# Synthesis of tropyrated azomethines exhibiting biological activity

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**Abstract.** The article presents the results of the synthesis of tropylated azomethines without the use of toxic solvents. The proposed synthesis methods make it possible to speed up the process of obtaining substances, as well as bypass the process of their purification. Biological activity has been proven in laboratory model experiments on wheat and pea seeds. A decrease in infection of wheat seeds by 1.2-1.6 times with the fungus *Mucor sp.* was established. Tropylated azomethine exhibits a growth-stimulating effect on peas. Soaking pea seeds for 1 hour in an azomethine solution with a concentration of $1\times10^{-2}$% improves plant development in the initial period of growth and development. There was an increase in plant height relative to the control by 3.4%, aboveground mass by 41.4%, soluble protein content in aboveground mass by 16.8%, in roots by 22.7%, peroxidase activity in sprouts by 117.6%, in the roots by 551.1%.

## 1 Introduction

Obtaining stable crop yields in changing soil and climatic conditions is the main task of food security for both individual regions and the country as a whole. The shortage of yields is associated with many factors, one of which is the low quality of seed material, namely its high contamination. As of April 5, 2020, specialists from the Chuvash branch of the FSBI "Rosselkhoztsentr" analyzed 31.61 thousand tons of spring grain and leguminous crops. According to the results of phytoexpertise, all batches of seeds are infected with various pathogens. The overall percentage of seed contamination ranged from 21 to 100% [1]. As of November 9, 2023, the Perm branch of the FSBI "Rosselkhoztsentr" inspected more than 29 thousand tons of seeds of spring grain and leguminous crops. 14.065 thousand tons of tested seeds comply with regulatory documentation for seeds. The percentage of quality standardized seeds was 63.9% of the tested seeds. Does not meet the requirements of regulatory documentation - 10.756 thousand tons of seeds (36.1% of tested seeds) [2]. The data presented in only two regions of the country indicate the relevance of the identified problem. Every year, 1-2% of the total cost of growing crops is spent on pre-sowing seed treatment. Epiphytotic development of root and radical rots causes yield losses of grain crops in the Perm region from 15% to 40% [3]. The use of plant growth regulators makes it possible to create an ecological system for protecting plants from pathogens. These drugs stimulate the immune system and nonspecific resistance of plants to various diseases, so the search for

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new compounds with antifungal properties is relevant. There are natural compounds that inhibit the activity of fungal plant pathogens, these include compounds containing the tropylium cycle, such as: thuic acid, tropolone, thujaplicines [4]. Interesting in this area of agriculture are tropylated amines and azomethines, containing the biologically active tropylium cycle, which exhibit growth activity on agricultural plants [5-7].

The purpose of the study is the synthesis of tropylated azomethines that exhibit biological activity in a simple accessible way without the use of toxic solvents.

## 2 Materials and methods

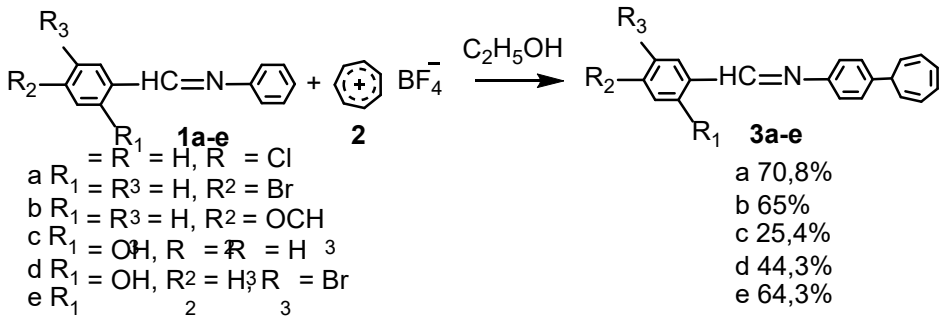
The presented work is a continuation of studies [6, 8], where the possibility of replacing the more toxic solvent tetrahydrofuran with the less toxic ethanol was shown. During the research, the following well-known reactions were studied: 1) tropylation of azomethines with tropylium tetrafluoroborate [9]; 2) multicomponent one-pot synthesis (One-pot synthesis) [10], but instead of the more toxic tetrahydrofuran, ethanol was used as a solvent. The 1H NMR spectrum was recorded on a Mercury 300 (300 MHz) instrument (Varian, USA), internal standard HMDS. Chromatomass spectrum was obtained using an Agilent Technologies 6890N/5975B sampler, HP-5ms column (30×0.25mm,0.25mkm, carrier gas – helium, electron impact ionization 70 eV, column thermostat temperature 100 °C. Evaporator temperature – 280 °C).

