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SYNTHESIS AND ANALGESIC ACTIVITY OF 6-AMINO-2-PIPERAZINYLQUINAZOLINES

6-Amino-2-piperazinylquinazolin-4-one and 2-piperazinyl-4-phenylquinazoline derivatives were synthesized. The analgesic activity of the obtained series of derivatives was investigated and it was found that the synthesized compounds showed high analgesic activity. ED₅₀ values range from 0.46 to 0.88 mg/kg.

Key words: 2-piperazinyl-3*H*-quinazolin-4-ones, 2-piperazinyl-4-phenylquinazolines, analgesic activity.

Pain is a physiologically important protective mechanism that occurs as a reaction of the body to the effects of harmful, destructive irritations [1]. At the same time, pain of extreme strength and prolonged pain irritation and a corresponding severe pain sensation lead to a decrease in the quality of life, invalidation, as well as significant economic losses.

To date, nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation and pain. However, along with a high rate of clinical effectiveness, their use is accompanied by a number of undesirable side effects. [2]. In this regard, there is a need to search for new potential drugs that would be superior to existing ones in terms of effectiveness and/or level of safety.

Quinazoline derivatives are known to have a wide range of pharmacological properties and are widely used as the base for the creation of new biologically active compounds [3–4]. In our previous works, the antiplatelet activity of 2,6-substituted derivatives of quinazolin-4-one and 4-phenylquinazoline have been studied [5, 6]. It should be noted that among the derivatives of quinazolin-4-ones and 4-phenylquinazoline there are compounds having analgesic and anti-inflammatory properties [2, 7, 8].

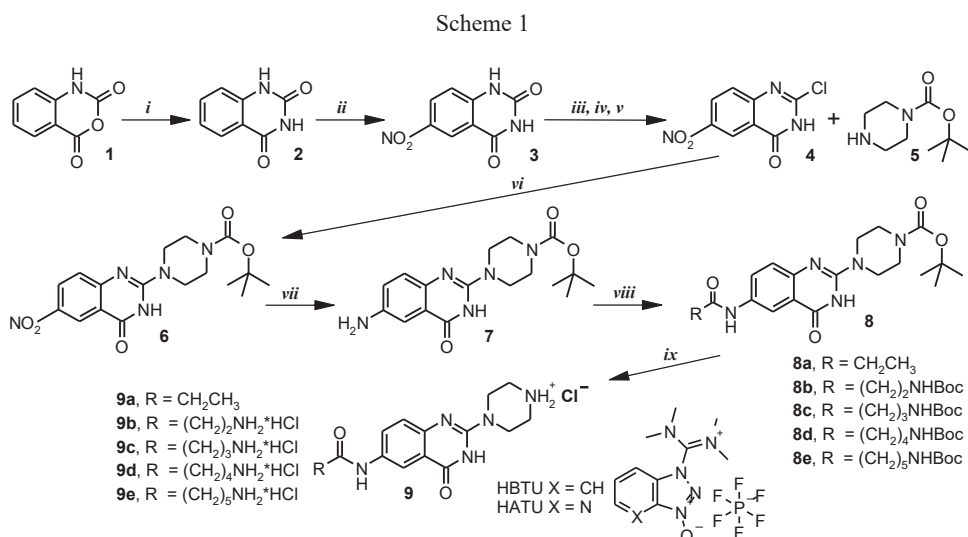
The aim of this work is the synthesis and study of the analgesic activity of a number of compounds derived from 2,6-substituted quinazolin-4-one and 4-phenylquinazoline.

DISCUSSION OF THE RESULTS

The syntheses of the main building block of 6-amino-2-piperazinquinazolin-4-one (**7**) and its derivatives (**9b-9c**) were carried out according to the previously described method [5], derivatives (**9a**, **9d-9e**) were obtained similarly (Scheme 1). As a result of a five-step synthesis 6-amino-2-(4-Boc-piperazin-1-yl)-3*H*-quinazolin-4-one was obtained. At first, by condensation of isatoic anhydride (**1**) with urea quinazoline-2,4-dione (**2**) was synthesized. Further, by the action of the nitrating mixture to compound **2**, 6-nitroquinazolin-2,4-dione was obtained (**3**). Compound **3**

was chlorodesoxygenated using phosphorus oxychloride, resulting in the formation of 6-nitro-2,4-dichloroquinazoline, which was then hydrolyzed to form 6-nitro-2-chloro-3*H*-quinazolin-4-one (**4**). The piperazine ring was introduced into the second position of the quinazoline core by condensation of compound **4** with mono-Boc-piperazine (**5**), and this led to the formation of compound **6**. The last stage of the synthesis of amine **7** was applied to reduction the nitro group in the sixth position of the quinazoline cycle of compound **6**. The reduction was carried out with hydrogen over a palladium catalyst.

