in reflux iso propyl alcohol yielded the N.N-disubstituted thiourea 5 (Scheme 1). It is known the present of a nitro group and amino groups in the heterocyclic systems often improve their biocidal properties (14). Thus, nitro aroylnation and amination of compound 2 by reflux with 3,5-dinitrobenzoyl chloride (pyridine) and cyanamide (pyridine) afforded derivative 6 and N-substituted guanidine 7 (scheme 2). α-Amino acids have a vital role in the biological routs in binding of various proteins within a vital-cells (15). Thus, α-Amino acids derivative 8 was obtained from treatment of compounds 2 monochloroacetic acids in aq. NaOH. The compound 8 also produced from the interaction between compound 2 with chloroacetonitriel in warm DMF to give the cyano methylamino derivative 9 which upon acidic hydrolysis by warm with aq. HCl yielded the compound 8. Pyrolysis of 8 via reflux with aq. K_2CL_3 afforded the N-methyl amine derivative 10 (scheme 3). The former structures of new compound obtained were established from their correct elemental analysis and spectral measurements. IR absorption spectrum of compound 2 showed both NH_2 and NH of 1,2,4-triazinone at 3500-3200 (broad band) and 3100 cm^{-1}, in addition C=O, C=N at 1670, 1600 cm^{-1}, while that of compound 3-6 recorded a lacks of both NH_2. Also, ^{1}HNMR spectrum of 2 showed at 5.5 and 3.4 ppm for two-NH_2 protons with at 13.4 ppm for NH of 1,2,4-triazinone, in addition of aromatic protons 8.6, 7.66.9 ppm, while that of 3-6 showed a lack 's of both NH_2 protons. Only the compound 7 recorded two NH_2 and four NH groups at 3.7, 3.4 ppm and 14.59, 13.71, 12.48 and 11.26 ppm, with at 10.29 ppm for OH of 1,2,4-triazine. N-Alkyl amino-derivatives 8-10 recorded the presence of –CH_2-protons at 4 3 ppm and at 66.9 ppm for ^{13}CNMR. IR spectrum of compound 8 showed a characteristic band at 3500, 3100, 3050 and 2920 cm^{-1} attribute to OH, NH, aromatic and aliphatic groups. Also, ^{1}HNMR recorded at 14.60 and 10.95 ppm for NH and OH protons. ^{13}CNMR showed at 173, 171, 154 and 146 ppm for C=O (carboxylic acid), C=O (1,2,4-triazine), 154 (C=N) and (carbon of 1,2,4-triazine). Mass spectrometry of compound 5 and 6 showed a molecular ion peak at low intensity with a base peak at m/e 95 and /or 167 attribute for C_6H_4F and /or C_6H_3 $(NO_2)_2$ radicals.

EXPERIMENTAL SECTION

BIOLOGICAL IN-VITRO EXPERIMENT

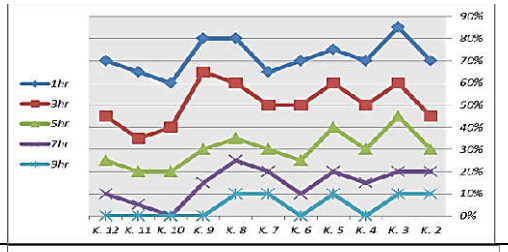
- **Materials preparation:** Triazine-synthesized compounds derivatives as a chemical compound were collected from the "Chemistry Dept.", that to conduct an *in-vitro* experiment.
- **Microorganisms used:** Monitor effects were tested on major pathogenic bacteria and fungi as anti-microbial. These microorganisms under study considered as major pathogenic besides they had multiple resistant drug activities (MADA), also made bid community health problem.

  Bacterial methods: Pure bacterial strains *Staph. aureus* and *E. coli* were collected from the "Research Center" and subculture them on "Molar Hinton Agar". Bacteria had suspended with "Sterile Salt Solution" in sterile "Screw Capped Wizerman Tubes". Same quantity of triazine synthesized compounds derivatives had added to the bacterial suspension and had leaved in incubator at 37ºC. Samples for bacterial inoculation were taken from the mixtures every (1, 3, 5, 7 and 9) hr and were spread on "Mannitol Salt and MacConkey Agar" Inoculated plates had placed in incubator for 24 hr at 37 ºC. Tube turbidity were read by "McFarland Standards" every (1, 3, 5, 7 and 9) hr. Bacterial growth colony were recorded and were calculated in percent pictures by an equation "(Colony account / 300 X 100)" (Abdel-Rahman, 1991).