To establish the antifungal effect of the obtained substances, a model laboratory experiment was carried out in rolls of filter paper according to the method [11]. The object was the seeds of spring wheat (*Triticum aestivum* L.) of the Irgina variety. The seeds were placed in Petri dishes, where they were soaked in 0.01% aqueous-alcohol suspensions of the test compounds for 24 hours. A water-alcohol solution served as the control.

To establish the growth-stimulating effect and the optimal concentration of azomethine for seed treatment, a model laboratory experiment was carried out according to the following scheme: 1. Control (distilled water); 2. $1\times10^{-5}$% aqueous solution of the substance; 3. $1\times10^{-4}$% aqueous solution of the substance; 4. $1\times10^{-3}$% aqueous solution of the substance; 5. $1\times10^{-2}$% aqueous solution of the substance. The variants were repeated six times in the experiment. Plants were grown in 150 $ml^3$ plastic containers containing 120 g of air-dried soil. The crop under study was peas of the Agrointel Substrate variety, on which peas and calcined sand were grown. Before sowing, pea seeds were soaked for 1 hour in the test substance according to the experimental design. 5 pea plants were planted in a vessel. Plants were grown in vessels for 14 days at a temperature of 20-25 °C, watering was carried out to a humidity of 60% full moisture capacity. Harvesting was carried out using the direct method simultaneously from all vessels. Simultaneously with the harvesting of the experiment, the biometric parameters of pea seedlings were analyzed in each repetition: the height of the sprouts, the weight of the sprouts and roots, as well as the amount of dry matter in them [12]. The following parameters were measured in plant samples of roots and sprouts: peroxidase activity [13], soluble protein content [14]. Mathematical processing of the research results was carried out using the method of analysis of variance.

## 3 Results and discussion

It was found that when azomethines 1a-e react with tropylium tetrafluoroborate 2 in a 1:1 ratio of the starting reagents, tropylated azomethines **3a-e** are not formed. The target products were isolated only when the proportion of azomethine was doubled - using the starting reagents 2:1 (Scheme 1).



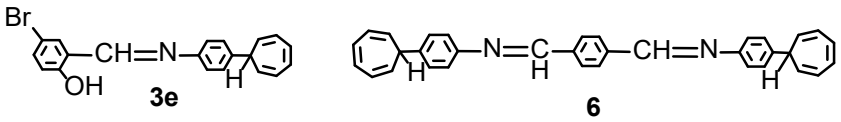

**Scheme 1**. Tropylation of azomethines with tropylium tetrafluoroborate

However, unlike works [9, 10], where the reaction time should be 3 hours, reaction products **3a-e** are already formed after 5-7 minutes. The formation of reaction products is observed by the precipitation of crystals in the reaction mass. The convenience of the developed method is that it is possible to significantly reduce the reaction time, simplify the procedure for isolating the target products (there is no need to fill the reaction mass with water, neutralize it to pH = 7 and allow time for the "ripening" of the reaction product, which can range from 2 to 24 hours) and obtain pure compounds without additional purification methods, it is enough to filter and recrystallize the target products. The yield of compounds **3a–e** depends on the substituent in the aldehyde moiety of azomethines. Thus, when using electron-withdrawing substituents (Cl, Br), the yield of tropylated azomethines **3a** and **3b** increases significantly and amounts to 70.8% and 65%, respectively. When using electron-donating substituents (OH and $OCH_3$), the yield of the target products decreases and amounts to 44.3% and 25.4%, respectively. The yield of compound **3e** containing both electron-donating and electron-withdrawing substituents was 64.3%.

General procedure for preparing compounds **3a-e**: 4 ml of ethanol was added to azomethine **1a-e**, then tropylium tetrafluoroborate (azomethine: tropylium totrafluoroborate in a molar ratio of 1:1), stirred for 30 minutes, the resulting crystals were filtered and recrystallized from hexane. Physical constants and spectra of compounds **3a-d** (**3a**. N-4-chlorophenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline; **3b**. N-4-bromophenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline; **3c**. N-4-methoxyphenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline; **3d**. N-2-hydroxyphenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline correspond to literature data [9, 10].

