ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Recommended by Doctor of Pharmacy, professor K.G.Schokina

UDC 615.076:001.891.5:615.213/.214:547.856:547.583.5

THE PHARMACOLOGICAL ACTIVITY OF DERIVATIVES OF 4-OXO-3,4-DIHYDROQUINAZOLINE AND ANTHRANILAMIDES CONTAINING A FRAGMENT OF GLYCINE

Yu.O.Ovsyanikova, D.V.Levashov, V.M.Kravchenko, V.P.Chernykh, L.A.Shemchuk

National University of Pharmacy

Key words: quinazolone; anthranilamide; antidepressant effect; anticonvulsant activity; hypnotic activity

Derivatives of 4-oxo-3,4-dihydroquinazoline are known as a promising class of compounds due to their wide spectrum of the pharmacological activity. Taking into account the PASS data for the substituted anthranilamides synthesized, as well as derivatives of 4-oxo-3,4-dihydroquinazoline containing an "in-built" fragment of amino acid glycine as a pharmacophore, the decision to study their central neurotropic effects was made. While studying the hypnotic, anticonvulsant and antidepressant activities the highest antidepressant properties of N-(1,1-diphenyl-1-hydroxyet-2-yl)-N'-diphenylhydroxyacetylanthranilamide (compound 4) have been determined, this compound is slightly inferior the reference drug Imipramine. N-(phenylhydrazidoacetyl)-N'-succinamidoanthranilamide (compound 1) reveals high anticonvulsant properties and is not inferior the classical anticonvulsant drug Depakine. When studying the hypnotic effect the antagonism with Barbamyl for 2-(4-oxo-3,4-dihydro-3-quinazolinyl)acetohydrazide (compound 5) has been found. Methyl-(2-methylcarbonyloxymethyl-4-oxo-3,4-dihydro-3-quinazolinyl)acetate (compound 8) decreased the latent period of "falling asleep" for animals in 1.6 and 1.7 times in the doses of 20 and 200 mg/kg, respectively (the same level with the reference drug), and therefore, it is a promising compound for further research of the hypnotic activity. The analysis of the "structure-activity" relationship gives the possibility to assume that such pronounced pharmacological activity is due to the presence of substituents in position 2 of the quinazoline nucleus. Therefore, the data obtained prove that the study of these derivatives is promising for further search of new biologically active substances with hypnotic, anticonvulsant and antidepressant properties.

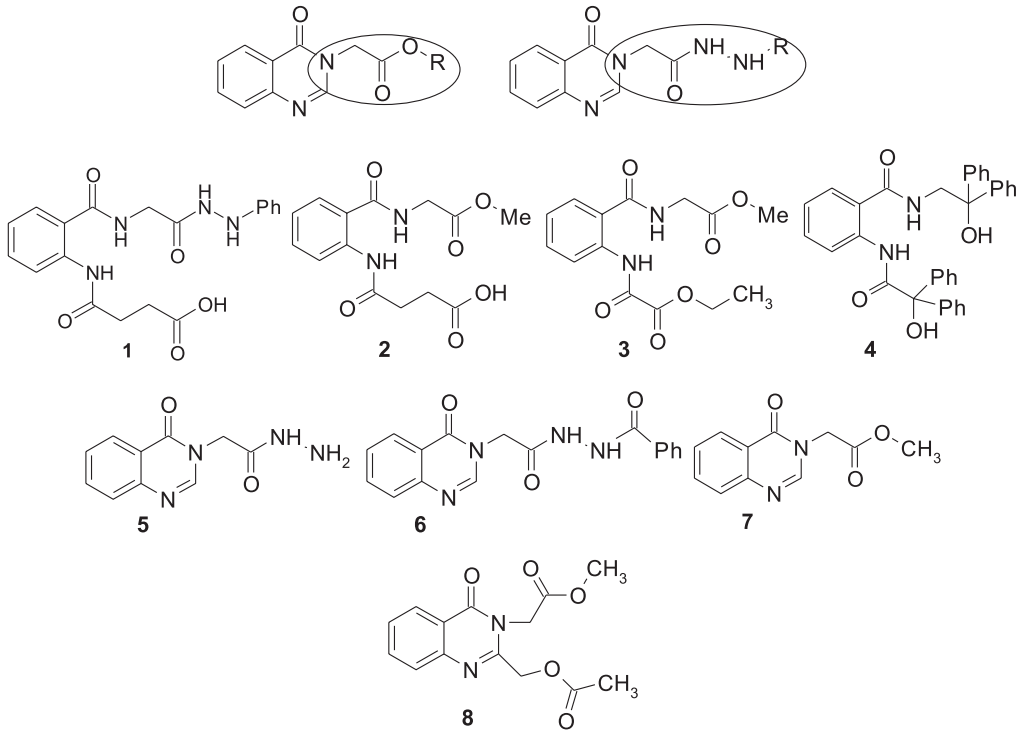
Derivatives of 4-oxo-3,4-dihydroquinazoline are known as a promising class of compounds due to their wide spectrum of the pharmacological activity. In particular, depending on the substituents in the quinazoline nucleus derivatives of this heterocyclic structure reveal the hypnotic, anticonvulsant, antibacterial, anticholinesterase, vasodilating activities [3, 4, 6, 9, 10]. Previously, derivatives of quinazoline and substituted anthranilamides (used as starting materials for quinazoline synthesis) containing an "in-built" fragment of amino acid glycine as a pharmacophore were synthesized [7, 8] (Fig.).

