Egypt. J. Chem. **Vol. 64,** No. 5 pp. 2653 - 2661 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis and evaluation of *in vitro* biological activity of new series of quinazolinone and benzoxazinone derivatives

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Abstract

A convenient synthesis for 1,3,4-thiadiazolyl quinazolinone derivatives is described via facile cyclization of anthranilic acid with succinic anhydride and upon reaction with glycine afforded 3-glycinyl quinazolinone derivative **(2)**, which then treated with thiosemicarbazide and produced 1,3,4-thiadiazolyl quinazolinone derivative **(3)**. Also, the key intermediate isothiocyanatobenzoazinone **(13)** is synthesized and checked for the synthesis of triazolyl-**(14)**, oxooxazolidinyl-**(15)** and triazinanyl-**(16a,b)** benzoxazinone scaffolds by reaction with phenylhydrazine, glycine and urea and/ or thiourea, respectively.

Antimicrobial activity of the synthesized compounds were evaluated against selected bacterial and fungal strains and compared with penicillin and compounds **6, 7b, 10b, 11, 14** and **16b** exhibited promising activity as compared to the tested standard. The structures of the products were assigned and confirmed on the basis of their elemental analyses as well as spectral data (IR, MS and ^{1}H NMR).

Keywords: Thiadiazolylquinazolinones, isothiocyanato benzoxazinone, *N*-heterocycles, scaffolds, antimicrobial activity.

1. Introduction

Quinazoline nucleous considered one of the most important classes of pharmaceuticals, which exhibit various activities as anti-tumor [1,2], antimalarial [3], anti-inflammatory [4], and antimicrobial [5,6]. It occurs extensively in nature and range of natural building blocks as alkaloids, and found across the plant and animal kingdoms as well as various microorganisms such as *Peganum nigellastrum* [7], *Bouchardatia neurococca* [8], *Dichroa febrifuga* [9] and *Bacillus cereus*[10].

2,4-Diaminoquinazoline analogues of folic acid have attracted a significant interest as potential chemotherapeutic agents, and found a remarkable antimalarial effects, also in treatment of pneumocystis carimi infection in AIDS patients [11,12], and In diabetes patients, Quinazoline inhibits the tyrosine kinase [13] activity which is an important and urgent matter.

Also, functionalized quinazolines possess diverse biological, and pharmaceutical activities as anti-bacterial [14], antioxidant [15], antiviral [16], anti-HIV [17], anticonvulsant [18], anti-obesity [19], antituberculosis [20], inflammatory [21], analgesic [22], anti-diabetes [23], anti-psychotic [24], anti-cytotoxin [25], and anti-hypertension [26], and insecticidal [27]. In addition, substituted 1,3,4-thiadiazole derivatives have received significant attention during the last years, and have been investigated increasingly due to their various therapeutic and industrial applications, the biological activities of 1,3,4-thiadiazole derivatives is supposed due to the presence of =N−C−S− moiety [28,29].

It has been reported that 1,3,4-thiadiazole scaffolds known to be associated with diverse pharmacological activities including antimicrobial [30], anti-bacterial [31], antioxidant [32], anti-inflammatory [33], anticancer [34], anti-HIV [35], antiviral [36], anti-tuberculosis [37], anticonvulsant [38], antidepressant [39], kinesin inhibitors [40], etc.

These growing and diverse applications prompted us to continue our ongoing interest on synthesis of these biologically active scaffolds [41-45]. Herein, we describe an efficient synthesis of 1,3,4-thiadiazolyl quinazolinone, triazolyl-, oxooxazolidinyl- and triazinanyl- benzoxazinone scaffolds and evaluation of their anti-microbial activities.

2. Experimental

2.1. Materials

Melting points were measured in open capillaries by Gallen kamp melting point apparatus and are uncorrected. Elemental analyses were carried out at micro analytical unit, Cairo university as well as ^{1}H NMR, IR and Mass spectra were carried out by

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Receive Date: 03 February 2021, Revise Date: 18 February 2021, Accept Date: 24 February 2021

DOI: 10.21608/EJCHEM.2021.61385.3319

micro analytical unit at Cairo University and Mansoura University. IR spectra (KBr disk) of the synthesized compounds were recorded on JASCO 600 plus spectrometer.

^{1}H NMR spectra were recorded on a Bruker a vance 400 (400 MHZ) using deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) as a solvent. Mass spectra were recorded on a Shimadzu (GCMS-QP) 100 EX mass spectrophotometer.

All reaction were monitored by thin layer chromatography TLC and carried out on 0.1 mm silica gel 60f254 (mark) plates.

Antibacterial and antifungal activities were carried out in the Micro Analytical Center, Faculty of Science, Cairo University.

2.2. Methods

Synthesis of 3-(4-oxo-4*H*-benzo[*d*][1,3]oxazin-2-yl)propanoic acid (1).

A mixture of anthranilic acid (0.01mol) and succinic anhydride (0.01 mol) in (20 mL pyridine) was refluxed for 4 hrs. After that, cooled, and poured on ice/HCl, the precipitated solid filtered off, dried and recrystallized from ethanol. (*c.f.* Table 1).

Synthesis of 3-(3-(carboxymethyl)-4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid (2).

Equimolar quantities of Compound **1** (0.01 mol) and Glycine (0.01 mol) in in pyridine (15 mL) was refluxed for 4h. The reaction mixture was left to cool and deposited solid was filtered off, washed several times, dried and recrystallized from ethanol/H_2O. (*c.f.* Table 1).

Synthesis of 2-(2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl)-3-((5-amino-1,3,4-thiadiazol-2-yl)methyl)quinazolin-4(3*H*)-one (3).