**N-5-bromo-2-hydroxyphenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline 3e**. Yellow crystals with a melting point of 138-139 °C. 1H NMR spectrum ($CDCl_3$), δ, ppm (*J*,Hz): 2.78 (1H, t, *J*=5.4, $C^7H$ in $C_7H_7$); 5.39-5.44 (2H, d.d., $J_{1,2}$=5.4, $J_{2,3}$=5.7, $C^{1,6}H$ in $C_7H_7$); 6.26-6.30 (2H, m, $C^{2,5}H$ in $C_7H_7$); 6.75 (2H, t, $J_{1,2}$=3.3, $J_{2,3}$=2.7 $C^{3,4}H$ in $C_7H_7$); 6.93 (1H, d, *J*=8.7, meta-$C_6H_3$-CH); 7.25-7.31 (2H, m, *ortho*-$C_6H_4$-N); 7.42-7.46 (3H, m, *meta*-$C_6H_4$-N + *meta*-$C_6H_3$-CH); 7.50-7.52 (1H, m, *para*-$C_6H_3$-CH); 8.58 (1H, s, CH=N). 13.36 (1H, br. s. OH). Mass spectrum, m/z (Irel.%): 366 [M]+(73), 366 (100), 364 (57), 348 (8), 274 (4), 194 (6), 181 (8 ), 167 (99), 166 (28), 165 (61).

Fungicidal activity was studied on wheat seeds of the Irgina variety for azomethines **3e** and **6**. The choice is justified by the fact that compound **3e** contains bromine (it is known that fungicidal agents often contain halogens), and substance **6** contains two biologically active tropylium cycles (Fig. 1). The research results are presented in the table below.




**3e**. N-5-bromo-2-hydroxyphenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl) aniline; **6**. 4*n*-phthalidene-bis-[4-(7-cyclohepta-1,3,5-trienylphenylimine)]

**Fig. 1.** Formulas of the studied compounds

The biological effectiveness of new substances was tested using bio- and phytotesting methods. During the experiment, it was revealed that the dominant fungal infection on wheat seeds is the fungus *Mucor sp*. Studies have shown that compounds **3e** and **6** at a concentration of 0.01% slightly inhibit the activity of the fungus *Mucor sp*. The degree of infection of wheat seeds with fungal infection for compound **3e** was 20%, and for compound **6** – 15%. The degree of contamination when using synthesized substances decreases by 5-10% compared to the control.

When treating pea seeds with azomethine, it was found that the height of plants was positively affected by a concentration of $1\times10^{-2}$%, the elongation of the sprout compared to the control was 0.6 cm (Table 1).

**Table 1.** Effect of tropylated azomethine on seedling peas

| Indicators | Control | $1\times10^{-5}$ % | | $1\times10^{-4}$ % | | $1\times10^{-3}$ % | | $1\times10^{-2}$ % | |
|---|---|---|---|---|---|---|---|---|---|
| | $X\pm m_x$ | $X\pm m_x$ | ±, % | $X\pm m_x$ | ±, % | $X\pm m_x$ | ±, % | $X\pm m_x$ | ±, % |
| Plant height, cm | 17.5±0.2 | 16.6±0.2 | -5.1 | 16.9±0.2 | -3.4 | 17.3±0.3 | -1.1 | 18.1±0.4 | 3.4 |
| Mass of sprout, g | 0.29±0.02 | 0.26±0.01 | -11.5 | 0.28±0.00 | -3.4 | 0.28±0.02 | -3.4 | 0.41±0.02 | 41.4 |
| Mass of radicle, g | 0.83±0.01 | 0.83±0.01 | 0.0 | 0.73±0.01 | -12.0 | 0.52±0.03 | -37.3 | 0.39±0.01 | -53.0 |

Note: $SSD_{05}$ for plant height pea = 0,3 cm, for the mass of sprouts and roots – 0,02 g

Other concentrations of the substance studied had a negative effect. The reduction in sprout length at a concentration of $1\times10^{-3}$% has not been mathematically proven. The studied substance had an ambiguous effect on the biomass of pea plants (Table 1). A positive mathematically proven effect on the weight of pea sprouts was noted in the variant with an azomethine concentration of $1 \times 10^{-2}$%; it increased compared to the control by 0.12 g. The negative effect of azomethine on this indicator was noted in the variant with a concentration of $1 \times 10^{-5}$ %, the weight decrease was 0.03 g. In variants with concentrations of $1\times10^{-3}$% and $1\times10^{-4}$%, changes in the mass of the sprout were not mathematically proven. A very close direct correlation has been established between the weight of the pea sprout and the concentrations of azomethine used (r = 0.983). Azomethine had a mathematically reliable negative effect on the root mass of peas. The decrease in root mass relative to the control was 0.11-0.44 g.