According to the data of the PASS software for compounds **1-8** the determination of some types of the pharmacological activity with probability higher than 0.75 was expected. Among them central neurotropic effects, namely antidepressant and anticonvulsant effects (for anthranilamides **1-4**) and hypnotic effect (for quinazolines **5-8**) prevailed. Therefore, the aim of this study was to conduct the pharmacological research of derivatives of 4-oxo-3,4-dihydroquinazoline and starting anthranilamides *in vivo*.

Materials and Methods

The antidepressant activity of compounds **1-4** was determined in white mice. For the depressive behaviour simulation the Porsolt behavioural despair test was used [5]. The test substances were injected intragastrically as water suspensions in the doses of 20 and 200 mg/kg 30 min prior to the experiment. Melipramin (Imipramine) was selected as a reference drug. It was injected intraperitoneally in the dose of 25 mg/kg. The control group received intragastrically the same volume of purified water. The total time of animal's immobile fixation and the number of immobility acts observed for 6 min were used as indicators of the antidepressant activity. The results obtained are given in Tab. 1.

The anticonvulsant activity was determined in white mice under conditions of experimental pentylenetetrazol convulsions [1]. The test substances were injected intragastrically as water suspensions in the doses of 20 and 200 mg/kg 30 min prior to the experiment. Depakine (sodium valproate) was selected as a reference drug.



Figure

Table 1

The effect of the test substances on the depressive behaviour of mice in the fixation test

Group of animals / test compound	Dose, mg/kg	Duration of immobile fixation, s	Number of immobility acts
Control	-	114.14+/-16.06	19.71+/-3.01
Compound **1**	20	71.20+/-10.26	15.40+/-1.40
	200	120.80+/-59.56	17.00+/-3.15
Compound **2**	20	99.20+/-29.57	13.60+/-2.62
	200	75.00+/-23.71	13.40+/-2.58
Compound **3**	20	94.40+/-37.85	17.60+/-2.29
	200	71.80+/-19.20	16.60+/-1.50
Compound **4**	20	80.20+/-20.23	14.00+/-2.07
	200	60.00+/-12.40*	18.20+/-3.32
Melipramin	25	64.30+/-14.10*	7.60+/-1.71*

Note: *significant differences in relation to the control ($p<0.05$).

It was injected intragastrically in the dose of 300 mg/kg. The control group received intragastrically the same volume of purified water. The aqueous solution of pentylenetetrazol (Sigma) in the dose of 80 mg/kg was injected subcutaneously. The anticonvulsant activity was assessed by the following indicators: the latent period of clonic or tonic convulsions, the number of clonic and tonic paroxysms per 1 mouse and mortality. The results obtained are given in Tab. 2.

The hypnotic activity of compounds **5-8** was assessed using the model of Barbamyl anesthesia in mice [2]. The test substances were injected intragastrically as water suspensions in the preventive mode in the doses of 20 and 200 mg/kg 30 min prior to the experiment. Animals of the comparison group received intragastrically the aqueous solution of Donormyl (doxylamine hydrochloride) in the dose of 20 mg/kg. The control group received intragastrically the same volume of purified water. Barbamyl in the dose of 50 mg/kg was injected intraperitoneally. The hypnotic activity was estimated by the following indicators: the latent period of anesthesia beginning ("falling asleep"), duration of the anesthesia sleep and the number of mice that were anesthetized. The results obtained are given in Tab. 3.