Reaction of Compound **2** (0.01 mol) with thiosemicarbazide (0.02 mol) in $POCl_3$ (15 mL) was heated for 7hr, then the reaction mixture was cooled, poured on crushed ice, and the produced solid was filtered off, and recrystallized from ethanol. (*c.f.* Table 1).

General procedure of Synthesis of compounds (4) and (5).

To solution of compound **3** (0.01 mol) in ethanol (15 mL), acetyl chloride and/ or acetic anhydride (0.02 mol) was added and boiled for 6h. After cooling, the precipitated solid obtained was poured on ice, filtered off, and recrystallized from ethanol. (*c.f.* Table 1).

***N*-(5-((2-(2-(5-acetamido-1,3,4-thiadiazol-2-yl)ethyl)-4-oxo quinazolin-3(4*H*)-yl)methyl)-1,3,4-thiadiazol-2-yl)acetamide (4).** (*c.f.* Table 1).

***N,N*-(5-((2-(2-(5-acetamido-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin- 3(4H)-yl)methyl)-1,3,4-thiadiazol-2-yl)acetamide (5).** (*c.f.* Table 1).

Synthesis of 1-(5-(2-(4-oxo-3-((5-(3-phenylureido)-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydroquinazolin-2-yl)ethyl)-1,3,4-thiadiazol-2-yl)-3-phenylurea (6).

A mixture of compound **3** (0.01 mol) and phenylisocyante (0.02 mol) in dry benzene (20 mL), and in presence of catalytic amount of triethylamine was refluxed for 5hrs. The produced solid was filtered off and recrystallized from n-butanol. (*c.f.* Table 1).

Synthesis of 2-(2-(5-(benzylideneamino/4-chlorobenzylideneamino)-1,3,4-thiadiazol-2-yl)ethyl)-3-((5-(benzylideneamino/4-chlorobenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)quinazolin-4(3*H*)-one (7a,b).

To a stirred solution of compound **3** (0.01 mol) in absolute ethanol (15 mL) was added to benzaldehyde and/ or 4-chlorobenzaldehyde (0.02 mol). The mixture was refluxed for 3h, then cooled. The deposited solid was collected and recrystallized from methanol. (*c.f.* Table 1).

General procedure for synthesis of compounds (8) and (9):

A (0.01 mol) of compound **3** in THF (15 mL) was treated with (0.02 mol) of *N*- tosylglycine, and/or *N*-methyl alanine and in presence of DCCI was stirred for 24 h. The produced solid was filtered off, washed with H_2O and recrystallized from a proper solvent. (*c.f.* Table 1).

Synthesis of 2-((4-methylphenyl)sulfonamido)-*N*-(5-((2-(2-(5-(2-((4-methylphenyl)sulfonamido)acetamido)-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin-3(4*H*)-yl)methyl)-1,3,4-thiadiazol-2-yl)acetamide (8).(*c.f.*Table 1).

Synthesis of 2-(methylamino)-*N*-(5-((2-(2-(5-(2-(methyamino)propanamido)-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin-3(4*H*)-yl)methyl)-1,3,4-thiadiazol-2-yl)propanamide (9). (*c.f.* Table 2).

General procedure for synthesis of 10a,b:

A mixture of compound **3** (0.01 mol) and *N*-phathaloyl glycine, and/ or *N*-Phathaloyl phenylalanine (0.02 mol) in n–butanol (20 mL) was refluxed for 5h. The produced solid was filtered off, and recrystallized from a proper solvent.

Table (1). physical data of compounds (1-8).

Compd. No	M.F. M. wt.	M. P. °C Colour	Yield (%)	Solvent	Analysis Calc. (Found) %		
					C	H	N
1	$C_{11}H_9NO_4$ 219.19	160-162 White	80	Ethanol	60.27 (60.14)	4.14 (4.09)	6.39 (6.42)
2	$C_{13}H_{12}N_2O_5$ 276.24	165-167 Pale yellow	78	ethanol/H_2O	56.52 (56.48)	4.38 (4.25)	10.14 (10.09)
3	$C_{15}H_{14}N_8OS_2$ 386.07	172 – 174 Pale yellow	70	Ethanol	46.62 (46.53)	3.65 (3.61)	29.00 (28.93)
4	$C_{19}H_{18}N_8O_3S_2$ 470.09	168-170 Yellow	91	Ethanol	48.50 (48.44)	3.86 (3.79)	23.81 (23.72)
5	$C_{23}H_{22}N_8O_5S_2$ 554.12	180-182 Yellow	80	Ethanol	49.81 (49.65)	4.00 (3.90)	20.20 (20.31)
6	$C_{29}H_{24}N_{10}O_3S_2$ 624.70	157-159 Brown	72	n-butanol	55.76 (55.71)	3.87 (3.75)	22.42 (22.46)
7a	$C_{29}H_{22}N_8OS_2$ 562.67	144-146	76	Methanol	61.90 (61.84)	3.94 (3.88)	19.91 (19.94)
7b	$C_{29}H_{20}Cl_2N_8OS_2$ 631.56	150-152 Yellow	81	Methanol	55.15 (55.04)	3.19 (3.16)	17.74 (17.69)
8	$C_{33}H_{32}N_{10}O_7S_4$ 808.92	183-185 Pale yellow	78	n-butanol	49.00 (48.92)	3.99 (4.04)	17.32 (17.27)

Synthesis of 2-(1,3-dioxoisoindolin-2-yl)-N-(5-((2-(2-(5-(2-(1,3-dioxoisoindolin-2-yl)acetamido)-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin-3(4*H*)-yl)methyl)-1,3,4-thiadiazol-2-yl)acetamide (10a) (*c.f.* Table 2).

Synthesis of (1,3-dioxoisoindolin-2-yl)-N-(5-((2-(2-(5-(2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanamido)-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin-3(4*H*)-yl)methyl)-1,3,4-thiadiazol-2-yl)-3-phenylpropanamide (10b) (*c.f.* Table 2).