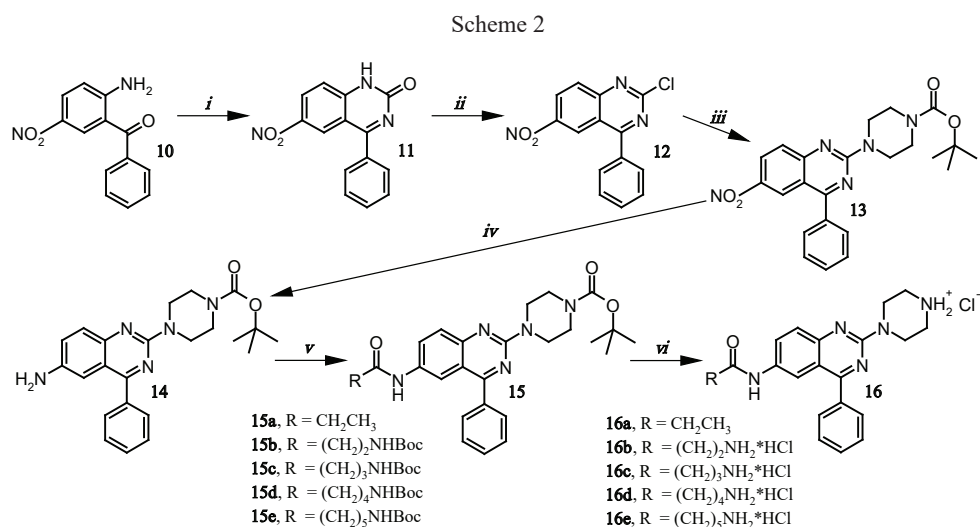
HBTU or HATU coupling reagents were used to condense 6-amino-2-(4-Boc-piperazin-1-yl)-3*H*-quinazolin-4-one (**7**) with Boc-protected amino acids (**b-e**). Synthesis with propionic anhydride (**a**) was carried out without the use of condensation agents. As a result, series of intermediate compounds of the general formula **8** was obtained (Scheme 1). At the next stage of the synthesis, the acidolytic removal of the Boc-protecting groups of compounds **8** was carried out to obtain the hydrochlorides of the target derivatives of general formula **9**.



Reagents and conditions: **(i)** (H₂N)₂CO; **(ii)** HNO₃, H₂SO₄; **(iii)** POCl₃; **(iv)** NaOH, H₂O; **(v)** H⁺; **(vi)** NEt₃; **(vii)** H₂, Pd(C); **(viii)** (CH₃CH₂CO)₂O (**a**) or *N*-Boc-acid (**b-e**), NEt₃, HBTU or HATU; **(ix)** HCl_(gas), CH₂Cl₂.

A group of derivatives based on 6-amino-2-piperazine-4-phenylquinazoline scaffold (**16a-16e**) was obtained using 2-amino-5-nitrobenzophenone (**10**) as the starting compound (Scheme 2). As a result of condensation of the starting benzophenone **10** with urea, 6-nitro-4-phenylquinazolin-2-one was obtained (**11**). Further preparation of chloro derivative (**12**), condensation with mono-Boc-piperazine (**13**), and reduction of the nitro compound to the amino compound (**14**) were carried out similarly to the corresponding reactions for quinazolin-4-one.

The condensation of 6-amino-2-(4-Boc-piperazin-1-yl)-4-phenylquinazoline (**14**) with propionic anhydride (**a**) was carried out directly, and the condensation with Boc-protected amino acids (**b-e**) was performed using the HATU coupling reagent with obtaining the corresponding intermediates of general formula **15** (Scheme 2). Acidolytic removal of the Boc-protecting groups of compounds **15** made it possible to obtain series of target derivatives of general formula **16** in the form of crystalline hydrochlorides.



Reagents and conditions: (i) (H₂N)₂CO, 180–190 °C, 2 h; (ii) POCl₃, PCl₅, 6 h; (iii) N-Boc-piperazine (**5**, see Scheme 1), NEt₃, 6 h; (iv) H₂, Pd(C), C₆H₆-MeOH (1:1), 2–3 h; (v) (CH₃CH₂CO)₂O (**a**) or N-Boc-acid (**b-e**), NEt₃, HATU; (vi) HCl_(gas), CH₂Cl₂.

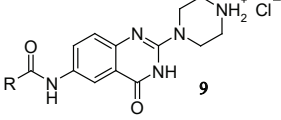
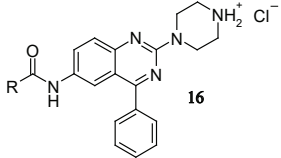
Thus, two series of 6-amino-2-piperazinylquinazoline derivatives containing substituents with various carbon chain lengths near the amino group at the 6th position of the quinazoline heterocycle, were obtained (Table 1).

The analgesic activity of the synthesized compounds was tested on a model of peripheral pain *in vitro* according to the “writhing” method in mice, which were induced by intraperitoneal administration of a 0.75% solution of acetic acid. The classic NSAID diclofenac sodium was used as a comparison preparation. The synthesized compounds showed high analgesic activity. ED₅₀ values are in the range from 0.46 to 0.88 mg/kg (for diclofenac sodium, the found ED₅₀ value is of 10.00 ± 1.80 mg/kg). Among the investigated derivatives, compound **16b** containing the residue of 3-aminopropionic acid, was found to be the most active. As a result of the experiment, the doses at which the compounds showed distinct pharmacological properties were determined. The ED₅₀ values of the test compounds are shown in the table (see Table 1).

When studying the effect of the substituent in the 4th position of the quinazoline heterocycle on the analgesic activity, it was found that compounds with the phenyl substituent (**16**) generally showed higher activity compared to 4-oxoquinazoline derivatives (**9**), the levels of activity of the latter are almost the same for all studied analogues (Table 1).