Results and Discussion

When studying the antidepressant activity only *N*-(1,1-diphenyl-1-hydroxyet-2-yl)-*N'*-diphenylhydroxyacetylanthranilamide (compound **4**) in the dose of 200 mg/kg reliably decreased the general duration of immobile fixation of animals in 1.9 times compared to the control group (at the same level as Imipramine). However, it had no effect on the number of immobility acts. The results ob-

Table 2

The effect of the test substances on pentylenetetrazol convulsions in mice

Group of animals / test compound	Dose, mg/kg	The latent period, min	Number of clonic and tonic paroxysms per 1 mouse	Mortality, %
Control	–	6.34+/-0.79	2.86+/-0.51	71
Compound **1**	20	6.43+/-0.71	2.60+/-0.81	40
	200	11.05+/-1.97*	1.40+/-0.25*	20**
Compound **2**	20	6.12+/-1.03	2.80+/-0.49	60
	200	4.14+/-0.44	3.00+/-0.55	80
Compound **3**	20	4.40+/-1.03	2.40+/-0.75	80
	200	5.56+/-1.41	2.00+/-0.32	80
Compound **4**	20	5.45+/-0.64	2.20+/-0.58	60
	200	5.55+/-0.95	2.60+/-0.40	60
Depakine	300	12.16+/-1.88*	1.20+/-0.41*	17**

Note: significant differences in relation to the control ($p<0.05$): * – by the Student's t-criterion; ** – by the Fisher angular transformation.

Table 3

The effect of the test substances on the Barbamyl anesthesia in mice

Group of animals / test compound	Dose, mg/kg	The latent period, min	Duration of anesthesia, min	% of mice that were anesthetized
Control	–	21.56+/-2.38	45.50+/-4.44	100
Compound **5**	20	–	0.00+/-0.00***	0##
	200	32.20	11.25*	20##
Compound **6**	20	18.53+/-1.69	15.95+/-3.12***	100
	200	9.47+/-0.48**	15.40+/-6.00**	100
Compound **7**	20	14.46+/-3.46	25.02+/-6.36*	100
	200	12.00+/-2.72*	34.11+/-6.08	100
Compound **8**	20	13.68+/-2.02*	45.82+/-7.54	100
	200	12.61+/-2.80*	43.60+/-6.75	100
Donormyl	20	11.72+/-1.46*	50.36+/-5.27	100

Note: significant differences in relation to the control: * – by the Student's t-criterion ($p<0.05$); ** – by the Student's t-criterion ($p<0.01$); *** – by the Student's t-criterion; # – by the Fisher angular transformation ($p<0.05$); ## – by the Fisher angular transformation ($p<0.01$).

tained confirm significant and dose-dependent antidepressant properties of this compound; they are slightly inferior the reference drug Imipramine.

Compounds **1-3** in both doses (except for compound **1** in the dose of 200 mg/kg) revealed a tendency to decrease the total duration of immobile fixation and the number of immobility acts. However, this difference did not reach the level of statistical significance because of high depression of data. The high activity of compound **4** can be associated with the presence of two fragments of benzylic (diphenylhydroxyacetic) acid in the structure of a molecule.

The results of the anticonvulsant activity study presented in Tab. 2 show that *N*-(phenylhydrazidoacetyl)-*N'*-succinamidoanthranilamide (compound **1**) in the dose of 200 mg/kg revealed significant anticonvulsant properties: against the background of its administration there was a statistically significant lengthening of the latent period of the clonic and tonic convulsions in 1.7 times, decrease in the number of paroxysms per 1 mouse more than twice, and also reduction of the animals' mortality by 51% ($p<0.05$) compared to the control group. The anticonvulsant effect of this compound is dose-dependent: in the dose of 20 mg/kg compound **1** does not affect the latent period and the number of convulsions per 1 mouse, and it only insignificantly reduces the mortality index in the group by 31% compared to the control group. In general, the anticonvulsant effect of this compound in the dose of 200 mg/kg is not inferior the classical anticonvulsant drug Depakine in the dose of 300 mg/kg. It caused a significant prolongation of the latent period of the first paroxysm development in the group in 1.9 times, as well as decrease in the number of convulsions in 2.4 times and the mortality index (54%) compared to the control group.

As can be seen from Tab. 3, a well-proven hypnotic drug Donormyl (doxylamine hydrochloride) in the dose of 20 mg/kg statistically significantly reduced the latent period of animals' anesthesia beginning in 1.8 times compared to the control group; however, it had no effect on the sleep duration.