Synthesis of 2-(5-((2-(2-(5-(1,3-dioxoisoindolin-2-yl)-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin-3(4*H*)-yl)methyl)-1,3,4-thiadiazol-2-yl)isoindoline-1,3-di-one (11).

A (0.01 mol) of compound **3** was refluxed with (0.02 mol) of phthalic anhydride in (20 mL) benzene, and in presence of catalytic amount of triethylamine for 3hrs. The produced solid was filtered off, washed with H_2O and recrystallized from methanol. (*c.f.* Table 2).

Synthesis of 1-(5-((2-(2-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,3,4-thiadiazol-2-yl)ethyl)-4-oxoquinazolin-3(4H)-yl)methyl)-1,3,4-thiadiazol-2-yl)-1H-pyrrole-2,5-dione (12).

A mixture of compound **3** (0.01 mol) and maleic anhydride (0.02 mol) in ethanol (20 mL), was refluxed for 5hrs. After that, the produced solid was filtered off, and recrystallized from n-butanol. (*c.f.* Table 2).

Synthesis of 3-(4-oxo-4*H*-benzo[*d*][1,3]oxazin-2-yl)propanoyl isothiocyanate (13).

To a stirred solution of the acid chloride (0.01 mol) in dry acetone (50 mL), a solid ammonium thiocyanate (0.01 mol) was added. And the reaction mixture was stirred for one hour at room temperature. Ammonium chloride was precipitated during the progress of the reaction, and separated by filtration leaving a clear solution of isothiocyanatobenzoazinone.

Synthesis of 2-(2-(2-phenyl-5-thioxo-2,5-dihydro-1H-1,2,4-triazol-3-yl)ethyl)-4H-benzo[d][1,3]oxazin-4-one (14).

A solution of isocyanatobenzoxazinone **(13)** (0.01 mol) and phenyl hydrazine (0.01 mol) in dry acetone (30 mL), and in presence of catalytic amount of pyridine was heated under reflux for 2 hrs, and concentrated. Then treated with a proper solvent to give solid crystals. (*c.f.* Table 2).

Synthesis of 3-(4-oxo-4H-benzo[d][1,3]oxazin-2-yl)-N-(5-oxooxazolidin-2-yl)propanamide (15).

To a solution of benzoxazinone isothiocyanate **(13)** (0.01 mol) in dry acetone (30 mL), glycine (0.01 mol) and few drops of pyridine were added as a catalyst. The reaction mixture refluxed for 2 hrs, after cooling, a solid product was precipitated, filtered off, washed with water and recrystallized from ethanol to give compound **(15).** (*c.f.* Table 2).

General procedure for synthesis of 16a,b:

To a stirred solution of benzoxazinone isothiocyanate **(13)** (0.01 mol) in dry acetone (30 mL), Urea and /or thiourea (0.01 mol) was added, and in presence of pyridine as a base catalyst. After that allowed to stir for one hour at room temperature. The solid precipitated filtered off, dried and then

crystallized from proper solvent to get compounds **16a,b**.

2-(2-(4-oxo-6-thioxo-1,3,5-triazinan-2-yl)ethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one (16a). (*c.f.* Table 2).

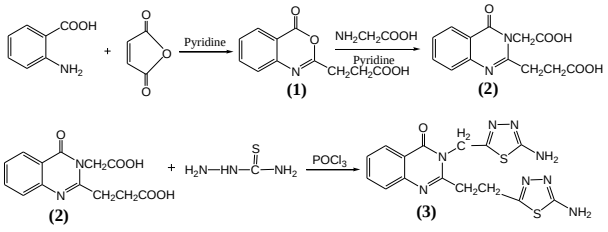
2-(2-(4,6-dithioxo-1,3,5-triazinan-2-yl)ethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one (16b). (*c.f.* Table 2).

Table (2). Physical data of compounds (9-16).

Compd. No	M.F. M. wt.	M. P. °C Colour	Yield (%)	Solvent	Analysis Calc. (Found) %		
					C	H	N
9	$C_{23}H_{28}N_{10}O_3S_2$ 556.66	188-190 Brown	85	Ethanol	49.63 (49.52)	5.07 (5.01)	25.16 (25.09)
10a	$C_{35}H_{24}N_{10}O_7S_2$ 760.13	193-195 White	76	Methanol	55.26 (55.19)	3.18 (3.11)	18.41 (18.35)
10b	$C_{49}H_{36}N_{10}O_7S_2$ 940.22	186-188 Yellow	77	n-butanol	62.54 (62.49)	3.86 (3.81)	14.88 14.81
11	$C_{31}H_{18}N_8O_5S_2$ 646.66	175-177 Yellow	76	Benzene	57.58 (57.52)	2.81 (2.98)	12.37 (12.29)
12	$C_{23}H_{14}N_8O_5S_2$546.54	182-184 White	74	n-butanol	50.07 (50.36)	2.58 (2.88)	20.50 (20.35)
14	$C_{18}H_{14}N_4O_2S$ 350.08	132-134 Yellow	75	Toluene	61.70 (61.84)	4.03 (4.12)	15.99 (15.63)
15	$C_{14}H_{13}N_3O_5$ 303.27	125-127 Pale yellow	67	Ethanol	55.45 (55.83)	4.32 (4.49)	13.86 (13.66)
16a	$C_{13}H_{12}N_4O_3S$ 304.32	136-138 Pale yellow	78	Ethanol	51.31 (51.25)	3.97 (3.90)	18.41 (18.35)
16b	$C_{13}H_{12}N_4O_2S_2$ 320.39	120-122 Yellow	75	Ethanol	48.74 (48.69)	3.78 (3.71)	17.49 (17.42)

3. Results and discussion:

Initially, 3,1-benzoxazin-4-one-3-propanoic acid (1) is easily prepared when succinic anhydride was allowed to react with anthranilic acid in refluxed pyridine, which then was treated with glycine and afforded 3-glycinyl quinazolinone derivative (2) in good yield (Scheme 1).



