



# Creation of New Analogues of Natural Phytohormones for Increasing the Yield of Agricultural Crops

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## Abstract

Synthesis of new potential plant growth regulators (analogues of natural phytohormones) is accomplished by mixing an equimolar mixture of aryloxypropin and 1-methylpiperidin-4-one under the conditions of Favorsky's reactions. New derivatives of acetylene aminoalcohols - the ZhOT series - have been obtained. The structure of the synthesized compounds was established by IR and NMR 1H spectroscopy. Screening of their water-soluble forms, on wheat and barley seeds, two preparations (ZhOT-4 and ZhOT-7) were selected, which exceed both the control and standards of known phytohormones, such as heteroauxin (indolyl-3-acetic acid), 6-BAP (6-benzylaminopurine) for further testing. Conducted in-depth laboratory and demonstration field tests on cereals (wheat and barley) showed high efficiency of ZhOT-4 and ZhOT-7, the indicators of which exceeded the well-known reference preparation - Agrostimulin (Ukraine). The growth regulating activity of synthesized compounds was determined. Processing of wheat seeds ZhOT-4 and ZhOT-7 increased the field germination of seeds, plant height, leaf area, the mass of the aerial part and the total area. The conducted studies showed that the biological activity of the tested compounds largely depends on the concentration and the best growth regulator is ZhOT-4. The activity of the synthesized growth regulatory compounds has been determined. Treatment of seeds of with ZhOT-4 and ZhOT-7 increased seed germination, height of plants, leave areas, weight of an elevated part and the total area, weight of an elevated part and the total area. Studies have shown that the biological activity of tested compounds is largely dependent on the concentration and the best growth regulator is ZhOT-4. Thus, the advanced laboratory and demonstration (small-scale) comparative tests of ZhOT-4 and ZhOT-7 and the well-known reference preparation "Agrostimulin" (Ukraine) for cereals (wheat and barley) showed high efficiency of new synthesized preparations. The use of ZhOT preparations increases the germination and germination energy, the number of spikelets, the mass of grains, the bushiness and the yield of wheat and barley, leads to the accumulation of dry mass in both the ground and underground parts of plants. The advantages of ZhOT-4 and ZhOT-7 are: high efficiency, a wide range of cultures, good solubility in water, low application dose of 0.0001% by active ingredient (1g per 1t of water) or 13-50 mg per hectare, comparable to natural phytohormones, long shelf life, safety, high yield of target products, sequential 3-stage reaction in one reactor.

**Keywords:** agriculture, analogs of phytohormones, regulators of plants growth.

## 1. Introduction

Despite the fact that Kazakhstan is historically an agrarian country, the problems connected with the increase in the productivity and quality of agricultural products, including cereals, the increase in resistance to unfavorable climatic conditions, weeds, various diseases and insects remain unresolved. As intensification of cultivation of grain crops, it becomes necessary to use large amounts of protective measures, which leads to a significant increase in the cost of production. In a plant organism built of a large number of different cells, tissues and organs, in which a variety of biochemical processes occur, a complex system is functioning that ensures the coordination of the functioning of its individual parts, as well as regulation at the level of the whole plant. From the modern science point of view, such regulation is carried out by the system of hormonal regulation with the help of endogenous chemical compounds called phytohormones or phyto regulators. Natural phytohormones have not received wide practical application because of their low content in plants, the complexity and high cost of their isolation from producer organisms, and the

multidirectional effect of light metabolic deactivation by plant enzymes. Application of PGR was possible only after the creation of synthetic analogues of phytohormones, more stable in the plant body, because there are no enzymes that cause their destruction. Therefore, the creation of new high-efficient and low-cost synthetic analogues of natural phytohormones (PGR) with complex properties (regulating, anti-stress, immunostimulating, etc.) is of particular urgency. The need for highly effective phyto regulators is increasing every day [1, 2]. In urgent need of preparations for various purposes for crop production Kazakhstan currently does not produce them, but imports them. PGR are imported from 14 countries: Russian Federation, Ukraine, Germany, Switzerland, etc. [3]. In Kazakhstan, only 14 PGRs have been added to the list of approved pesticides for agriculture. Of these, only 3 are domestic. But they also passed only the registration stage and were not brought to wide use (due to the complexity of obtaining), they have limited permission to use (only for vegetable crops) [4]. In addition, despite the fact that Kazakhstan is an important grain exporter in the international market and cereal crops occupy over 80% (16.5 million hectares out of 21.5 million hectares) of the crop area, there are not any



allowed to use domestic PGR for cereals. Therefore, the development of economically viable, low-stage, highly effective methods for the synthesis of domestic preparations for crop production, including PGR, increasing productivity and improving the quality of crop production is relevant.

