DOI: 10.5281/zenodo.1050953
UDC: 615.212:547.29

# Experimental study of new 3-(2-$R^1$-6-$R^2$-4-oxyquinoline-3(4H)-yl)alkyl (alkaryl-, aryl) carboxylic acid derivative (PC-66 compound)

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**Abstract**

**Background:** Screening studies have revealed in new 3-(2-R1-6-R2-4-oxyquinoline-3(4H)-yl)alkyl (alkaryl-, aryl) carboxylic acid derivative (PC-66 compound) expressive analgesic properties without any damaging effects on the stomach. Therefore, in-depth study of the pharmacological properties of PC-66 compound as a pain management agent is considered topical. Objective of the study – to evaluate a pain-killing effect of PC-66 compound compared to ketorolac and diclofenac sodium on various rat pain models.

**Material and methods:** In experiments on 101 Wistar male rats (180-210 g) of somatic model (tail-flick) and neuropathic pain model (ligation of the sciatic nerve), and formalin test (5% formalin solution, 0.1 ml subplantarly) we investigated the antinociceptive activity of the PC-66 compound (1.0 mg/kg) versus ketorolac (2.4 mg/kg) and diclofenac (4.0 mg/kg) administered intraperitoneally.

**Results:** In the tail-flick model, PC-66 compound presented significant growth of PT at Hour 1 and Hour 2 by 40.6% and 50.6%, respectively. The analgesia effect of the test compound was superior to the one of diclofenac sodium, but inferior to ketorolac at Hour 1 and Hour 2, yet surpassed it by duration of action. In the formalin test model, analgesic effect of compound PC-66 was the most evident in the first (central) phase, and slightly changed the latent period and duration of the second phase of the test, while diclofenac mostly influenced Phase II (inflammatory) of the formalin test. In the model of neuropathic pain, compound PC-66 also demonstrated pronounced pain-killing effect: PT of subject rat limb grew on average by 46.7% in 2 hours following intraperitoneal administration. For this activity, PC-66 was slightly inferior to ketorolac, which caused PT growth by 51.9%.

**Conclusions:** new 3-(2-R1-6-R2-4-oxyquinoline-3(4H)-yl) alkyl (alkaryl-, aryl) carboxylic acid derivative (PC-66 compound) presented distinct analgesic effect both in somatic and neuropathic pain models.

**Key words:** carboxylic acid derivative (PC-66 compound), analgesic effect, rat pain models.

## Introduction

Search for and development of highly efficient and safe medicines for management of pain of various natures is still one of the priorities of modern pharmacology [1, 2]. 4-oxo (amino-) quinazoline derivatives have proved to be quite promising among the classes of chemicals. According to the research literature [3, 4], this class of compounds have shown cerebro- and actoprotective and antinociceptive properties, and appeared to be low-toxic substances [5]. In previous screening studies of pain models caused by electrical (pulse) stimulation of rectal mucosa and thermal stimulation of rat tail, we found two leading compounds (PC-66 and PC-199) with pronounced analgesic effect within a range of new 4-oxo (amino-) quinazolin derivatives, which appeared to be equivalent to diclofenac sodium and ketorolac both in the efficacy and effect duration [6]. However, the PC-66 compound revealed to be much safer for the gastric mucosa than PC-199 [7]. This became the basis for further in-depth study of the pharmacological properties of PC-66 compound as an anesthetic medicine.

Study objective – to evaluate the analgesic effect of the above substance compared with ketorolac and diclofenac sodium in various rat pain models.

## Material and methods

Experiments were conducted on 101 male 180-210-gram Wistar rats bred at vivarium of DU 'Instytut farmakolohii ta toksykolohii NAMN Ukrainy' (State institution "Institute of Pharmacology and Toxicology of AMS of Ukraine"), kept in standard conditions of vivarium on traditional diet with 12 –hour light-and-dark regime and access to water ad libitum. All experiments were carried out in compliance with the required bioethical standards. The following nociception models were used in the study: thermal stimulation model (tail-flick) for registration of changes in the latent period of tail flicks against the focused beam of light (pain threshold, PT) measured in seconds [9]; comparative benchmarks for duration of latent period and its changes in 1, 2, 4 and 6 hours after administration of test compound and reference medicine; and the model of neuropathic pain after ligation of the sciatic nerve [9]. The intensity of mechanical hyperalgesia was evaluated on Day 14 after surgery using Dolorimeter Baseline, USA. The pain threshold (PT) in subject and intact limbs was taken as minimal pressure on rat foot (g/$mm^2$), which caused painful reaction in animals (vocalization and/or foot flick). More information about central and peripheral components of analgesic response was obtained after the formalin test (5% formalin solution, 0.1 ml subplantarly). The monitoring lasted 60 minutes after algogenic substance administration. We registered I and II phase latent period duration, and the total duration of pain in each phase of the test. Single dose of PC-66 compound, ketorolac and diclofenac were administered intraperitoneally. Animals in the control group received equivolumic quantities of solvents. The results were processed by methods of variation statistics using STATISTICA 8.0 software and nonparametric methods of analysis [10].