Table 1

**Analgesic activity of derivatives of 4-oxo(4-phenyl)6-amido-2-piperazinylquinazolines
(compounds 9a-e, 16a-e)**

Structure	Compound	R	Analgesic activity ED ₅₀ * [mg/kg]*
	9a	CH ₃ CH ₂ -	0.73±0.18
	9b	HCl*NH ₂ CH ₂ CH ₂ -	0.80±0.24
	9c	HCl*NH ₂ (CH ₂) ₂ CH ₂ -	0.80±0.25
	9d	HCl*NH ₂ (CH ₂) ₃ CH ₂ -	0.83±0.26
	9e	HCl*NH ₂ (CH ₂) ₄ CH ₂ -	0.79±0.25
	16a	CH ₃ CH ₂ -	0.52±0.16
	16b	HCl*NH ₂ CH ₂ CH ₂ -	0.46±0.14
	16c	HCl*NH ₂ (CH ₂) ₂ CH ₂ -	0.53±0.17
	16d	HCl*NH ₂ (CH ₂) ₃ CH ₂ -	0.77±0.24
	16e	HCl*NH ₂ (CH ₂) ₄ CH ₂ -	0.88±0.25

*Comparison preparation – diclofenac sodium, ED₅₀ = 10.0±1.8 mg/kg

It is necessary to note certain regularities in the dependence of the analgesic activity of the compounds on their structure, namely, the influence of the carbon chain near the amido group in the 6th position of the quinazoline heterocycle. In particular, for compounds with shorter substituents **16a-16c**, the activity is 1.5–2 times higher than for derivatives with residues of 5-aminopentanoic **16d** and 6-aminohexanoic acid **16e**.

It was also found that the presence of an amino group at the end of the carbon chain gives a increase in analgesic activity at the conversion from the propanamide derivative **16a** to the 3-aminopropanamide derivative **16b**.

EXPERIMENTAL PART

The reaction progress and the purity of the obtained compounds were monitored by thin layer chromatography (TLC) on ALUGRAM® Xtra SIL G/UV254 (MACHEREY-NAGEL) plates in a solvent system – benzene: acetone: acetic acid (100:50:1). Substances were detected on chromatograms using ninhydrin reagent or UV light at $\lambda = 254$ nm. Mass spectra of the compounds were recorded by the FAB method on a VG 7070 mass spectrometer, nitrobenzyl alcohol was used as a matrix, ionization was carried out by a beam of Ar atoms with energy of 8 kV. The (¹H) and (¹³C) NMR spectra were recorded on a Bruker Avance DRX instrument with operating frequencies of 500 MHz and 125 MHz, respectively, in DMSO-d₆ solutions (99.9%); internal standard was TMS at 25 °C. The melting temperature was determined on a PTP-1 device in sealed capillaries, the heating rate was 1 °C/min. The synthesis of compounds **2-7**, **8b-8c**, **9b-9c** was carried out according to the previously described method [5]. Compounds **8a**, **8d-8e**, **9a**, **9d-9e** were synthesized in a similar manner.

N-[2-(4-*Boc*-piperazin-1-yl)-4-oxo-3*H*-quinazolin-6-yl]propanamide (**8a**).

Yield 56%. White solid. R_f 0.39. M.p >250 °C. $^1\text{H NMR}$ (400 MHz, DMSO-d_6), δ , ppm: 1.09 (t, $J=7.4$ Hz, 3 H) 1.42 (s, 9 H) 2.32 (q., $J=7.4$ Hz, 2 H) 3.40 (s, 4 H), 3.56 (s, 4 H) 7.26 (d, $J=8.3$ Hz, 1 H) 7.74 (d, $J=7.9$ Hz, 1 H) 8.26 (s, 1 H) 9.95 (s, 1 H) 11.34 (br.s, 1 H). FAB-MS m/z : 402 $[\text{M}+\text{H}]^+$.

5-*N*-*Boc*-amino-*N*-[2-(4-*Boc*-piperazin-1-yl)-4-oxo-3*H*-quinazolin-6-yl]pentanamide (**8d**).

Yield 71.5%. White solid. R_f 0.37. M.p 234–235 °C. $^1\text{H NMR}$ (400 MHz, DMSO-d_6), δ , ppm: 1.36 (s, 10 H), 1.41 (s, 10 H), 1.56 (t, $J=6.5$ Hz, 2 H), 2.29 (t, $J=5.6$ Hz, 2 H), 2.92 (dd, $J=12.1$, 5.6 Hz, 2 H), 3.40 (s, 4 H), 3.56 (s, 4 H), 6.80 (br.s, 1 H), 7.27 (d, $J=7.5$ Hz, 1 H), 7.73 (d, $J=8.4$ Hz, 1 H), 8.27 (s, 1 H), 9.98 (s, 1 H), 11.43 (br.s, 1 H). FAB-MS m/z : 545 $[\text{M}+\text{H}]^+$.

6-*N*-*Boc*-amino-*N*-[2-(4-*Boc*-piperazin-1-yl)-4-oxo-3*H*-quinazolin-6-yl]hexanamide (**8e**).

Yield 65%. White solid. R_f 0.4. M.p 239–240 °C. $^1\text{H NMR}$ (500 MHz, DMSO-d_6), δ , ppm: 1.22–1.45 (m, 4 H), 1.35 (s, 9 H), 1.41 (s, 9 H), 1.55–1.61 (m, 2 H), 2.29 (t, $J=7.5$ Hz, 2 H), 2.90 (dd, $J=12.1$, 6.5 Hz, 2 H), 3.40 (s, 4 H), 3.56 (s, 4 H), 6.76 (s, 1 H), 7.26 (d, $J=8.4$ Hz, 1 H), 7.73 (d, $J=8.4$ Hz, 1 H), 8.26 (s, 1 H), 9.96 (s, 1 H), 11.43 (br.s, 1 H). FAB-MS m/z : 559 $[\text{M}+\text{H}]^+$.

N-[2-(4-Piperazinium-1-yl)-4-oxo-3*H*-quinazolin-6-yl]propanamide chloride (**9a**).

