Pharmaceutical Chemistry Journal, Vol. 57, No. 3, June, 2023 (Russian Original Vol. 57, No. 3, March, 2023)

SYNTHESIS OF 6-SUBSTITUTED 3(H)-QUINAZOLIN-4-ONES AND THEIR ANTIMICROBIAL ACTIVITY

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Original article submitted July 31, 2021.

Three-step synthesis of 6-amino-3(H)-quinazolin-4-ones has been performed. Initially, the condensation of 3(H)-quinazolin-4-one was carried out in the presence of anthranilic acid and formamide in a 1:3 ratio. Then, hydrogen atom in state 6 of the resulting 3(H)-quinazolin-4-one was replaced by nitro group using a nitriding compound and 6-nitro-3(H)-quinazolin-4-one reduction was performed using $SnCl_2 \cdot 2H_2O$ as a reducing agent. Acylation reactions were accomplished on the synthesized 6-amino-3(H)-quinazolin-4-one. The structures of synthesized compounds were determined based on their IR and ^{1}H NMR spectra. The obtained compounds were evaluated for antibacterial and antifungal activities by the agar disk diffusion test. The results indicated that 6-nitro-3(H)-quinazolin-4-one exhibited remarkable antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* bacterial strains.

Keywords: 3(H)-quinazolin-4-one; nitration; reduction; acylation; antimicrobial activity

1. INTRODUCTION

heterocyclic quinazoline compounds are considered biologically active substances [1 – 6]. A significant number of natural and synthetic derivatives of quinazolin-4-one with high biological activity are known [7 – 10]. The best known natural compound containing a quinazoline heterocyclic nucleus is peganine (vazicine) - an alkaloid contained in harmala seeds (*Peganum harmala*). Its hydrochloride is used as an anticholinesterase agent for treating myopathy and myasthenia [11 – 13]. In addition, synthetic derivatives of quinazoline exhibited psychotropic, antitumor [14 – 16], diuretic (quinetasone), cardiovascular (prazosin), antiviral (quinazoline analogs of efavirenz), anti-inflammatory, anticonvulsant [14], antibacterial [17 – 21], and antidepressant properties [22 – 24] and other types of biological action.

Therefore, the synthesis of 3(H)-quinazolin-4-one (I) and its 6-substituted (6-nitro, -amino, and -acylamino) derivatives and determination of their antimicrobial activity are considered an actual task. In this work, we have synthesized 3(H)-quinazolin-4-one (6-nitro, -amino and some acetylamino) derivatives and investigated their antimicrobial activity.

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2. EXPERIMENTAL

2.1. Equipment

The product structures waere confirmed by IR and ^{1}H NMR spectra. The IR spectra were recorded on a System 2000 IR Fourier spectrometer in KBr pellets. The ^{1}H NMR spectra were taken on a Varian Unity 400+ instrument (operating frequency 400 MHz, internal standard TMS, δ scale in ppm) using CD_3COOD as solvent. Melting points of the synthesized compound were determined on a Boetius heating table (Germany).

2.2. Synthesis

3(H)-quinazolin-4-one (I). 13.7 g (0.1 mol) of anthranilic acid and 16 ml of formamide were added to a 50 ml reflux flask (ρ = 1.13 g/sm^3). The reaction was heated in an oil bath at 140(C for 3 h. The resulting reaction mixture was immediately poured into an ice container. The precipitate was filtered and dried at room temperature. The product recrystallized in water.

Compound **I**: yield, 12.87 g (88.17%); m.p., 217 – 218°C. R_f = 0.61 (chloroform: benzene: methanol, 5 :

3 : 1); IR spectrum (ν_{max}, cm^{-1}): 1664(C=O), 3436 (NH), 1612 (C=N), 1468 (C–N), 1558 (C=C). ^{1}H NMR spectrum in CD_3COOD (δ, ppm; J, Hz): 8.35 (s, 1H, H-2), 8.20 (dd, 1H, J_1=1.3 Hz, J_2 = 8.0, Hz, H-5), 7.80 – 7.76 (m, 1H, H-7), 7.71 (exp.d, 1H, J = 8.0 Hz, H-8), 7.51 – 7.49 (m, 1H, H-6).