## 2. Synthesis of High Effective Plant Growth Regulators

By the methods of fine organic and combinatorial synthesis, the modification of the aromatic radical (naphthyl, phenyl, n-chlorophenyl-) and the tertiary nitrogen atom of the piperidine ring of the agent (hydrochloric acid, methyl iodide, succinic acid) synthesized derivatives of acetylene aminoalcohols (Fig. The synthesis consisted of three stages: 1 - synthesis of propargyl esters by Williamson reaction, 2 - synthesis of tertiary acetylenic alcohols according to Favorsky reaction and 3 - quaternization of amino alcohols with the aim of obtaining water-soluble salts. Combinatorial synthesis of the compounds was carried out in order to study the effect of the replacement of the radicals: phenyl on naphthyl, phenyl on chlorophenyl, phenyloxy on phenylamino, and also on salt-forming radicals (hydrochloride-iodomethylate-succinic acid).

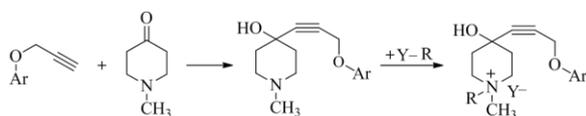


Fig.1 Synthesis of aryloxypropargylpiperidols

The conditions of all stages of synthesis have been optimized and it has been established that the propargylation of phenols and naphthols with propargyl bromide is more effective at an equimolar ratio of reagents, in acetone in the presence of potash, at a temperature of 60 °C. And propargylation of p-chloroaniline in methanol, with the participation of a condensing agent, potassium acetate, at a temperature of 50°C. Condensation of aryloxy- and arylaminopropargils with 1-methylpiperidin-4-one is more efficient in diethyl ether in the presence of a 3-fold excess of potassium hydroxide at room temperature. Quaternization of acetylenic alcohols with practically quantitative yield proceeds in the medium of absolute alcohol with the addition of ethereal hydrochloric acid or an equimolar amount of methyl iodide or succinic acid at a temperature of 50 °C. Under these conditions, crystalline substances, readily soluble in water, are formed, white, light yellow in color. The structure of the compounds is identified by IR and NMR <sup>1</sup>H and <sup>13</sup>C spectroscopy. To test them, the synthesized piperidols were converted to water-soluble salts and they were assigned codes ZhOT-1-ZhOT-12, respectively [5-7].

### 1.1 Bioscreening of Compounds on Growth and Development of Wheat Seeds

The effect of the growth regulating activity of synthesized acetylene amino alcohols studied at the Biological Faculty of Al-Farabi KazNU on cultures callus of wheat (*Triticum aestivum* L.), obtained from "Otan". Isolated explants cultured on an Agar medium of Murashige-Skoga (MS) at a temperature of 22-24 °C under dark conditions for a month. The results of the experiments evaluated by the gain of the callus tissue, which calculated by the formula:  $Kr = (\text{final weight} - \text{initial weight}) / \text{initial weight}$ .

The hormone-like effect of synthesized compounds confirmed by its inducing effect on the process of rhizogenesis (root formation) in the callus tissue of wheat. Adding ZhOT-1 in the concentration of 0.1-1 mg·L<sup>-1</sup> stimulates root formation in callus tissue up to 80%, whereas in control (MS medium without hormones) rhizogenesis did not exceed 60%. Thus, the conducted studies revealed the stimulating effect of synthesized acetylenic amino alcohols