Yield 97%. White hygroscopic solid. $^1\text{H NMR}$ (500 MHz, DMSO-d_6), δ , ppm: 1.08 (t, $J=7.5$ Hz, 3 H), 2.36 (q, $J=7.5$ Hz, 2 H), 3.26 (s, 4 H), 4.07 (s, 4 H), 7.89–7.92 (m, 2 H), 8.44 (s, 1 H), 9.82 (s, 2 H), 10.41 (s, 1 H); ^1H (400 MHz, D_2O), δ , ppm: 1.07 (t, $J=7.5$ Hz, 3 H), 2.32 (q, $J=7.4$ Hz, 2 H), 3.46 (t, $J=4.8$ Hz, 4 H), 3.98 (t, $J=4.5$ Hz, 4 H), 7.24 (d, $J=9.0$ Hz, 2 H), 7.53 (dd, $J=8.9$, 2.1 Hz, 1 H), 7.83 (d, $J=1.8$ Hz, 1 H). $^{13}\text{C NMR}$ (126 MHz, D_2O), δ , ppm: 9.11 (1 C), 29.82 (1 C), 42.30 (2 C), 43.26 (2 C), 115.15 (1 C), 116.81 (1 C), 118.39 (1 C), 128.82 (1 C), 135.30 (1 C), 135.52 (1 C), 149.92 (1 C), 163.13 (1 C), 176.43 (1 C). FAB-MS m/z : 302 $[\text{M}+\text{H}]^+$.

5-Ammonium-*N*-[2-(4-piperazinium-1-yl)-4-oxo-3*H*-quinazolin-6-yl]pentanamide dichloride (**9d**).

Yield 95%. White hygroscopic solid. $^1\text{H NMR}$ (500 MHz, DMSO-d_6), ppm: 1.59–1.68 (m, 4 H), 2.41 (t, $J=5.8$ Hz, 2 H), 2.78 (dd, $J=11.3$, 5.8 Hz, 2 H), 3.27 (br.s, 4 H), 4.12 (br.s, 4 H), 7.96 (d, $J=8.7$ Hz, 1 H), 8.01 (d, $J=8.4$ Hz, 1 H), 8.10 (s, 3 H), 8.48 (s, 1 H), 9.97 (s, 2 H), 10.72 (s, 1 H); $^1\text{H NMR}$ (400 MHz, D_2O), δ , ppm: 1.61–1.64 (m, 4 H), 2.37 (t, $J=6.1$ Hz, 2 H), 2.91 (t, $J=6.0$ Hz, 2 H), 3.44 (t, $J=4.8$ Hz, 4 H), 3.99 (t, $J=4.8$ Hz, 4 H), 7.37 (d, $J=9.0$ Hz, 1 H), 7.63 (dd, $J=8.9$, 2.4 Hz, 1 H), 7.95 (d, $J=2.3$ Hz, 1 H). $^{13}\text{C NMR}$ (126 MHz, D_2O), δ , ppm: 21.40 (1 C), 25.72 (1 C), 35.18 (1 C), 38.66 (1 C), 41.76 (2 C), 43.10 (2 C), 114.87 (1 C), 117.13 (1 C), 118.01 (1 C), 129.10 (1 C), 134.59 (1 C), 135.08 (1 C), 149.15 (1 C), 161.74 (1 C), 174.33 (1 C). FAB-MS m/z : 345 $[\text{M}+\text{H}]^+$.

6-Ammonium-*N*-[2-(4-piperazinium-1-yl)-4-oxo-3*H*-quinazolin-6-yl]hexanamide dichloride (**9e**).

Yield 98%. White hygroscopic solid. $^1\text{H NMR}$ (500 MHz, DMSO-d_6), δ , ppm: 1.34 (ddd, $J=15.0$, 7.8, 7.7 Hz, 2 H), 1.56–1.63 (m, 4 H), 2.37 (t, $J=7.3$ Hz, 2 H), 2.75 (q, $J=6.7$ Hz, 2 H), 3.25 (br.s, 4 H), 4.05 (br.s, 4 H), 7.85–7.94 (m, 2 H), 8.04 (br.s, 3 H),

8.45 (d, $J=1.0$ Hz, 1 H), 9.84 (br.s, 2 H), 10.54 (s, 1 H); ^1H NMR (400 MHz, D_2O), δ , ppm: 1.32 (ddd, $J=15.0, 7.8, 7.6$ Hz, 2 H), 1.55–1.63 (m, 4 H), 2.34 (t, $J=7.4$ Hz, 2 H), 2.88 (t, $J=7.5$ Hz, 2 H), 3.42 (t, $J=5.0$ Hz, 4 H), 3.97 (t, $J=5.0$ Hz, 4 H), 7.33 (t, $J=8.8$ Hz, 1 H), 7.61 (dd, $J=9.0, 2.3$ Hz, 1 H), 7.92 (d, $J=2.3$ Hz, 1 H). ^{13}C NMR (126 MHz, D_2O), δ , ppm: 24.07 (1 C), 24.68 (1 C), 25.97 (1 C), 35.68 (1 C), 38.82 (1 C), 41.81 (2 C), 42.85 (2 C), 114.92 (1 C), 117.06 (1 C), 118.07 (1 C), 128.90 (1 C), 134.87 (1 C), 135.09 (1 C), 149.50 (1 C), 162.65 (1 C), 174.96 (1 C). FAB-MS m/z : 359 $[\text{M}+\text{H}]^+$.

The synthesis of compounds **11–12** was carried out similarly to the procedure described in [9]; the synthesis of compounds and derivatives **13–16 (a–e)** was carried out in accordance with the previously described procedure [5].

6-Nitro-4-phenylquinazolin-2-one (11)

A mixture of 87 g of 5-nitro-2-aminobenzophenone and 22 g of carbamide was kept at 180–190 °C until the evolution of ammonia ceased, about 2 hours. Next, the melt mixture was transferred to a porcelain cup, cooled, grinded and triturated with water. The precipitate was filtered off and recrystallized from DMF. Yield 22 g (23%). Orange crystalline precipitate. M.p. >300 °C. FAB-MS m/z : 268 $[\text{M}+\text{H}]^+$.