Scheme 1. Synthesis of compounds (1-3)

IR-spectrum of compound (1) showed νOH at 3424 cm^{-1}, νC-H aromatic centered at 3048, in addition to νC=O'^{S} at 17**6**5, 1708 cm^{-1}. Its mass spectrum showed molecular ion peak ($M^{\cdot+}$-1) at 218 (2.80%), and the base peak at 57 (100%), and other fragments, which confirm the given structure in chart 1.

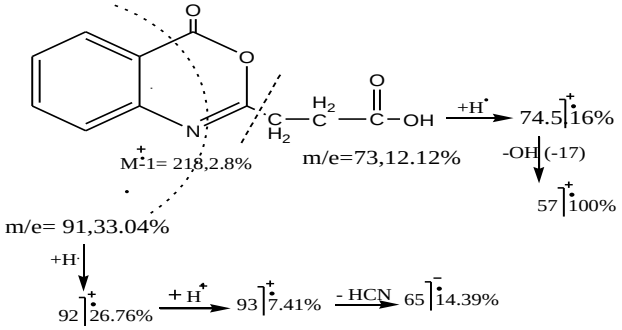


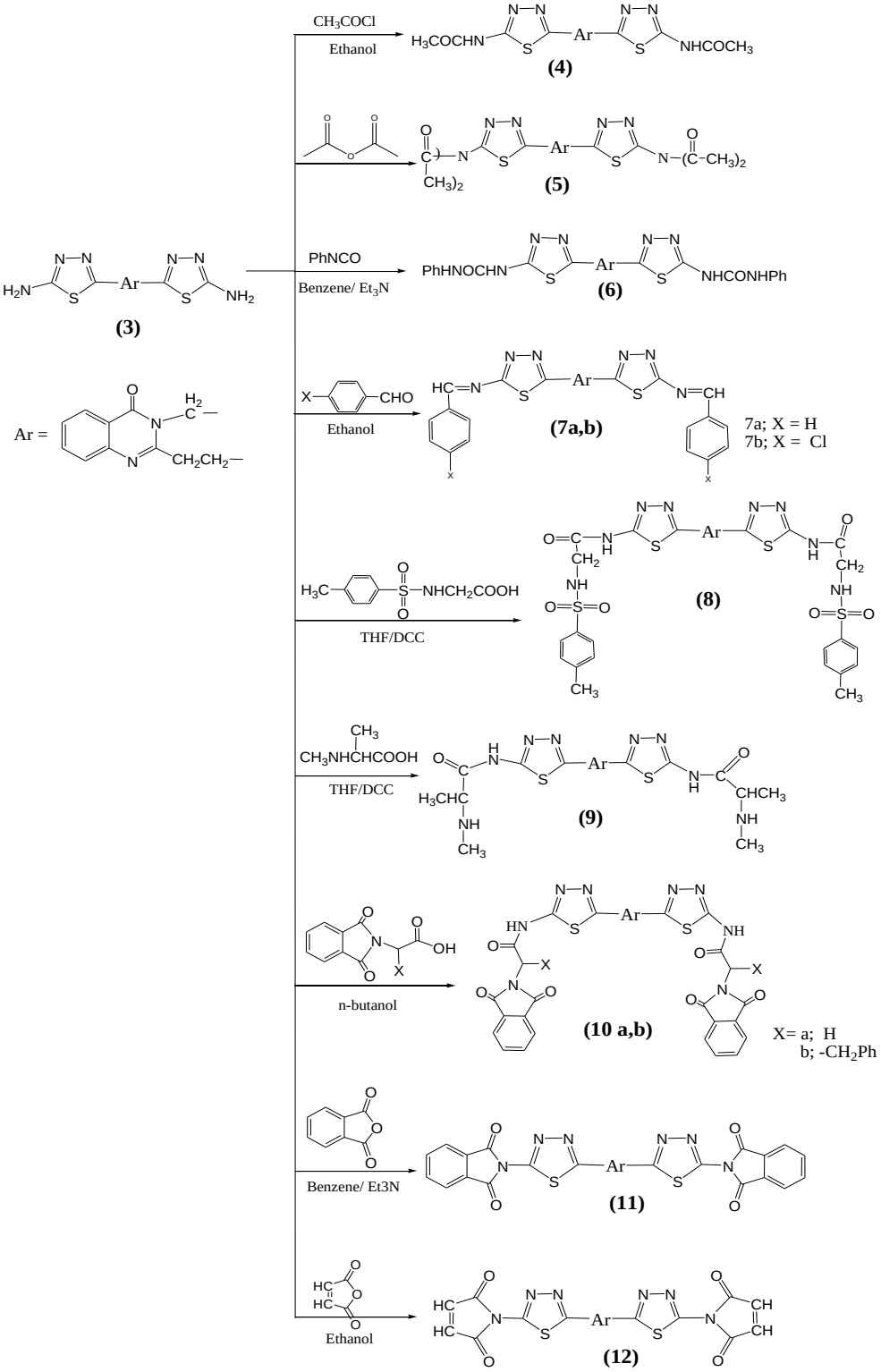
Chart 1. Mass fragmentation pattern of compound (1)

The structure of quinazolinone derivative (2) was inferred from its IR-spectrum which showed νOH at 3422 cm^{-1}, and frequency due to acid, and quinazolinone carbonyls νC=O'^{S} at 1716, and 1680 cm^{-1}, respectively.

After that, treatment of quinazolinone derivative (2) with thiosemicarbazide in $POCl_3$, furnished the key compound thiadiazolylquinazolinone derivative (3),

which clearly implied from disappearance of carbonyl and hydroxyl bands of acid in its IR spectrum.