Compound **5** revealed the antagonism to Barbamyl: in the dose of 20 mg/kg none of the animals were anes-

thetized, behavioural indicators were in the normal range, visual signs of retardation and depression of the CNS were not found. In the group of animals received the water solution of compound **5** in the dose of 200 mg/kg the development of classic drug-induced sleep was observed only in one animal. The results obtained indicate the stimulating effect of compound **5** on the CNS, and it requires the additional study.

Compounds **6** and **7** in the dose of 20 mg/kg had no significant effect on the latent period of animals' anesthesia beginning. However, they decreased duration of anesthesia, and it could be the evidence of the sleep structure disorder. Against the background of administration of compounds **6** and **7** in the dose of 200 mg/kg there was a significant reduction of the latent period of anesthesia beginning in 2.3 and 1.8 times compared to the control group. At the same time compound **6** showed statistically significant decrease of the sleep duration in 2.6 times.

Compound **8** in both doses reliably decreased the latent period of "falling asleep" of animals in 1.6 and 1.7 times, respectively, at the same level as the reference drug. However, it has no effect on duration of anesthesia and remained at the level of the similar value in the control group.

Therefore, it is methyl-(2-methylcarbonyloxymethyl-4-oxo-3,4-dihydro-3-quinazolinyl)acetate (compound **8**) that is a promising compound for further research of the hypnotic activity since it substantially decreases the time of "falling asleep" and does not affect duration of sleep. The analysis of the "structure-activity" relationship gives the possibility to assume that such pronounced pharmacological activity is due to the presence of substituents in position 2 of the quinazoline nucleus.

CONCLUSIONS

1. For N-(1,1-diphenyl-1-hydroxyet-2-yl)-N'-diphenylhydroxyacetylanthranilamide (compound **4**) the highest level of the antidepressant activity has been determined, it is slightly inferior than that for the reference drug Imipramine.

2. N-(phenylhydrazidoacetyl)-N'-succinamidoanthranilamide (compound **1**) reveals high anticonvulsant properties and is not inferior the action of the classical anticonvulsant drug Depakine.

3. It has been found that 2-(4-oxo-3,4-dihydro-3-quinazolinyl)acetohydrazide (compound **5**) shows the antagonistic effect in relation to Barbamyl.

4. Methyl-(2-methylcarbonyloxymethyl-4-oxo-3,4-dihydro-3-quinazolinyl)acetate (compound **8**) decreases the latent period of "falling asleep" for animals, and therefore, it is a promising biologically active substance for further research of the hypnotic activity.

REFERENCES

1. *Головенко М.Я., Громов Л.О. Доклінічне вивчення специфічної активності потенційних протисудомних препаратів: Метод. рекоменд. – К.: Авіценна, 2003. – 26 с.*
2. *Миронов А.Н., Кукес В.Г. Руководство по проведению доклинических исследований лекарственных средств. Ч. I. – М.: Гриф и К, 2012. – 244 с.*
3. *Manoj K., Srivastava S., Bharati M., Nizamuddin N. // Ind. J. Chem. – 2001. – Vol. 40. – P. 342-344.*
4. *Perumal P., Bilal A.R., Dontireddy R.S.R., Natesh R.K. // Eur. J. of Med. Chem. – 2009. – Vol. 44. – P. 2328-2333.*
5. *Porsolt R.D., Lenegre A., Elliot J.M. et al. // Experimental Approaches to Anxiety and Depression. – Chichester New York, 1992. – P. 73-85.*
6. *Sarvesh K.P., Abhishek S., Ashutosh S. // Eur. J. Med. Chem. – 2009. – Vol. 44. – P. 1188-1197.*
7. *Shemchuk L.A., Al-Asri Jamil M., Levashov D.V. et al. // Вісник фармації. – 2011. – №3. – С. 30-32.*
8. *Shemchuk L.A., Levashov D.V., Al-Asri Jamil M. et al. // ЖОФХ. – 2011. – Т. 8, №9. – С. 36-39.*
9. *Shisokoo J., Shirsath S., Rathod J.S., Yande O. // Eur. J. Med. Chem. – 2000. – Vol. 35, №3. – P. 351-358.*
10. *Usifoh C.O., Scriba K.E. // Med. Chem. – 2000. – Vol. 333. – P. 261-266.*