6-Nitro-3(H)-quinazolin-4-one (II). Reactions were carried out in a three-necked flask equipped with a mechanical stirrer and reflux condenser. 3(H)-quinazolin-4-one (22.5 g) was dissolved in 78 ml of concentrated sulfuric acid and heated to 30(C for 1 hour. A nitrating mixture (21 ml of nitric acid and 18 ml of concentrated sulfuric acid) was added dropwise to the solution with vigorous stirring. The reaction mixture was stirred for another 1 h, maintaining the temperature not higher than 30(C and then for another 1 h at room temperature. On stirring at room temperature, 45 mL of nitric acid was added dropwise over 1 h and The reaction mixture was left at room temperature for 10 h. The content of the flask was poured onto ice, the precipitated crystals were filtered off, washed with water, and dried. Technical 6-nitro-3(H)-quinazolin-4-one was recrystallized from ethyl alcohol with the addition of activated carbon.

Compound **II**: yield, 25.7 g (87.4 %); m.p., 287 – 289°C. R_f = 0.48 (acetone:benzene, 3:2). IR spectrum (ν_{max}, cm^{-1}): 1668 (C=O), 3417 (NH), 1618 (C=N), 1467 (CN), 1514 (C-NO_2). ^{1}H NMR spectrum in CD_3COOD (δ, ppm; J, Hz): 8.98 (dd, 1H, J_1 = 2.66 Hz, J_2 = 0.41 Hz, H-5), 8.55 (dd, 1H, J_1 = 9 Hz, J_2 = 2.66 Hz, H-7), 7.9 (dd, 1H, J_1 = 8.99 Hz, J_2 = 0.42 Hz, H-8).

6-Amino-3(H)-quinazolin-4-one (III). A three-nozzle flask was fitted with a mechanical stirrer and a drip funnel. Cooled in an ice bath (0 + 2°C) 12.6 g (0.056 mol) of tin(II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$) was added to 16.98 mL of concentrated (36%) HCl. After that, for 10–15 min with constant stirring of the reaction mixture were added 3 g (0.016 mol) of 6-nitro-3(H)-quinazolin-4-one, 20 mL of ethyl alcohol and 7 mL of HCl as a suspension. The reaction was carried out for 10 – 15 min at 0 and +2°C, 30 min at room temperature, and 2 h in a water bath (90 + 95°C). Thn, the reaction mixture was left overnight at room temperature, diluted with water and brought to a strong alkaline condition (pH 10 – 11) in the presence of 10% sodium hydroxide. The expected 6-amino-3(H)-quinazolin-4-one has been dissolved so that it was brought to a neutral in the presence of hydrochloric acid. Then precipitated when transferred to an alkaline condition with ammonia. The precipitate was filtered, washed with water until it reached a neutral state, and dried at room temperature.

Compound **III**: yield, 6.67 g (88.1 %); m.p., 316 – 318°C. R_f = 0.53 (acetone : benzene, 3 : 2). IR spectrum (ν_{max}, cm^{-1}): 1672 (C=O), 3411 (NH), 1602 (C=N), 3022 (C-H), 1485 (C-C), 1392 (C-N). ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm; J, Hz): 7.71 (s, 1H, J = 2.24 Hz, H-2), 7.14 (d, 1H, J = 2.68 Hz, H=5), 7.33 (d, 1H, J = 8.64 Hz, H-7), 7.03 (d, 1H, J = 8.71 Hz, H=8).

N-(4-oxo-3,4-dihydroquinazolin-6-yl)acetamide (IV). To 0.5 g (0.001 mol) of 6-amino-3(H)-quinazolin-4-one was added 3.0 mL (0.0525 mol) of acetic acid in 50 mL round-bottomed flask and the mixture was heated for 10 – 15 min at 35 – 40°C. After that the reaction mixture was cooled to room temperature, 7.0 mL (ρ = 0.7893 g/sm^3, 96.0%) of ethyl alcohol (0.115 mol) was poured into the precipitate and heated for 2 – 3 min at 35 – 40°C. Finally, the precipitate was filtered and dried at room temperature.