Next, the nucleophilicity of NH_2 in the thiadiazolyl quinazolinone derivative (3) has been checked for alternate synthesis of some other derivatives (4-12). (Scheme 2)



Scheme (2). Synthesis of compounds (4-12)

Acetylation of the thiadiazoloquinazolinone derivative **(3)** using acetyl chloride and / or acetic anhydride readily provide the acetylated products **(4)** and **(5)**, respectively. Compound **(4)** was elucidated on the basis of its IR-spectrum which showed νNH'^{S} at 3407, 3230 cm^{-1}, νC-H aromatic at 3050, and νC-H aliphatic at 2925 cm^{-1}, besides the frequency due to carbonyls at 1710, and 1683 cm^{-1}, and its ^{1}H NMR spectrum showed signals at δ'^{S} ppm at 1.8 (s, 6H , 2 CH_3), 2.8 (t, 4H , 2 CH_2), 3.3 (s , 2H , 1CH_2), 7-8 (m , 4H , Ar-H), and 8.57 (s, 2H , NH'^{S}), while IR-spectrum of compound **(5)** showed νC=O'^{S} in range of 1710-1685cm^{-1} and disappearance of absorption bands due to NH'^{S} and ^{1}H NMR spectrum showed signals at δ'^{S} ppm at 1.9 (s, 4CH_3 ,12H), 2.8 (t, 4H, 2CH_2), 3.4 (s, 2H, 1CH_2), 7.4-8.2 (m, 4H , Ar-H). Its Mass spectrum showed molecular ion peak (M$^{\cdot+}$-1) at 553 (0.18%), (M$^{\cdot+}$-2) at 552(0.43%), and the base peak at 57(100%).

Addition of phenyl isocyanate to the thiadiazoloquinazolinone derivative **(3)** produced (5-(2-(4-oxo-3-((5-(3-phenylureido)-1,3,4-thiadiazol-2-yl)methyl)-1,3,4-dihydroquinazolin-2-yl)ethyl)-1,3,4-thiadiazol-2-yl)-3-phenylurea **(6)**. The structure of this compound was inferred from its IR-spectrum which showed νNH'^{S} at 3424cm^{-1}, νC-H aromatic at 3049, and νC-H aliphatic at 2922 cm^{-1}, and νC=O'^{S} at 1719, and 1685 cm^{-1}, and ^{1}H NMR spectrum, which showed signals δ'^{S} ppm at 3.3 (s , 6 H , 3CH_2) , 7.4-8.1 (m, 14H, Ar –H), and 8.6 (s, 4H, NH'^{S}).

Also, the Schiff's base derivatives **(7a)** and **(7b)**, were obtained in good yields by condensation of thiadiazoloquinazolinone derivative **(3)** with different aldehydes such as benzaldehyde and/or 4-chlorobenzaldehde, respectively. IR-spectrum of compound (**7a)** showed νC=O at 1693, and νC=N at 1635 cm^{-1}, besides the other characteristic peaks of the compound.

Also, the structure of the schiff's base derivative **(7b)** was characterized from correct analytical data as well as its IR-spectrum which showed νC=O at 1685, and νC=N at 1630 cm^{-1}, and frequency due to *p*-disubstituted at 956 and 884.

Furthermore, and in order to obtain amino acid derivatives of thiadiazolo quinazolinone. *N*-tosylglycine and/ or *N*-methyl alanine was added to the thiadiazoloquinazolinone derivative **(3)** in refluxed THF, and in presence of DCC and yielded compound **8**, and **9**, respectively. IR spectrum of compound **8** showed νNH'^{S} at 3382 cm^{-1}, νC=O'^{S} at 1710, and 1685 cm^{-1}, νC=N at 1630 cm^{-1}, and absorption band at 1338 cm^{-1} due to (S=O) absorption, while IR spectrum of compound **9** showed ν NH'^{S} at 3384 cm^{-1}, and νC=O'^{S} at 1711 and νC=N'^{S} at 1632 cm^{-1}, in addition to other characteristic peaks of the compound.

Incorporation of dioxoisoindoline moiety in the thiadiazoloquinazolinone **(3)** was also achieved by treatment with *N*- phathaloylglycine, and/ or *N*-Phathaloyl phenylalanine in boiling n-butanol, and afforded compounds **10a** and **10b**, respectively. IR-spectrum of **(10a)** showed νNH'^{S} at 3466 cm^{-1}, and νC=O'^{S} at 1773, 1724 and 1665 cm^{-1}, and ^{1}H NMR spectrum showed signals δ'^{S} ppm at 2.4 (t, 4H, 2CH_2), 3.3 (s, 6H, 3CH_2), 7.4-8.2 (m, 12H, Ar-H), and 8.6 (s, 1H, NH'^{S}). The mass spectrum of compound **(10a)** showed molecular ion peak (M$^{\cdot+}$-1) at 759 (0.14%), and the base peak at 91 (100%).

While, the structure obtained from incorporation of *N*-Phathaloyl phenylalanine into thiadiazoloquinazolinone **(10b)** was characterized from IR-spectrum which showed νNH'^{S} at 3327 cm^{-1}, besides the νC=O'^{S} at 1778, 1716 and 1686 cm^{-1}.