2-Chloro-6-nitro-4-phenylquinazoline (12)

A mixture of 19 g of 6-nitro-4-phenylquinazolin-2-one, 100 ml of fresh POCl_3 , previously distilled, and 14 g of PCl_5 were refluxed for about 5 hours. The reaction mixture was cooled, after which phosphorus oxychloride was evaporated under reduced pressure. The residue was poured onto ice (100 g) and carefully adjusted to a neutral pH with the aqueous solution of ammonia. The resulting precipitate was filtered off, washed with plenty of water. The product was recrystallized from ethanol. Yield 76%. Beige solid. R_f 0.93. M.p. 185–186 °C. FAB-MS m/z : 286, 288 $[\text{M}+\text{H}]^+$.

6-Nitro-2-(4-Boc-piperazin-1-yl)-4-phenylquinazoline (13)

To a solution of 4.5 g of 2-chloro-6-nitro-4-phenylchloroquinazoline (0.016 mol) in 200 ml of acetonitrile, 3 g of N-Boc-piperazine and 2.2 ml of NEt_3 was added. The solution was boiled with stirring for 4 hours. Then the resulting mixture was cooled in the refrigerator. The precipitate was filtered off, washed with small amounts of water and cold acetonitrile, and dried.

Yield 75.5%. Yellow solid. R_f 0.8. M.p. 179–180 °C. FAB-MS m/z : 436 $[\text{M}+\text{H}]^+$.

6-amino-2-(4-Boc-piperazin-1-yl)-4-phenylquinazoline (14)

A solution of 1 g of 6-amino-2-(4-Boc-piperazin-1-yl)-4-phenylquinazoline in 100 ml of EtOH was subjected to catalytic hydrogenation at room temperature in the presence of 5% palladium-carbon (0.05 g) for 1–1.5 hours (the progress of the reactions was monitored by TLC). After completion of the reaction, a helium stream was passed through the solution for 20 minutes. The solution was filtered through a zeolite layer; the filtrate was evaporated to dryness under reduced pressure. The amino derivative was used directly in the next stage.

Yield 89%. Yellow solid. R_f 0.75. FAB-MS m/z : 406 $[\text{M}+\text{H}]^+$.

N-[2-(4-Boc-piperazin-1-yl)-4-phenylquinazolin-6-yl]propanamide (15a)

To a solution of 0.002 mol of amine in 25 ml of methylene chloride, 0.26 ml (0.002 mol) of propionic anhydride was added. The mixture was refluxed for 2 hours. Then the solution was cooled and washed successively with water (40 ml), 1M HCl solution (40 ml) and 5% NaHCO_3 solution (40 ml). The organic phase was dried over anhydrous

sodium sulfate, and the solvent was evaporated to dryness under reduced pressure. The residue was purified using silica column chromatography (5% MeOH/CHCl₃). Yield 53%. Yellow solid. R_f 0.76. M.p. 193–194 °C. ¹H NMR (500 MHz, CDCl₃), δ, ppm: 1.22 (t, *J*=7.4 Hz, 3 H), 1.50 (s, 9 H), 2.37 (q, *J*=7.1 Hz, 2 H), 3.54 (s, 4 H), 3.97 (s, 4 H), 7.45–7.55 (m, 4 H), 7.62 (d, *J*=7.4 Hz, 1 H), 7.73–7.75 (m, 3 H), 8.12 (s, 1 H). FAB-MS *m/z*: 462 [M+H]⁺.

General method for the synthesis of derivatives with various amino acids

0.002 mol of Boc acid was dissolved in anhydrous acetonitrile (25 ml). The solution was cooled to –5 °C and 0.28 ml (0.002 mol) of triethylamine was added, followed by HBTU (0.76 g, 0.002 mol) or HATU (0.76 g, 0.002 mol). The mixture was stirred for 1 hour at –5 °C, and then 0.002 mol of amine was added. The mixture was then stirred for 7 hours at 50 °C. The residual amounts of activated ester and the initial Boc acid were destroyed by the addition of a few drops of *N,N*-dimethylpropane-1,3-diamine. The solvent was evaporated to dryness under vacuum. The residue was dissolved in 100 ml of chloroform and washed successively with water (40 ml), 1M HCl solution (40 ml) and 5% NaHCO₃ solution (40 ml). The organic phase was dried over anhydrous sodium sulfate and concentrated by rotary evaporation. The resulting products **15b–15e** were purified by silica column chromatography (5% MeOH/CHCl₃).

3-N-Boc-amino-N-[2-(4-Boc-piperazin-1-yl)-4-phenylquinazolin-6-yl]propanamide (15b)

Yield 25%. Yellow solid. R_f 0.69. M.p. 190–191.5 °C. ¹H NMR (500 MHz, CDCl₃), δ, ppm: 1.40 (s, 9 H), 1.50 (s, 9 H), 2.59 (t, *J*=5.5 Hz, 3 H), 3.48 (dd, *J*=11.3, 5.5 Hz, 2 H), 3.55 (s, 4 H), 3.98 (s, 4 H), 5.14 (br.s, 1 H), 7.53–7.56 (m, 3 H), 7.64 (s, 1 H), 7.75 (d, *J*=4.4 Hz, 2 H), 7.82 (d, *J*=8.5 Hz, 1 H), 8.10 (br.s, 1 H), 8.14 (s, 1 H). FAB-MS *m/z*: 577 [M+H]⁺.