## Results and discussion

The results of screening studies of PC-66 compound and reference medicines allowed us to define conditionally-effective antinociceptive doses of these substances administered intraperitoneally, which were 1.0, 2.4, and 4.0 mg/kg for PC-66, ketorolac and diclofenac sodium, respectively. Therefore, these same doses of studied substances were used for further study of analgesic effect.

The first phase of thermal stimulation test (tail-flick) investigated the extent and duration of PC-66 effect compared to ketorolac and diclofenac sodium. The results presented presumable overrun of rat PT with quinazoline derivative well in an hour after administration (40.6%). Maximum growth of antinociceptive effect was registered at Hour 2 (+ 50.6%), which decreased gradually and at Hour 6 was 20.4% compared to the baseline (fig. 1). The analgesia effect of the test compound was found superior to diclofenac at all times of the study, but inferior to ketorolac at Hour 1 and 2 after administration, yet exceeding the duration of the above effect (at Hour 6, the PT growth influenced by PC-66 was 20.4%, versus 8.93% influenced by ketorolac).

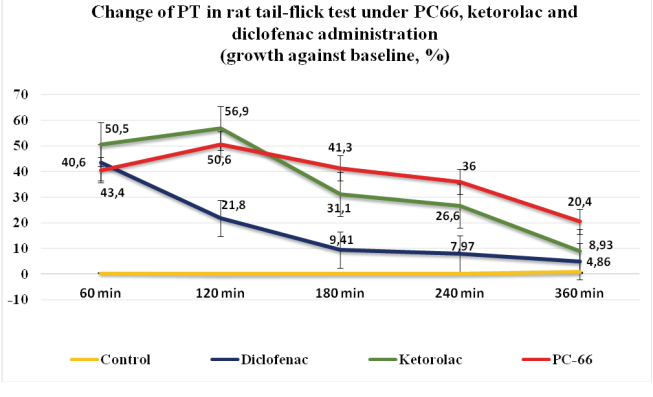


**Fig. 1. Behaviour of analgesic effect of PC-66, ketorolac and diclofenac sodium administered intraperitoneally at suppositive – effective doses.**

The obtained data led to further in-depth studies of analgesic effect in formalin test on rats, which allowed distinguishing central and peripheral components of antinociceptive effect in more details. The results showed that subplantar administration of formalin caused pain reaction in intact animals that started in average 0.69 ± 0.36 minutes following the procedure. Pain reaction manifested in raising and swinging the subject leg (Phase 1), as well as in biting and licking (Phase 2). Total duration of pain was 5.02 ± 0.42 minutes. After that, the animals calmed down, and about 20 minutes did not present any change in behavior. Then again the signs of pain reaction returned, clinically manifested in two phases with total duration of 31.9 ± 2.36 minutes. At the end of the monitoring period (60 minutes), signs of pain in considerable number of rats did not abate. The animals, intraperitoneally administered solvents (control group) instead of study medicines, presented all above-mentioned clinical signs of pain reaction, not different in severity and duration of Phase I and II latent periods from intact animals (tab. 1).

**Table 1**

**Analgesic activity of PC-66 (1 mg/kg IP) and diclofenac (4 mg/kg IP) in rat model formalin test (M ± m, n = 10)**

| Group of animals | Phase I | | Phase II | |
|---|---|---|---|---|
| | Latent period, min | Phase duration, min | Latent period, min | Phase duration, min |
| Intact animals | 1.69±0.36 | 5.02±0.45 | 21.3±2.45 | 31.9±2.36 |
| Control (solvent) | 1,67±0.29 | 5.14±0.61 | 21.2±1.21 | 32.0±1.09 |
| Diclofenac | 1.79±0.16 | 5.64±0.28 | 27.3±2.19* | 18.7±2.58* |
| PC-66 | 2,41±0,28# | 4,48±0,29# | 22,2±1,04# | 29,4±1,15# |

*Notes:* * – statistically significant differences against the control ($p<0.05$), # – statistically significant differences against diclofenac ($p<0.05$).