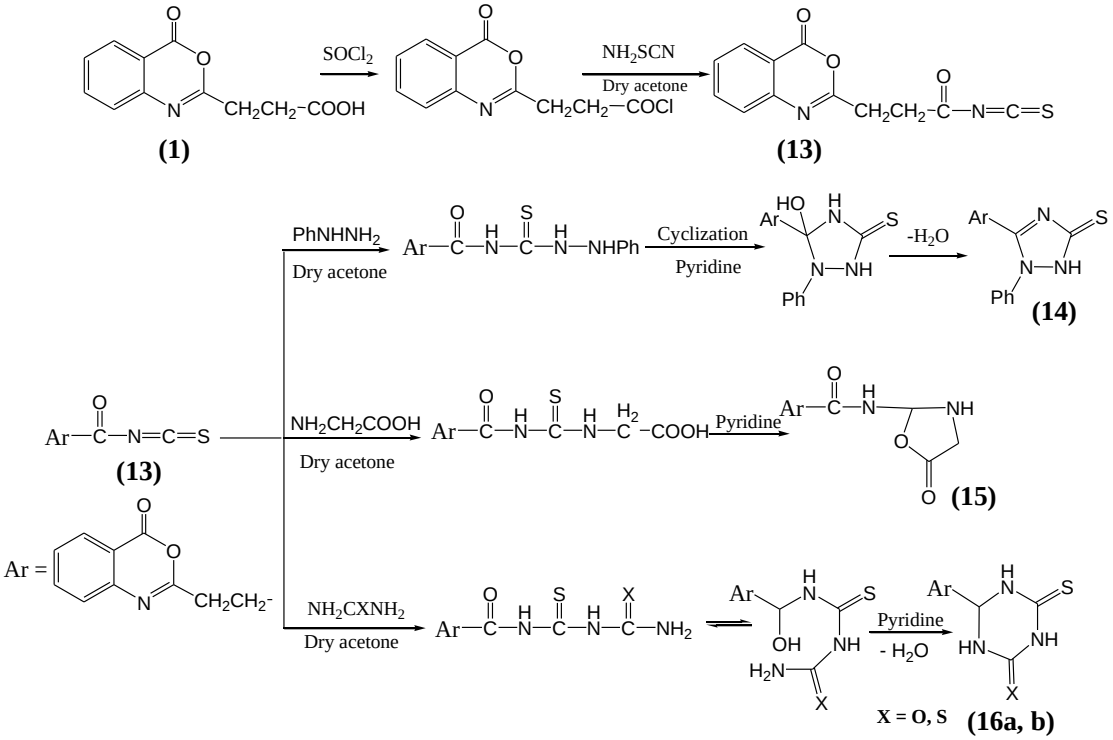
Bis-(1,3-dioxoisoindolinyl/(2,5-dioxo-2,5-dihydro-pyrrolyl)thiadiazolyl quinazolinone derivative **(11)** and **(12)** were also achieved, respectively, via reaction of the thiadiazoloquinazolinone derivative **(3)** with phthalic anhydride and / or maleic anhydride. IR-spectrum of compound **(12)** showed disappearance of aldehydic carbonyls absorption bands, and appearance of νC=O'^{S} in range of 1720-1685cm^{-1}, besides the other characteristic peaks of the compound. Its ^{1}H NMR spectrum showed signals at δ'^{S} ppm at 4.3 (s, 2H, 3CH_2), 5.5 (dd , 4H , 2 CH = CH), 7.4-8.2 (m ,12H, Ar-H).

On the other side, when isothiocyanato benzoxazinone **(13)** solution in acetone (prepared in situ) was treated with phenyl hydrazine gave intermediate, which undergoes cyclization followed by dehydration and furnished 2-(2-(2-phenyl-5-thioxo-2,5-dihydro-1H-1,2,4-triazol-3-yl)ethyl)-4H-benzo[d][1,3]oxazin-4-one **(14)**. **(Scheme 3).**

IR-spectrum of compound **(14)** showed absorption bands for νNH'^{S} at 3447, 3422, and 3168 cm^{-1}, νC-H aromatic at 3049, νC-H aliphatic at 2926 cm^{-1}, νSH at 2066 cm^{-1}, νC=O at 1765 cm^{-1}, νC=N at 1621cm^{-1}, and νC=S at 1402 cm^{-1}.

Also, glycine reacted with 3,1-benzoazinone isothiocyanate **(13)** and produced the thiourea derivative, which cyclized in the presence of pyridine to 3-(4-oxo-4H-benzo[d][1,3]oxazin-2-yl)-N-(5-oxooxazolidin-2-yl)propenamide **(15)**. IR-spectrum showed absorption bands for νNH'^{S} at 3420 cm^{-1}, νSH at 2066 cm^{-1}, νC=O'^{S} at 1767, 1742, and 1662 cm^{-1}, and νC-O-C (ether) at 1128, 1075cm^{-1}, ^{1}H NMR spectrum showed signals δ'^{S} ppm at 2.4 (t, 4H, 2CH_2), 3.3 (s, 2H, 1CH_2), 7.4-8.2 (m ,4H, Ar-H), and 8.6 (s, 2H, NH'^{S}).

Finally, Addition of urea and/or thiourea to the isothiocyanato benzoxazinone **(13)** afforded thioxo/ dithioxo-1,3,5-triazinanyl benzoxazinone **(16a)** and **(16b)**, respectively. IR-spectrum of **(16a)** revealed νNH~OH at 3410-3300 cm^{-1}, νC=O's at 1760, 1710 cm^{-1} and νC=S at 1380 cm^{-1}.,while IR-spectrum of **(16b)** showed absorption bands for νNH's at 3410, 3141 cm^{-1}, νSH at 2064 cm^{-1}, νC=N at 1630, and νC=S at 1400 cm^{-1} beside the other characteristic peaks of the compound.



Scheme (3). Synthesis of compounds (13-16)

3.2 Antimicrobial Activity:

The antimicrobial activity of the synthesized compounds was screened invitro using cup plate method [46,47] at concentration (50 mg/mL) against different strains of Gram positive bacteria (*Streptococcus sp.*) and Gram negative bacteria (*Escherichia coli*). In addition, to fungi strains as *Aspergillus Niger* and *Penicillium sp.*

Studies on the biological activity of the outlined synthesized compounds led to the fact that these derivatives are biologically active against the tested microorganisms. The results are given in Table **(3)**.