under *in vitro* conditions, which manifested itself in the accumulation of callus biomass of wheat, as well as in the induction of rhizogenesis in callus tissue of wheat. The work was carried out in accordance with generally accepted methods. Tap water was used as a control. Phytohormones - indolyl-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) "Sigma". The experiments was carried out as follows: screening of drugs was carried out according to biometric parameters; selection of the most effective synthesized piperidine derivatives possessing growth-regulatory activity was conducted by growing wheat plants of different varieties in four concentrations of the studied compounds (0.01%; 0.001%; 0.0001% and 0.00001%). The control experiment was tap water, phytohormones - indolyl-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) "Sigma". Comparison of biometric parameters of wheat plants of different varieties and barley showed that in the case of preliminary soaking of plant seeds in solutions of derivatives, amino alcohols have higher values than those grown directly in a solution of the same concentration. The maximum growth of wheat plants of different varieties observed at the concentration of 0.0001%. Further decrease in concentration leads to the decrease in the length of the stem of the plants. The use of higher concentrations of synthesized compounds (0.01% and 0.001%) inhibits the growth of wheat plants. The study of their influence on the growth of wheat of different varieties showed that many of them have a growth regulating activity. On the wheat seeds, three compounds were selected (activity above the standards of 30-38%) for further in-depth tests to increase yield, drought resistance, accelerate the growth and development of wheat. Comparison of the treated compounds and the known registered reference preparation "Agrostimulin" shows that these compounds increase the biometric characteristics of the ground part of plants and have a strong root-forming ability. The most effective preparations are ZhOT-4 and ZhOT-7, biometric indicators of which exceed both the control and the standard.

### 1.2 Field Demonstration Tests on Growth of Wheat and Barley

Place of testing: "Experimental base of Kazakh National Research Institute of agriculture and plant growing" (KazNRI), Almaty region, Kazakhstan. Culture, variety: spring wheat «Kazakhstan 10», barley «Baishoshek». Soil: dark chestnut, medium loamy, humus 3.0 - 3.5%, pH 7.0. Plowing of the soil surface - by 20-22 cm, pre-sowing cultivation, packing after sowing. Sowing is manual, the seeding rate is 3.5 million pieces per ha, depth is 5-6 cm. Variants of the experiment: preparations - 0.0001% solutions of ZhOT-4 and ZhOT-7; the standard - "Agrostimulin"; control - untreated seeds. Type of experience, area of experimental plots, repeatability: field plot, plot size - 2.0 m<sup>2</sup>, repetition - four-fold. Terms and methods of application of preparations: seed dressing before sowing. Field experiments have confirmed that all synthesized compounds in the optimal concentration have a growth stimulating effect. Moreover, the combined use of synthesized PGR with disinfectants proved to be more effective (Table 1).

Table 1: Effect of production on the field germination of wheat seeds of the grade "Kazakhstan 10"

Variant	Field germination, pcs·m <sup>-2</sup>				
	I	II	III	IV	Density of shoots, pcs·m <sup>-2</sup>
ZhOT-4	69	105	98	97	396
ZhOT-7	99	100	95	96	388
Agrostimulin	94	93	98	86	371
Control	88	84	81	96	349

The tested synthesized derivatives of amino alcohols contribute to an increase in the yield of wheat, barley and their biometric indicators above the control and the standard - Agrostimulin. The highest rates were observed in ZhOT-4 and ZhOT-7. Thus, the advanced laboratory and demonstration (small-scale) comparative tests of ZhOT-4 and ZhOT-7 and the well-known reference preparation "Agrostimulin" (Ukraine) for cereals (wheat and barley)

showed high efficiency of new synthesized preparations, which exceeded the indicators of "Agrostimulin". The use of ZhOT preparations increases the germination and germination energy, the number of spikelets, the mass of grains, the bushiness and the yield of wheat and barley, leads to the accumulation of dry mass in both the ground and underground parts of plants. The advantages of ZhOT-4 and ZhOT-7 are: high efficiency, a wide range of cultures, good solubility in water, low application dose of 0.0001% by active ingredient (1g per 1t of water) or 13-50 mg per hectare, comparable to natural phytohormones, long shelf life, safety, high yield of target products, sequential 3-stage reaction in one reactor.

### 3. Conclusion

New derivatives of acetylene amine alcohols have been obtained - the ZHOT series. The structure of the synthesized compounds was determined by IR spectroscopy and NMR spectroscopy. Screening of their water-soluble forms for seeds of wheat and barley with two preparations (ЖОТ-4 and ЖОТ-7), which exceed both the control and standards of known phytohormones such as heteroauxin (indolyl-3-acetic acid), 6-BAP (6-benzylaminopurine) for further testing. Conducted in-depth laboratory and demonstration field tests for cereals (wheat and barley) showed high efficiency of ZhOT-4 and ZhOOT-7, the indicators of which exceeded the well-known reference preparation - Agrostimulin (Ukraine). The regulatory activity of growth of synthesized compounds was determined. Processing of wheat seeds ZhOT-4 and ZhOT-7 increased field germination, plant height, leaf area, air mass and total area.

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