Instead, administration of PC-66 and diclofenac sodium, significantly changed pain behavior of animals. PC-66 significantly (44.3%) extended the duration of latent period of phase I in formalin test and statistically significantly (12.8%) reduced the duration of this phase, which substantionally characterized central mechanisms of antinociceptive action. Unlike PC-66, diclofenac sodium practically did not influence the duration of this phase. Having analyzed the influence of PC-66 and diclofenac on phase II of the formalin test, you can see that the tested compound influenced the duration of the inflammatory phase much weaker than diclofenac. Thus, the increase in average latency period and reduction of the total duration of pain reaction changed insignificantly (4.7 and 8.1%, respectively), while the latent period influenced by diclofenac demonstrated statistically significant growth by 28.8%, while duration of pain decreased by 41.5% compared to control ($p<0.05$).

The received data showed that PC-66 demonstrated analgesic properties in the rat model formalin test, manifested mostly in the Phase I of the model pathology, which involved mainly central antinociceptive mechanisms and slightly reduced latent period and duration of phase II of the test. These properties were different from non-steroid anti-inflammatory effect of diclofenac sodium, which altered Phase II (inflammatory), which mechanisms were influenced by mediators of inflammatory response, mainly prostaglandins, leukotrienes, etc.

Another important aspect of analgesic action of biologically active compounds is their effect on the neuropathic pain model. According to the results of the study, on Day 14 after nerve ligation, animals developed a chronic pain syndrome manifested in behavioral reactions of rats and

**Table 2**

**Analgesic activity of PC-66 compared to ketorolac under conditions of intraperitoneal administration in rat neuropathic pain model (M ± m, n = 7)**

| Groups | PT, g/mm² | | | | |
|---|---|---|---|---|---|
| | Intact limb | Subject limb | Changes in pain perception threshold* | In 2 hours after compound administration | Changes in pain perception threshold** |
| Control | 406.4±19.9 | 390.7±22.4 | -3.86% | 392.1±20.5 | +0.35% |
| PC-66 1 mg/kg | 380.7±20.4 | 298.6±19.7# | -21.5% | 437.9±29.4& | +46.7% |
| Ketorolac 2.4 mg/kg | 407.1±5.76 | 309.3±17.4# | -24.0% | 470.0±24.1& | +51.9% |

*Notes:* 1. Control – sham-operated animals that received equivolumic quantities of solvents; 2. * – compared to intact limb; 3. ** – compared to subject limb before compound administration; 4. # – statistically significant differences ($p<0.05$) compared to intact limb; 5. &- statistically significant differences ($p<0.05$) compared to subject limb before administration of compounds.

decrease of pain threshold in subject limbs by 21-24%, in average, compared to intact limbs (tab. 2). Under these conditions, PC-66 demonstrated expressed analgesic effect, as evidenced by increase of PT in subject limbs 2 hours after intraperitoneal administration by average 46.7%, compared with the index before administration of the substance. For this activity, PC-66 was slightly inferior to ketorolac, which presented PT growth by 51.9% ($p <0.05$).

## Conclusions

Thus, we have received information that confirmed pretty high analgesic effect of new 3-(2-$R^1$-6-$R^2$-4-oxyquinoline-3(4*H*)-yl)alkyl-(alkaryl-, aryl-) derivative of carboxylic acid (PC-66 compound) at both somatic and neuropathic pain models. The antinociceptive efficacy of this compound was found similar to ketorolac and diclofenac ones, and even surpassed them in the activity. It is worthy to note that effect of the compound was the most evident in Phase I of the formalin test, while its influence on the second (inflammatory) phase was negligible. Having compared the actual findings with those from the previous studies, according to which the compound did not present any damaging effect on the stomach both in subchronic and chronic administration [7, 8], one may think of the lack of mechanisms of any significant effect on prostaglandin system and high probability of involvement of other antinociceptive mechanisms, which requires further research of the issue. Conducting in-depth studies of mechanisms of analgesic action of 4-oxo (amino-) quinazoline derivative (PC-66 compound) and its other effects may help provide possible indications for its use as a medicine with distinctive analgesic effect.

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