The results of biological activity of the synthesized compounds depicted in table **(3)** showed that most of the synthesized compounds exhibit from moderate to good antimicrobial activity.

The synthesized compounds showed a promising inhibitory effect against both bacterial and fungal strains compared to the starting quinazolinyl propanoic acid **(2)**. The biological activity was enhanced by incorporation of thiadiazole moiety to the quinazoline ring.

The quinazolinone derivatives **6, 7b, 10b, 11, 14 and 16b** showed high activity against both *Streptococcus sp.* and *Escherichia Coli* and lower inhibitions against the two species of fungal strains which suggest that they have more comprehensive bacterial inhibitory than fungicidal activity, which may be due to the presence of the thiadiazole (N=C=S) moiety[28,29], and incorporation of some groups such as –Cl, $–SO_2$ and $–CH_2Ph$. These results are in agreement with previously reported results for thiadiazole derivatives [30,31].

Also, the presence of phthaloyl amino acids in the Thiadiazolyl quinazolinone scaffolds **10a,** and **10b** improved both antibacterial and antifungal activities.

On the other hand, compounds **11 , 12, 15** and **16a** showed moderate to high inhibitory effect towards the tested bacteria and fungi *Aspergillus Niger* and showed no activity against *Penicillium sp*

Table (3): Biological activities of the synthesized compound.

Compound No.	*Streptococcus sp.*	*Escherichia Coli*	*Aspergillus. Niger*	*Penicillium sp.*
penicillin	+++	+++	++	+++
1	+	++	+	-
2	++	++	-	+
3	++	+++	+	++
4	+	++	++	+
5	+	+++	-	-
6	+++	++	++	+++
7a	++	++	+	-
7b	+++	+++	++	++
8	+++	++	+++	++
9	++	+	++	+
10a	++	++	+++	++
10b	++	+++	++	++
11	++	+++	++	-
12	++	++	+	-
14	+++	++	+++	++
15	++	+++	++	-
16a	++	+	++	-
16b	++	+++	++	+++

Signals in table **(3)** represent the extent of the zone diameter (r mm) inhibition of either fungal growth or bacterial cells for each compound; (+), slightly active; (++), moderately active, (+++), highly active and (-), no inhibition was observed.

Conclusion

In conclusion, we have developed a new series of potent antimicrobial thiadiazolyl quinazolinone **(3-12)** and triazolyl-**(14)**, oxooxazolidinyl-**(15)** and triazinanyl-**(16a,b)** benzoxazinone scaffolds, which have been successfully synthesized in good yields. Most of compounds have potent inhibitory activities toward Gram (+) bacteria (*Streptococcus sp.*) and Gram (-) bacteria (*Escherichia coli*). In addition, to fungi strains as *Aspergillus Niger* as compared to penicillin and could be used as lead compounds for the development of antimicrobial agents.

Acknowledgement

Authors would like to thank Benha University, Faculty of Science for their supporting, also, they gratefully acknowledged the Chemistry Department for their technical assistance.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Falsini M., Squarcialupi L., Catarzi D., Varano F., Betti M., Di Cesare Mannelli L., Tenci B., Ghelardini C., Tanc M., Angeli A., Supuran C.T. and Colotta V., *J Med Chem.,* 60, 6428–6439(2017).
2. Wang C.H., Zeng H., Wang Y.H. et al., *J Asian Nat Prod Res.* 17, 595– 600 (2015).
3. Patel T.S., Bhatt J.D., Dixit R.B., Chudasama C.J., Patel B.D. and Dixit B. C., *Arch Pharmazie.*, 352-1900099(2019).
4. Fabiana P., Benedetta M., Giuseppe D., Demetrio R., *Eur J Med Chem.*, 2, 113070(2020).
5. Gatadi S., Gour J., Shukla M., Kaul G., Das S., Dasgupta A., Malasala S., Borra R. S., Madhavi Y. and Chopra S., *Eur. J. Med. Chem.*, 157, 1056–1067(2018).
6. Rohini R., Muralidhar Reddy P., Shanker K., Hu A., Ravinder V: *Eur J Med Chem.,* 45, 1200–1205(2010).
7. Badadhe P. V., Chate A. V., Hingane D.G., Mahajan P.S., Chavhan N.M., Gill C.H., *J. Korean Chem. Soc., 55*, 936(2011).
8. Kabri Y., Nadine A., Dume tre A. L., Hutter S., Laget M., Verhaeghe P., Gellis A., Vanelle P., *Eur. J. Med. Chem.* 45, 616(2010).
9. Rosowesy A., Mota C.E. and Queener S.F., *J. Heter. Chem.*,33, 1959(1996).