ФАРМАКОЛОГІЧНА АКТИВНІСТЬ ПОХІДНИХ 3,4-ДИГІДРО-4-ОКСОХІНАЗОЛІНУ ТА АНТРАНІЛАМІДІВ, ЩО МІСТЯТЬ ЗАЛИШОК ГЛІЦИНУ

Ю.О.Овсяникова, Д.В.Левашов, В.М.Кравченко, В.П.Черних, Л.А.Шемчук

***Ключові слова**: хіназолон; антраніламід; антидепресивний ефект; протисудомна активність; снодійна активність*

Похідні 3,4-дигідро-4-оксохіназоліну відомі як перспективний клас хімічних сполук, яким притаманний широкий спектр фармакологічної активності. Враховуючи дані PASS-прогнозу для одержаних нами заміщених антраніламідів та похідних 4-оксо-3,4-дигідрохіназолінів, що містять «вбудований» залишок амінокислоти гліцину в якості фармакофору, виникла підстава для дослідження центральних нейротропних ефектів. При вивченні снодійної, протисудомної та антидепресивної активності встановлені високі антидепресивні властивості N-(1,1-дифеніл-1-гідроксіет-2-іл)-N'-дифенілгідроксіацетилантраніламіду (сполука 4), який дещо поступається препарату порівняння іміпраміну. N-(фенілгідразидоацетил)-N'-сукцинамідоантраніламід (сполука 1) виявляє високі протисудомні властивості і не поступається дії класичного протисудомного засобу депакіну. При вивченні снодійного ефекту встановлено, що 2-(4-оксо-3,4-дигідро-3-хіназолініл)ацетогідразид (сполука 5) виявляє антагоністичний вплив відносно барбамілу. Метил-(2-метилкарбонілоксиметил-4-оксо-3,4-дигідро-3-хіназолініл)ацетат

(сполука 8) в дозах 20 та 200 мг/кг достовірно на рівні препарату порівняння зменшував латентний період «засинання» тварин у 1,6 та 1,7 рази відповідно і є перспективною БАР для подальших досліджень снодійної активності. Аналіз зв'язку «структура-дія» дає можливість припустити, що виразний прояв фармакологічної активності обумовлений наявністю замісників у положенні 2 хіназолінового ядра. Отримані дані дозволяють зробити висновок, що дослідження зазначених похідних є перспективним для подальшого пошуку нових біологічно активних речовин зі снодійною, протисудомною та антидепресивною властивостями.

ФАРМАКОЛОГИЧЕСКАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ 3,4-ДИГИДРО-4-ОКСОХИНАЗОЛИНА И АНТРАНИЛАМИДОВ, КОТОРЫЕ СОДЕРЖАТ ОСТАТОК ГЛИЦИНА

Ю.А.Овсяникова, Д.В.Левашов, В.Н.Кравченко, В.П.Черных, Л.А.Шемчук

***Ключевые слова**: хиназолон; антраниламид; антидепрессивный эффект; противосудорожная активность; снотворная активность*

Производные 3,4-дигидро-4-оксохиназолина известны как перспективный класс химических соединений, проявляющих широкий спектр фармакологической активности. С учетом данных PASS-прогноза для полученных нами замещенных антраниламидов и производных 4-оксо-3,4-дигидрохиназолинов, которые содержат «встроенный» остаток аминокислоты глицина в качестве фармакофора, возникли основания для исследования центральных нейротропных эффектов. При изучении снотворной, противосудорожной и антидепрессивной активности установлены высокие антидепрессивные свойства N-(1,1-дифенил-1-гидроксиэт-2-ил)-N'-дифенилгидроксиацетилантраниламида (соединение 4), который несколько уступает препарату сравнения имипрамину. N-(фенилгидразидоацетил)-N'-сукцинамидоантраниламид (соединение 1) проявляет высокие противосудорожные свойства и не уступает классическому противосудорожному средству депакину. При изучении снотворного эффекта установлено, что 2-(4-оксо-3,4-дигидро-3-хиназолинил)ацетогидразид (соединение 5) проявляет антагонистическое влияние относительно барбамила. Метил-(2-метилкарбонилоксиметил-4-оксо-3,4-дигидро-3-хиназолинил)ацетат (соединение 8) в дозах 20 и 200 мг/кг достоверно на уровне препарата сравнения уменьшает латентный период «засыпания» животных в 1,6 и 1,7 раза соответственно и является перспективным БАВ для дальнейших исследований снотворной активности. Анализ связи «структура-действие» дает возможность предположить, что выраженное проявление фармакологической активности обусловлено наличием заместителей в положении 2 хиназолинового ядра. Полученные данные позволяют сделать вывод, что исследования данных производных являются перспективными для дальнейшего поиска новых биологически активных веществ со снотворными, противосудорожными и антидепрессивными свойствами.