Table 1 and Graph (1 and 2): Mean prevalence of *Staph. aureus growth and non-growth after exposed to the compounds

*K. *No	Growth %					Non-growth %				
	1*hr	3hr	5hr	7hr	9hr	1hr	3hr	5hr	7hr	9hr
*K. 2	70%	45%	30%	20%	10%	30%	55%	70%	80%	90%
K. 3	85%	60%	45%	20%	10%	15%	40%	55%	80%	90%
K. 4	70%	50%	30%	15%	00%	30%	50%	70%	85%	100%
K. 5	75%	60%	40%	20%	10%	25%	40%	60%	80%	90%
K. 6	70%	50%	25%	10%	00%	30%	50%	75%	90%	100%
K. 7	65%	50%	30%	20%	10%	35%	50%	70%	80%	90%
K. 8	80%	60%	35%	25%	10%	20%	40%	65%	75%	90%
K. 9	80%	65%	30%	15%	00%	20%	35%	70%	85%	100%
K. 10	60%	40%	20%	00%	00%	40%	60%	80%	100%	100%
K. 11	65%	35%	20%	5%	00%	35%	65%	80%	95%	100%
K. 12	70%	45%	25%	10%	00%	30%	55%	75%	90%	100%

***Staph. Aureus: Staphylococcous aureus, *K *No: Key Number, *hr: Hour**



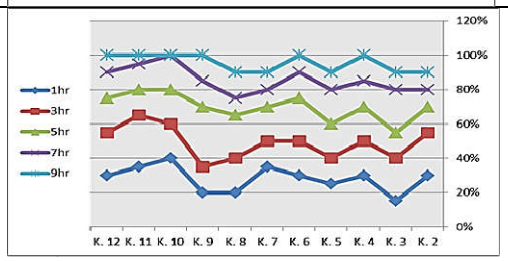
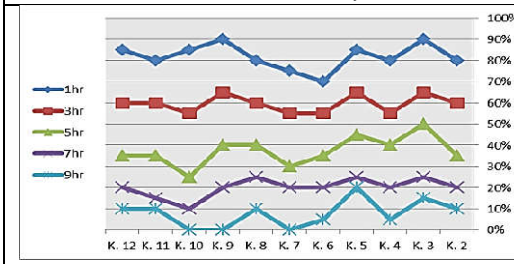


Table 2. and Graph (3 and 4): Mean prevalence of *E. coli growth and non-growth afterexposed to the compounds

*K. *No	Growth %					Non-growth %				
	1*hr	3hr	5hr	7hr	9hr	1hr	3hr	5hr	7hr	9hr
*K. 2	80%	60%	35%	20%	10%	20%	40%	65%	80%	90%
K. 3	90%	65%	50%	25%	15%	10%	35%	50%	75%	85%
K. 4	80%	55%	40%	20%	5%	20%	45%	60%	80%	95%
K. 5	85%	65%	45%	25%	20%	15%	35%	55%	75%	80%
K. 6	70%	55%	35%	20%	5%	30%	45%	65%	80%	95%
K. 7	75%	55%	30%	20%	00%	25%	55%	70%	80%	100%
K. 8	80%	60%	40%	25%	10%	20%	40%	60%	75%	90%
K. 9	90%	65%	40%	20%	00%	10%	35%	60%	80%	100%
K. 10	85%	55%	25%	10%	00%	15%	45%	75%	90%	100%
K. 11	80%	60%	35%	15%	10%	20%	40%	65%	85%	90%
K. 12	85%	60%	35%	20%	10%	15%	40%	65%	80%	90%

***E. coli: Escherichia coli, *K *No: Key Number, *hr: Hour**



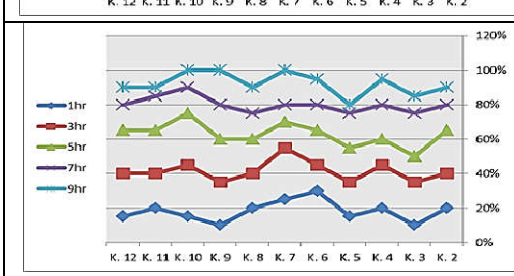
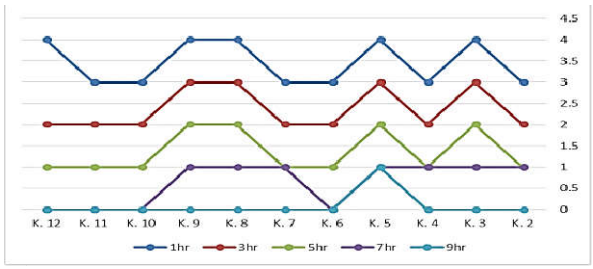
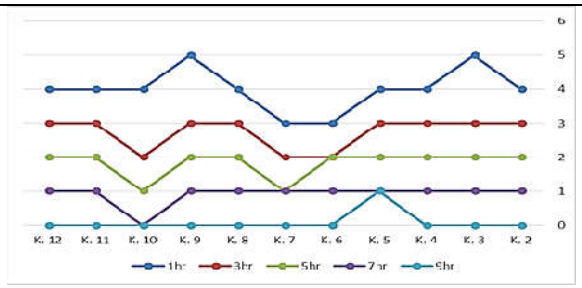