4-N-Boc-amino-N-[2-(4-Boc-piperazin-1-yl)-4-phenylquinazolin-6-yl]butanamide (15c)

Yield 65%. Yellow solid. R_f 0.59. M.p. 209–210 °C. ¹H NMR (500 MHz, CDCl₃), δ, ppm: 1.45 (s, 9 H), 1.50 (s, 9 H), 1.86 (ddd, *J*=11.7, 6.2, 6.0 Hz, 2 H), 2.37 (t, *J*=6.0 Hz, 2 H), 3.24 (dd, *J*=11.3, 6.0 Hz, 2 H), 3.55 (s, 4 H), 3.98 (s, 4 H), 4.81 (t, *J*=5.5 Hz, 1 H), 7.50–7.56 (m, 3 H), 7.64 (s, 1 H), 7.79 (d, *J*=6.3 Hz, 2 H), 7.89 (d, *J*=8.5 Hz, 1 H), 8.27 (s, 1 H), 9.12 (s, 1 H). FAB-MS *m/z*: 591 [M+H]⁺.

5-N-Boc-amino-N-[2-(4-Boc-piperazin-1-yl)-4-phenylquinazolin-6-yl]pentanamide (15d)

Yield 46%. Yellow solid. R_f 0.61. M.p. 138–139 °C. ¹H NMR (500 MHz, CDCl₃), δ, ppm: 1.40 (s, 9 H), 1.50 (s, 9 H), 1.55 (ddd, *J*=13.7, 6.9, 6.6 Hz, 2 H), 1.74 (ddd, *J*=14.1, 7.1, 7.0 Hz, 2 H), 2.39 (t, *J*=7.1 Hz, 2 H), 3.16 (dd, *J*=11.5, 6.0 Hz, 2 H), 3.55 (s, 4 H), 3.98 (s, 4 H), 4.67 (br.s, 1 H), 7.51–7.56 (m, 3 H), 7.62 (d, *J*=8.5 Hz, 1 H), 7.76–7.80 (m, 4 H), 8.15 (br.s, 1 H). FAB-MS *m/z*: 605 [M+H]⁺.

6-N-Boc-amino-N-[2-(4-Boc-piperazin-1-yl)-4-phenylquinazolin-6-yl]hexanamide (15e)

Yield 52%. Yellow solid. R_f 0.64. M.p. 94–95 °C. ¹H NMR (500 MHz, DMSO-*d*₆), δ, ppm: 1.21–1.27 (m, 2 H), 1.30–1.39 (m, 11 H), 1.43 (s, 9 H), 1.55 (m, *J*=7.8, 7.8, 7.8 Hz, 2 H), 2.27 (t, *J*=7.4 Hz, 2 H), 2.88 (q, *J*=6.6 Hz, 2 H), 3.45 (s, 4 H), 3.85 (t, *J*=4.9

Hz, 4 H), 6.74 (t, $J=4.3$ Hz, 1 H), 7.58–7.61 (m, 4 H), 7.72–7.74 (m, 2 H), 7.93 (dd, $J=9.1, 2.2$ Hz, 1 H), 8.24 (d, $J=1.7$ Hz, 1 H), 10.04 (s, 1 H). FAB-MS m/z : 619 $[M+H]^+$.

General procedure for acidolytic cleavage of Boc-protective groups

0.001 mol of compound **15** was dissolved in 100 ml of dried CH_2Cl_2 . Dry HCl was bubbled through the solution for 1 hour. The completeness of the reaction was monitored by TLC. The product precipitated from the solution in the form of hydrochloride. After completion of the reaction, the solvent was evaporated to dryness and the precipitate was dried at 40 °C under reduced pressure (2 mm Hg) for 2 hours.

N-[2-(4-piperazinium-1-yl)-4-phenylquinazolin-6-yl]propanamide chloride (16a)

Yield 98%. Yellow hygroscopic solid. 1H NMR (500 MHz, $DMSO-d_6$), δ , ppm: 1.05 (t, $J=6.8$ Hz, 3 H), 2.33 (q, $J=6.9$ Hz, 2 H), 3.24 (s, 4 H), 4.24 (s, 4 H), 7.61–7.67 (m, 3 H), 7.78 (d, $J=5.5$ Hz, 2 H), 7.94 (d, $J=6.6$ Hz, 1 H), 8.06 (d, $J=8.1$ Hz, 1 H), 8.44 (s, 1 H), 9.68 (s, 2 H), 10.40 (s, 1 H); 1H NMR (500 MHz, D_2O), δ , ppm: 0.99 (t, $J=7.7$ Hz, 3 H), 2.23 (q, $J=7.6$ Hz, 2 H), 3.34 (t, $J=4.9$ Hz, 4 H), 4.11 (t, $J=4.9$ Hz, 4 H), 7.39–7.48 (m, 5 H), 7.53–7.57 (m, 2 H), 7.91 (d, $J=2.0$ Hz, 1 H). ^{13}C NMR (126 MHz, D_2O), δ , ppm: 8.53 (1 C), 29.34 (1 C), 41.34 (2 C), 42.15 (2 C), 115.90 (1 C), 117.55 (1 C), 118.91 (1 C), 128.28 (2 C), 129.55 (1 C), 129.69 (2 C), 131.90 (1 C), 133.93 (1 C), 134.74 (1 C), 138.78 (1 C), 131.22 (1 C), 172.74 (1 C), 175.75 (1 C). FAB-MS m/z : 362 $[M+H]^+$.