Compound **IV**: yield, 0.56 g (88.9 %); m.p., 344 – 346°C. R_f = 0.39 (methanol:benzene, 1:3); IR spectrum (ν_{max}, cm^{-1}): 1690 (C=O), 3242 (NH), 1662 (C=N), 3022 (CH_3), 1491 (C-C), 1314 (C-N); ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm; J, Hz): 7.99 (s, 1H, H-2), 12.17 (s, 1H, NH), 8.44 (d, 1H, J =2.28 Hz, H-5), 7.89 (dd, 1H, J = 2.44 Hz, H-7), 7.62 (d, 1H, J = 8.78 Hz, H-8), 10.3 (s, 1H, NH), 2.08 (s, 3H, CH_3).

N-(4-oxo-3,4-dihydroquinazolin-6-yl)propionamide (V). To 0.161 g (0.001 mol) of 6-amino-3(H)-quinazolin-4-one was added 3.7 mL (0.049 mol) of propionic acid in 50 round-bottomed flask and the mixture was heated to140 – 145°C on an oil bath for 4 h. The reaction mixture was cooled to room temperature and the precipitate was filtered and recrystallized from ethyl alcohol.

Compound **V**: yield, 0.128 g (79.5 %); m.p., 272 – 274°C. R_f = 0.63 (benzene : acetone, 2 : 3); IR spectrum (ν_{max}, cm^{-1}): 1689 (C=O), 3282 (N-H), 1659 (C=N), 1415 (CH_3), 1481 (C-C), 1301 (C-N), 3058 (C-H), 1450 (CH_2); ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm; J, Hz): 7.56 (dd, 1H, J = 8.79 Hz, H-2), 12.06 (s, 1H, NH), 8.41 (t, 1H, J = 2.3 Hz, H-5), 7.92 – 7.96 (m, 2H, H -7, -8), 10.11 (s, 1H, NH), 2.36 (k, 2H, J = 7.46 Hz, $\underline{CH_2}CH_3$), 1.12 (t, 3H, J = 7.57 Hz, $CH_2\underline{CH_3}$).

2.3. Evaluation of Antimicrobial Activity

Antibacterial activity of the synthesized compounds and reference drugs was assessed using the modified agar-disk diffusion method according to published guidelines [25, 26]. Sterile nutrient agar was inoculated with test strain cells and poured into Petri dishes to give a solid medium. Forty microliters of test material (equivalent to 0.2 mg/per disc of individual compound dissolved in chloroform) was applied on sterile paper discs. According to the guidelines of Antimicrobial Susceptibility Testing, the ampicillin is used against Gram-positive bacteria, ceftriaxone against Gram-

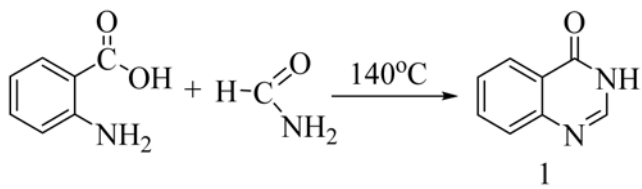

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Fig. 1. Reaction used to obtain 3(H)-quinazolin-4-one (I).

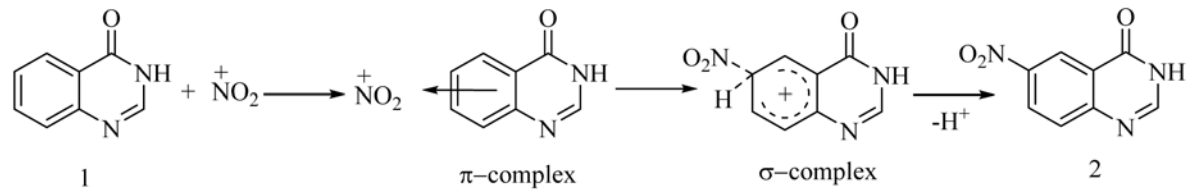

Fig. 2. Mechanism of the nitration reaction of 3(H)-quinazolin-4-one (**II**).