Table 3 and Graph (5 and 6): Mean prevalence of *Staph. aureus and *E. coli turbidity after exposed to the compounds

*K. *No	*Staph. aureus					*E. coli				
	1*hr	3hr	5hr	7hr	9hr	1hr	3hr	5hr	7hr	9hr
*K. 2	3	2	1	1	0	4	3	2	1	0
K. 3	4	3	2	1	0	5	3	2	1	0
K. 4	3	2	1	1	0	4	3	2	1	0
K. 5	4	3	2	1	1	4	3	2	1	1
K. 6	3	2	1	0	0	3	2	2	1	0
K. 7	3	2	1	1	0	3	2	1	1	0
K. 8	4	3	2	1	0	4	3	2	1	0
K. 9	4	3	2	1	0	5	3	2	1	0
K. 10	3	2	1	0	0	4	2	1	0	0
K. 11	3	2	1	0	0	4	3	2	1	0
K. 12	4	2	1	0	0	4	3	2	1	0

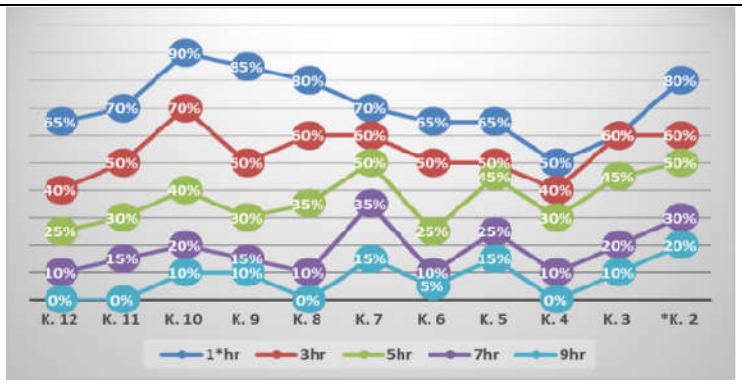
Staph. Aureus: Staphylococcous aureus, *E. coli: Escherichia coli, *K *No: Key Number, *hr: Hour





*K. *No	Growth %				
	1*hr	3hr	5hr	7hr	9hr
*K.	80%	60%	50%	30%	20%
K. 3	60%	60%	45%	20%	10%
K. 4	50%	40%	30%	10%	00%
K. 5	65%	50%	45%	25%	15%
K. 6	65%	50%	25%	10%	5%
K. 7	70%	60%	50%	35%	15%
K. 8	80%	60%	35%	10%	00%
K. 9	85%	50%	30%	15%	10%
K. 10	90%	70%	40%	20%	10%
K. 11	70%	50%	30%	15%	00%
K. 12	65%	40%	25%	10%	00%

*K *No: Key Number, *hr: Hour



- **Fungal methods:** Monitor effects were tested on major pathogenic fungi as anti-fungi. Pure fungi "*Candida albicans*" were collected from the "Research Center" and subculture them on "Sabaroud Agar". Fungi had suspended with "Sterile Salt Solution" in sterile "Screw Capped Wizerman Tubes". Same quantity of triazine synthesized compounds derivatives had added to the fungal suspension and had leaved in incubator at 37ϟC. Samples for fungi inoculation were taken from the mixtures every (1, 3, 5, 7 and 9) hr and were spread on "Sabaroud Agar". Inoculated plates had placed in incubator for 24 hr at 35 ϟC (Abdel-Rahman, 1991).
- **Data analysis:** All results had collected and had entered in "Simple Excel Program" to release as "Tables and Graphs". As well, that will reconnoiter the work prominence. Table 1 and graph (1 and 2) had showed mean prevalence of *Staph. aureus* growth and non-growth after exposed to the compounds, the experiment was carried out to follow the effect of compounds on the bacterial cell growth. It had shown that, the growth had decreased almost at 9 h and had increased number of non-growth bacteria. The most influential was the compound number 10 where it was killed all bacteria at 7 h. As well, compounds (4, 6, 9, 11 and 12) were completely killed bacterial cells at 9 hr. However, compounds number (4, 6, 9, 11 and 12), then (2, 3, 5, 7 and 8) where bacterial growth was very small until 9 hr. This had indicated the effect of these compounds under experiment on the bacterial growth and this had shown by the amount of growth and non-growth of bacterial cells. The ability of various compounds had capability to counteract bacterial cells was an alternative to antibiotics (Rajeesh *et al.,* 2018; Zhou, 2008; Sojib *et al.,* 2017; Julian, 2010; Ashraf *et al.,* 2018).