3-Ammonium-N-[2-(4-piperazinium-1-yl)-4-phenylquinazolin-6-yl]propanamide dichloride (16b)

Yield 95%. Yellow hygroscopic solid. 1H NMR (500 MHz, $DMSO-d_6$), δ , ppm: 2.78 (t, $J=6.6$ Hz, 2 H), 3.04 (q, $J=5.8$ Hz, 2 H), 3.22 (s, 4 H), 4.17 (s, 4 H), 7.61–7.64 (m, 3 H), 7.75–7.80 (m, 3 H), 8.01–8.06 (m, 4 H), 8.42 (s, 1 H), 9.56 (br.s, 2 H), 10.68 (s, 1 H); 1H NMR (500 MHz, D_2O), δ , ppm: 2.76 (t, $J=6.9$ Hz, 2 H), 3.21 (t, $J=6.6$ Hz, 2 H), 3.39 (t, $J=4.9$ Hz, 4 H), 4.20 (t, $J=4.9$ Hz, 4 H), 7.53 (t, $J=7.7$ Hz, 2 H), 7.62–7.67 (m, 4 H), 7.79 (dd, $J=9.3, 2.2$ Hz, 1 H), 8.08 (d, $J=2.2$ Hz, 1 H). ^{13}C NMR (126 MHz, D_2O), δ , ppm: 32.77 (1 C), 35.36 (1 C), 41.97 (2 C), 42.67 (2 C), 116.90 (1 C), 119.20 (1 C), 119.46 (1 C), 128.91 (2 C), 130.20 (2 C), 130.80 (1 C), 132.33 (1 C), 134.81 (1 C), 134.86 (1 C), 139.51 (1 C), 151.89 (1 C), 170.73 (1 C), 174.18 (1 C). FAB-MS m/z : 377 $[M+H]^+$.

4-Ammonium-N-[2-(4-piperazinium-1-yl)-4-phenylquinazolin-6-yl]butaneamide dichloride (16c)

Yield 98%. Yellow hygroscopic solid. 1H NMR (500 MHz, $DMSO-d_6$), δ , ppm: 1.90 (q, $J=7.4$ Hz, 2 H), 2.44–2.46 (m, 2 H), 2.77–2.83 (m, 2 H), 3.24 (s, 4 H), 4.25 (s, 4 H), 7.62–7.67 (m, 3 H), 7.77–7.80 (m, 2 H), 7.95 (d, $J=8.5$ Hz, 1 H), 8.09 (dd, $J=9.1, 1.6$ Hz, 1 H), 8.15 (br.s, 3 H), 8.46 (s, 1 H), 9.74 (br.s, 2 H), 10.68 (s, 1 H); 1H NMR (500 MHz, D_2O), δ , ppm: 1.86 (ddd, $J=15.4, 7.7, 7.5$ Hz, 2 H), 2.40 (t, $J=7.5$ Hz, 2 H), 2.92 (t, $J=7.5$ Hz, 2 H), 3.36 (dd, $J=5.2, 5.2$ Hz, 4 H), 4.16 (dd, $J=5.2, 5.2$ Hz, 4 H), 7.48 (t, $J=7.8$ Hz, 2 H), 7.55–7.62 (m, 4 H), 7.68 (dd, $J=9.1, 2.3$ Hz, 1 H), 8.02 (d, $J=2.1$ Hz, 1 H). ^{13}C NMR (126 MHz, D_2O), δ , ppm: 22.51 (1 C), 33.06 (1 C), 38.75 (1 C), 41.90 (2 C), 42.61 (2 C), 116.62 (1 C), 118.73 (1 C), 119.28 (1 C), 128.82 (2 C), 130.19 (2 C), 130.46 (1 C), 132.35 (1 C), 134.58 (1 C), 135.01 (1 C), 139.19 (1 C), 151.68 (1 C), 173.27 (1 C), 173.80 (1 C). FAB-MS m/z : 391 $[M+H]^+$.

5-Ammonium-N-[2-(4-piperazinium-1-yl)-4-phenylquinazolin-6-yl]pentanamide dichloride (16d)

Yield 94%. Yellow hygroscopic solid. ^1H NMR (500 MHz, DMSO-d_6), δ , ppm: 1.56–1.65 (m, 4 H), 2.37 (t, $J=5.9$ Hz, 2 H), 2.77 (dd, $J=11.3, 6.6$ Hz, 2 H), 3.24 (s, 4 H), 4.24 (s, 4 H), 7.61–7.67 (m, 3 H), 7.78 (d, $J=7.1$ Hz, 2 H), 7.94 (d, $J=8.0$ Hz, 2 H), 8.06–8.11 (m, 4 H), 8.47 (s, 1 H), 9.74 (s, 2 H), 10.61 (s, 1 H); ^1H NMR (500 MHz, D_2O), δ , ppm: 1.59–1.62 (m, 4 H), 2.35 (t, $J=6.9$ Hz, 2 H), 2.92 (t, $J=6.6$ Hz, 2 H), 3.39 (dd, $J=5.0, 4.9$ Hz, 4 H), 4.19 (dd, $J=5.0, 4.9$ Hz, 4 H), 7.51 (t, $J=7.7$ Hz, 2 H), 7.59–7.64 (m, 4 H), 7.71 (dd, $J=8.8, 2.2$ Hz, 1 H), 8.06 (d, $J=2.2$ Hz, 1 H). ^{13}C NMR (126 MHz, D_2O), δ , ppm: 21.83 (1 C), 26.23 (1 C), 35.70 (1 C), 39.12 (1 C), 41.92 (2 C), 42.67 (2 C), 116.75 (1 C), 118.86 (1 C), 119.55 (1 C), 128.86 (2 C), 130.19 (2 C), 130.54 (1 C), 132.32 (1 C), 134.72 (1 C), 135.00 (1 C), 139.63 (1 C), 151.96 (1 C), 173.82 (1 C), 174.67 (1 C). Mass spectrum (FAB-MS) m/z : 405 $[\text{M}+\text{H}]^+$.