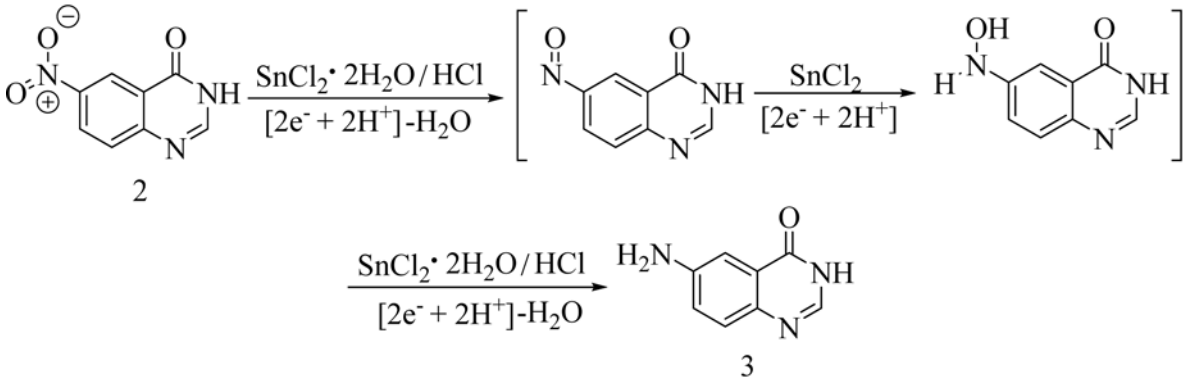

Fig. 3. The reaction mechanism of 6-amino-3(H)-quinazolin-4-one (**III**).

negative bacteria, and fluconazole against fungi [25]. Ampicillin (for Gram-positive bacteria), ceftriaxone (for Gram-negative bacteria) and fluconazole (for fungi) (Himedia Laboratories Pvt. Limited) were used as positive controls and the solvent as negative controls. The solvents were allowed to evaporate in a stream of air. The discs were deposited on the surface of inoculated agar plates. Plates were kept for 3 h in refrigerator to enable the diffusion of the substances into the agar. Plates with bacteria were incubated for 24 h at 37°C and plates with fungi for 48 h at 26°C. The inhibition zone (including the disc diameter) was measured and recorded after the incubation time. An average zone of inhibition was calculated for the three replicates in independent assays.

3. RESULTS AND DISCUSSION

3.1. Synthesis of 6-Amino-3(H)-quinazolin-4-ones

The synthesis of nitrogen-containing heterocycles, in particular, 3(H)-quinazolin-4-one and its derivatives is theoretically and practically important [27]. The 3(H)-quinazolin-4-one contains nitrogen atoms in the first and third positions, the carbonyl group in the fourth position and the benzene ring allow various electrophilic and nucleophilic reactions. For the synthesis of 3(H)-quinazolin-4-one derivatives, two main approaches were used: either 2-aminobenzaldehydes (2-aminophenyl ketones) or anthranilic acid (Niementovsky reactions) were used as source materials (Fig. 1). The reaction proceeded when anthranilic acid was heated in excess of formamide with the detachment of two water molecules.

The aim of this study was to develop the nitration, reduction of the 3(H)-quinazolin-4-one, and acylation reactions on the obtained amino group. Initially, 3(H)-quinazolin-4-on was synthesized by quantitative yield in the Nimentovsky method [28, 29]. The main factors influencing the reaction yield were studied. Due to the high negative charge value in the 6-position carbon atom in the 3(H)-quinazolin-4-one ring, the nitronium cation attacks the electrophile, forming first a π-complex, then a β-complex, and the proton separates and stabilizes. The reaction equation was as follows (Fig. 2).

The obtained nitro derivatives are important syntons and they are an important raw material for the chemistry of heterocyclic compounds. That is, the reduction of the nitro group leads to the formation of the corresponding 6-amino-3(H)-quinazolin-4-one. The reduction of nitro compounds to amines in various ways proceeds with the formation of intermediates 6-nitro-3(H)-quinazolin-4-one and 6-hydroxylamino-3(H)-quinazolin-4-one, since 6-nitroso-3(H)-quinazolin-4-one and 6-hydroxylamino-3(H)-quinazolin-4-one cannot be separated individually. The advantage of reduction to amines in the presence of tin(II) chloride dihydrate is that other functional groups are not reduced in the aromatic ring. According to the literature, $SnCl_2 \cdot 2H_2O$ structure occurs in the form $[Sn(H_2O)Cl_2] \cdot H_2O$ [30 – 32]. The reduction of nitro compounds in acidic conditions in the presence of metals begins with the merger of electrons. Because of the transition of the double electron of tin(II) chloride dihydrate to the