Table 2 and graph (3 and 4) had showed mean prevalence of *E. coli* growth and non-growth after exposed to the compounds, the experiment was conducted to follow the effect of compounds on the growth of bacteria had been shown to grow almost were inclined but less than *Staph. aureus*. The first compound was had the compound number (7, 9 and 10) at 9 h of the growth and increasing number of non-growth bacteria. There was no complete killed of bacteria at 7 h and the arrangement was after 95% for the compounds number (4 and 6); 90% for (2, 8, 11 and 12); 85% for compound number 3 and finally 80% for compound number 5. This had indicated the effect of these compounds were under experiment on the bacterial growth and this had shown by the amount of growth and non-growth. The ability of compounds to kill bacterial cells was an alternative to antibiotics (Rajeesh *et al.,* 2018; Zhou, 2008; Sojib *et al.,* 2017; Julian, 2010; Ashraf *et al.,* 2018). Table 3 and graph (5 and 6) had showed mean prevalence of Staph. aureus and E. coli turbidity after exposed to the compounds, by measuring the turbidity it was found to have gradually decreased the lack of bacterial cells and their death. Staph. aureus had affected by compounds number (6, 10, 11 and 12) at 7 h, which had led to clear the tubes as killing all cells. Moreover, the rest had killed at 9 h that did not have any turbidity. Which had indicated the death of bacteria except compound number 5 did not kill all cells. As well for E. coli, there was no turbidity for compound 10 at 7 h; the rest had no turbidity except for compound number 5.

This was indicated this compound had anti-bacterial activity, so could be used as sub-stationary for antibiotics, they were used as a natural remedy and are available from plant extracts (Rajeesh *et al.,* 2018; Zhou, 2008; Sojib *et al.,* 2017; Julian, 2010; Ashraf *et al.,* 2018). Table 4 and Graph 7 showed mean prevalence of Candida albicans growth after exposed to the compounds; the experiment was carried out to follow the effect of compounds on the fungal cell growth. It had shown that, the growth had decreased almost at 9 h. The most influential was the compound number (4, 8, 11 and 12). This had indicated the effect of these compounds under experiment on the fungal growth and this had shown by the amount of growth of fungal cells. The ability of various compounds had capability to counteract fungal cells was an anti-fungal effect and as substitution of chemical fungicidal (Rajeesh *et al.,* 2018; Zhou, 2008; Sojib *et al.,* 2017; Julian, 2010; Ashraf *et al.,* 2018).

CONCLUSION

Some new substituted amino-1,2,4-triazin-5-ones have been obtained by a simple route. The effect of c triazine-derivatives on Staphylococcus aureus (Staph. aureus) had revealed the growth had decreased at 9 h and increased non-growth bacteria. Compound number 10 had killed all bacteria at 7 h while compounds (4, 6, 9, 11 and 12) had completely killed at 9 h. However, compounds number (4, 6, 9, 11 and 12), then (2, 3, 5, 7 and 8) bacterial growth were very small until 9 h. Escherichia coli (E. coli) growth had revealed less than Staph. aureus. Compound number (7, 9 and 10) at 9 h of the growth and increasing number of non-growth bacteria. There was no complete killed at 7 h and the arrangement was after 95% for compounds number (4 and 6); 90% for (2, 8, 11 and 12); 85% for compound number 3 and finally 80% for compound number 5. The measuring turbidity had found gradually decreased in bacterial cells growth and had increased their death. Staph. aureus was affected by compounds number (6, 10, 11 and 12) at 7 h. Moreover, the rest was affected at 9 h, which did not have any turbidity, except compound number, 5 did not kill all cells. As for *E. coli*, there was no turbidity for compound 10 at 7 h; at the rest did not have turbidity except for compound number 5. *Candida albicans* growth had decreased almost at 9 h, that was for compound number (4, 8, 11 and 12). That concluded the newly prepared compounds showed good anti-microbial activity, also showed that may be a promising template for anti-bacterial activities. That recommended uses in substitution of resistant bacteria and fungi.

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