6-Ammonium-N-[2-(4-piperazinium-1-yl)-4-phenylquinazolin-6-yl]hexanamide dichloride (16e)

Yield 97%. Yellow hygroscopic solid. ^1H NMR (500 MHz, DMSO-d_6), δ , ppm: 1.32 (m, $J=7.5, 7.5, 7.5, 7.5$ Hz, 2 H), 1.57 (m, 4 H), 2.34 (t, $J=7.4$ Hz, 2 H), 2.73 (m, $J=6.1, 6.1, 6.1, 6.1$ Hz, 2 H), 3.23 (s, 4 H), 4.23 (s, 4 H), 7.61–7.65 (m, 3 H), 7.77–7.79 (m, 2 H), 7.91 (d, $J=8.7$ Hz, 1 H), 8.05 (s, 3 H), 8.08 (d, $J=9.2$ Hz, 1 H), 8.44 (s, 1 H), 9.72 (s, 2 H), 10.51 (s, 1 H); ^1H NMR (400 MHz, D_2O), δ , ppm: 1.30 (m, $J=8.2, 8.2, 8.2, 8.2$ Hz, 2 H), 1.53–1.61 (m, 4 H), 2.30 (t, $J=7.4$ Hz, 2 H), 2.88 (t, $J=7.4$ Hz, 2 H), 3.38 (t, $J=4.9$ Hz, 4 H), 4.18 (t, $J=4.4$ Hz, 4 H), 7.50 (t, $J=7.4$ Hz, 2 H), 7.59–7.63 (m, 4 H), 7.70 (dd, $J=9.3, 1.6$ Hz, 1 H), 8.05 (d, $J=1.6$ Hz, 1 H). ^{13}C NMR (126 MHz, D_2O), δ , ppm: 24.49 (1 C), 25.18 (1 C), 26.46 (1 C), 36.20 (1 C), 39.49 (1 C), 41.92 (2 C), 42.67 (2 C), 116.79 (1 C), 118.91 (1 C), 119.57 (1 C), 128.85 (2 C), 130.17 (2 C), 130.58 (1 C), 132.29 (1 C), 134.74 (1 C), 135.03 (1 C), 139.65 (1 C), 152.00 (1 C), 173.87 (1 C), 175.36 (1 C). FAB-MS m/z : 419 $[\text{M}+\text{H}]^+$.

Biological experiment

The study of the analgesic activity of the synthesized compounds was carried out on a model of peripheral pain, the basis of which is chemical pain irritation. The pain was induced by the intraperitoneal administration of 0.75% acetic acid solution, which leads to the occurrence of involuntary contractions of the abdominal muscles – “writhing”. Acetic acid solution was administered intraperitoneally 40 minutes after intraperitoneal administration of the test compounds in a dose range. Experimental animals were monitored for 20 minutes and the number of “writhing” for each animal was counted. Analgesic activity was evaluated by the ability of the compounds to reduce the number of “writhing” compared with the control group. The classic NSAID diclofenac sodium at a dose of 10 mg/kg was used as a comparison preparation. ED_{50} values were calculated according to the method of V.B. Prozorovsky.

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СИНТЕЗ ТА АНАЛЬГЕТИЧНА АКТИВНІСТЬ ПОХІДНИХ 6-АМІНО-2-ПІПЕРАЗИНІЛХІНАЗОЛІНІВ

Метою даної роботи є синтез та дослідження анальгетичної активності ряду похідних 2,6-заміщених хіназолін-4-ону і 4-фенілхіназоліну. В результаті проведеної роботи було отримано ряд похідних 2-піперазиніл-3*H*-хіназолін-4-ону (п'ять сполук) та 2-піперазин-4-фенілхіназоліну (п'ять сполук), що містять при аміногрупі 6-ого положення хіназолінового гетероциклу замісники з різною довжиною вуглецевого ланцюга. Будову синтезованих сполук підтверджували за допомогою методів ^1H - та ^{13}C - ЯМР спектроскопії та FАВ мас-спектрометрії. Анальгетичну активність синтезованих сполук досліджували *in vitro* на моделі периферичного болю за методом «корчів» у мишей, що викликали внутрішньочеревним введенням оцтової кислоти. Цільові сполуки проявили високу анальгетичну активність. Значення ED_{50} знаходяться в діапазоні від 0.46 до 0.88 мг/кг (для диклофенака натрію знайдене значення ED_{50} складає 10.0 ± 1.8 мг/кг). В якості препарату порівняння виступав класичний нестероїдний протизапальний препарат – диклофенак натрію. В результаті експерименту визначені дози, в яких сполуки проявили виразні фармакологічні властивості. При дослідженні впливу замісника 4-ого положення хіназолінового гетероциклу на анальгетичну активність, встановлено, що сполуки, які містять у своєму складі центральний 4-фенілхіназоліновий фрагмент, загалом проявили більшу активність в порівнянні з похідними до складу яких входить центральний 4-оксохіназоліновий каркас; для останніх рівень активності майже однаковий для всіх досліджених аналогів.

Ключові слова: 2-піперазиніл-3*H*-хіназолініл-4-они, 2-піперазин-4-фенілхіназоліни, анальгетична активність.

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СИНТЕЗ И АНАЛЬГЕТИЧЕСКАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ 6-АМИНО-2-ПИПЕРАЗИНИЛХИНАЗОЛИНОВ

Синтезированы производные 6-амино-2-пиперазинхиनाзолин-4-она и 2-пиперазин-4-фенилхиназолина. Изучена анальгетическая активность полученного ряда производных и обнаружено, что синтезированные соединения обладают высокой анальгетической активностью. Значения ED_{50} находятся в диапазоне от 0,46 до 0,88 мг/кг.

Ключевые слова: 2-пиперазинил-3*H*-хиназолин-4-оны, 2-пиперазинил-4-фенилхиназолины, анальгетическая активность.

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