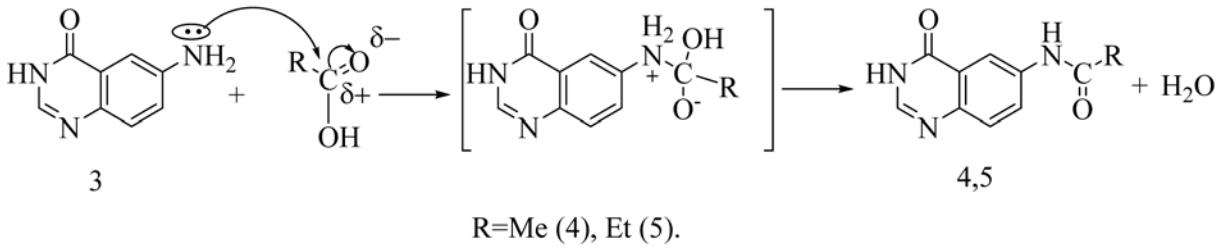

Fig. 4. Mechanism of N-(4-Oxo-3(H)-quinazolin-6-yl)acetamide (**IV**) and N-(4-oxo-3(H)-quinazolin-6-yl)-propionamide (**V**) formation.

TABLE 1. Antibacterial and Antifungal Effect Evaluated by the Diameter of Growth Inhibition Zone for 6-Substituted 3(H)-quinazolin-4-ones **I–V** in the Agar Disk-Diffusion Assay (Mean ± SD, P ≤ 0.05)

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi
	B. subtilis	*S. aureus*	*E.coli*	*P. aeruginosa*	*Candida albicans*
I	na*	na	na	na	na
II	15.04 ± 0.10	12.08 ± 0.12	10.08 ± 0.12	na	na
III	na	na	na	na	na
IV	na	na	na	na	na
V	10.04 ± 0.10	9.08 ± 0.12	6.08 ± 0.12	na	na
Ampicillin (10 μg/disc)	28.04 ± 0.10	27.08 ± 0.12	25.08 ± 0.12	nt	nt
Ceftriaxone (30 μg/disc)	nt*	nt	26.08 ± 0.12	28.12 ± 0.13	nt
Flucanazole (25 μg/disc)	nt	nt	nt	nt	30.04 ± 0.10

na* = not active; nt* = not tested.

electronegative oxygen element of the nitro group, the nitrogen-oxygen double bond is broken and an anion radical is formed. The general mechanism of the reaction is proposed as follows (Fig. 3).

The 6-amino-3(H)-quinazolin-4-one nucleophilic central amino group attacks the partially positively charged carbon atom in acetic acid and propionic acid, resulting in an acylation reaction of 6-amino-3(H)-quinazoline. The proposed approximate reaction mechanisms carry out as follows (Fig. 4).

3.2. Antibacterial and Antifungal Activity

The antimicrobial activity was evaluated using the strains of Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa,* and fungus *Candida albicans* by modified agar disk diffusion method described in our previous work [33]. Results of antimicrobial assay are presented as diameters of the zone of inhibition (Table 1). Overall, some tested compounds exhibited various degrees of antibacterial activity against Gram-positive bacteria and *E. coli,* whereas, the all compounds were not active against *P. aeruginosa* and pathogenic fungi *C. albicans.* The unsubstituted 3(H)-quinazolin-4-one (**I**) was not effective against all strains, but the substituted derivatives exhibited notable variation in their antibacterial activity. Among them, nitro-substituted (**II**) and propionamide-substituted (**V**) quinazollinones demonstrated remarkable antibacterial activity. Results have revealed that *B. subtilis, S. aureus* and *E. coli* are highly susceptible to the presence of 6-nitro group in 3(H)-quinazolin-4-one (**II**).

In concluding, 6-nitro derivative of 3(H)-quinazolin-4-one (**II**) exhibited remarkable antibacterial activity against three strains of test microorganisms and demonstrated potential for the development of new therapeutic agents against selected bacterial strains.

FUNDING

This research was funded by the Academy of Sciences of the Republic of Uzbekistan and the Ministry of Innovative Devlopment of the Republic of Uzbekistan.

CONFLICT OF INTEREST

The authors declare that they have neither competing financial interests nor personal conflict relationships in this work.

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