10. Gangjee A., vasudevan A. and Kisliuk R.L., *J. Heter. Chem.*,34, 1669 (1997).
11. Myers R.M., Setzer N.N., Spada P.A., Persons E.P., Martin C.Q.C.LY,Z.P., Allison L., Cheney L.D., Zilbestein A., Johnson E.S., Franks F.C. and Mitchell J. K., Bioorg. *Med. Lett.*, 7(4), 421(1997),.
12. Carroll S.S., Stahlhut M., Geib J., Olsen D. ., *Biolo J Chem.*, 269 (51), 32351-32357(1994).
13. Maria Teresa Conconi., Giovanni Marzaro., Luca Urbani., Ilenia Zanusso., Rosa Di Liddo., Ignazio Castagliuolo., Paola Brun., Francesca Tonus., Alessandro Ferrarese., Adriano Guiotto., Adriana Chilin., *Eur. J. Med. Chem.*, 67, 373-383(2013).
14. Gatadi S., Lakshmi TV., Nanduri S., *Eur. J. Med. Chem*., 170,157–172(2019).
15. AlSalahi R., Anouar E.H., Marzouk M., Taie H.A., Abuelizz H.A., *Future Med. Chem.*, 10, 379-390(2017).
16. Wang M., Zhang G., Wang Y., Wang J., Zhu M., Cen S., et al., *Bioorg. Med. Chem., Lett.* 30, 127143(2020).
17. Hajimahdi Z., Zabihollahi R., Aghasadeghi MR., Zarghi A., *Curr. HIV. Res.*, 17(3), 214-22(2019).
18.Jangam S.S., Wankhede S. B . and Chitlange S. S., *Chem. Intermed., Res.*, 45, 471–486(2019).
19. Shetha A., and Wijdan I. A ., *J. Chem. Pharm. Res.*, 5(7), 42–45(2013),.
20. Couturier C., Lair C., Pellet A., Upton A., Kaneko T., Perron C., Cogo E., Menegotto J., Bauer A., Scheiper B., Lagrange S., Bacqué E., *Bioorg. Chem. Let.*, 26(21), 5290-5299(2016).
21. Saravanan G., Pannerselvam P. and Prakash C. R., *Der Pharm. Letters.*, 2 (3), 216–226(2010).
22. Alagarsamy V., Solomon V.R., Sulthana M.T., Vijay M.S., Narendhar B., *Z Naturforsch.*,70(8), 1–8(2015).
23. Saeedi M., Mohammadi-Khanaposhtani M., Pourrabia P., . Razzaghi N., Ghadimi R., Imanparast S., Faramarzi M. A., Bandarian F., Esfahani E. N. and Safavi M., *Bioorg. Chem.*, 83, 161–16(2019).
24. Nucifo F. C. Jr., Mihaljevic M., Lee, B. J. & Sawa, A. , *Neurotherapeutics* 14, 750–761(2017).
25. Hieu D.T., Anh D.T., Hai P-T., Huong L-T-T., Park EJ., Choi J.E., Kang J.S., Dung P.T.P., Han S.B., Nam N.H., *Chem Biodivers*., 15, 1800027(2018)
26. Hess H.J., Cronin T.H., Scriabine A; *J. Med. Chem.*, 11, 130–136(1968).
Wang and Gao Chemistry Central Journal, 7(95), 12-15(2013).
27. Shuai Y., Qiuqin L., Fengwen L., Xunyuan J., Chen Z., and Hanhong Xu, *Pest Manag Sci.*, 77(2), 1013–1022(2021).
28. Holla B S., Poorjary K N., Rao B S., Shivananda M K., *Eur. J. Med. Chem.*, 37, 511–517(2002).
29. Zhang S., Liu XJ., Tang R., Wan HX., Liu HY., Liu YM., et al., *Chem Pharm Bull (Tokyo).*, 65, 950(2017).
30. Bhosale M., Yadav A., Magdum C., Mohite S., *Int. J. Curr. Adv. Res.*, 09(08)(A): 22894-22899(2020).
31. Yadav A., Mohite S., Magdum C., *Asian J. Res. Pharm. Sci.*, 10(3), 149-152 (2020).
32. Jakovljević K., Matić IZ., Stanojković T., Krivokuća A., Marković V., Joksović MD., Mihailović N., Nićiforović M., Joksovi L., *Bioorg. Med. Chem. Lett.*, 27, 3709–3715 (2017).
33. Bhosale M., Yadav A., Magdum C., Mohite S., *Int J Sci Res Sci & Technol.* 7(5), 51-62(2020).
34. Gomha S.M., Edrees M.M., Muhammad Z.A., El-Reedy A.A., *Drug. Des. Devel. Ther.*,12, 1511–1523(2018).
35. Khan M.H., Hameed S., Farman M., Al-Masoudi NA., Stoeckli-Evans H.Z., *Z Naturforsch* 70(8)b, 609–616(2015).
36. Yu L., Gan X., Zhou D., He F., Zeng S., Hu D., *Molecules* 22, 658–666(2017).
37. Tatar E., Kucukguzel S.G ., Karakus S., De Clercq E.; Andrei G., Snoeck, R., Pannecouque., C., OktemOkullu, S., Unubol, N., Kocagoz, T., et al. *Marmara Pharm. J.*, 19, 88–102(2015).
38. Sharma B., Verma A., Prajapati S, Sharma UK., *Int. J. Med. Chem.*, 348948(2013).
39. Khan I., Tantray MA., Hamid H., Alam MS., Kalam A., Dhulap A., *Bioorg Med. Chem. Lett.*, 26, 4020–4024(2016).
40. Bahmani Y., Bahrami T., Alabadi A. , *Indian J Pharm Sci.*,81(1), 63(2019).
41. Aly A. A., Behalo M.S., *J. Chem. Res.*, 35, 355-360(2011).
42. Behalo M.S., Aly A.A.; Wasfy A.F.; Rizk M. M., *Eur. J. Chem.*,4, 92-97(2013).
43. Abdelmajeid A., Aly A. A., Amine M.S. Ghith A.A., *Int. J. Nano. Chem.*, 2(2), 37-45(2016).
44. Abdelmajeid A., Amine M.S., Hassan R.A., *Int. J. Org. Chem*.,7, 346-368(2017).
45. Abdelmajeid A., Amine, M.S., Hassan R.A., *American J. Heter. Chem*. 4(2), 30-41(2018).
46. Bauer A. W., Kirby W.M., Sherris C., Turck M., *Am J Clin Pathol* 45, 493–496 (1966).
47. Liebowitz L. D., Ashbee H.R., Evans E. G. V., Chong Y., Mallatova N., zaidi M., Gibbs D., *Microbiol.Infect.Dis.*, 4, 27-33(2001).