In addition to the biometric parameters of plants, phytotesting includes observations of changes occurring inside plants [15, 16].

When treating pea seeds, azomethine did not have a mathematically significant effect on the accumulation of dry matter in the above-ground mass, however, there was a tendency for this indicator to increase relative to the control at a concentration of $1\times10^{-5}$ and $1\times10^{-4}$% (Table 2).

**Table 2.** Effect of tropylated azomethine on biochemical parameters in the seedlings peas

| Indicators | Control | $1\times10^{-5}$ % | | $1\times10^{-4}$ % | | $1\times10^{-3}$ % | | $1\times10^{-2}$ % | |
|---|---|---|---|---|---|---|---|---|---|
| | $X\pm m_x$ | $X\pm m_x$ | ±, % | $X\pm m_x$ | ±, % | $X\pm m_x$ | ±, % | $X\pm m_x$ | ±, % |
| Dry matter in the sprout, % | 11.6±0.6 | 12.1±0.7 | 4.3 | 13.6±1.2 | 14.7 | 10.5±1.0 | -9.5 | 11.5±0.6 | -0.9 |
| Dry matter in the radicle, % | 59.1±0.6 | 65.4±1.9 | 10.7 | 56.1±0.8 | -5.1 | 43.7±0.6 | -26.1 | 51.9±0.6 | -12.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Soluble protein content in the sprouts, % | 4.28±0.08 | 4.03±0.05 | -5.8 | 4.03±0.06 | -5.8 | 4.09±0.11 | -4.4 | 5.00±0.11 | 16.8 |
| Soluble protein content in the radicle, % | 5.38±0.05 | 5.59±0.03 | 3.9 | 6.46±0.17 | 20.1 | 6.87±0.03 | 27.7 | 6.60±0.16 | 22.7 |
| Peroxidase activity in the sprouts, $\frac{\text{optical density units}}{\text{g×min}}$ | 10.2±2.2 | 4.1±0.3 | -59.8 | 1.8±0.2 | -82.4 | 7.8±0.4 | -23.5 | 22.2±1.2 | 117.6 |
| Peroxidase activity in the sprouts, $\frac{\text{optical density units}}{\text{g×min}}$ | 3.6±0.0 | 0.8±0.3 | -77.8 | 10.5±2.6 | 191.7 | 22.6±4.1 | 527.8 | 22.0±1.0 | 511.1 |

Note: $SSD_{05}$ for dry matter in sprout = F act. < Ftabl., for dry matter in radicle – 1,5 %, for soluble protein content in the sprout – 0,11 %, for soluble protein content in the radicle – 0,14 %, for peroxidase activity in the sprouts – 1,6 $\frac{\text{optical density units}}{\text{g×min}}$, for peroxidase activity in the radicle – 3,1 $\frac{\text{optical density units}}{\text{g×min}}$.

In the variant with an azomethine concentration of $1\times10^{-5}$%, a positive effect of the compound on the accumulation of dry matter in the roots was noted; the increase relative to the control was 6.3%. In the remaining experimental variants studied, azomethine had a negative effect on this indicator; the decrease relative to the control was 3.0-15.4%. Treatment of seeds with azomethine led to a decrease in the content of soluble protein in pea sprouts at all concentrations; the decrease relative to the control was 0.19-0.25%. In the variant with the maximum concentration of azomethine, an increase in soluble protein was noted relative to the control by 0.72%. A very close direct correlation has been established between the concentrations of azomethine and soluble protein in pea sprouts (r = 0.961). The use of azomethine led to the accumulation of soluble protein in the roots, the increase relative to the control was 0.22-1.49%. The results of studies of peroxidase activity in pea sprouts showed that the test substance had a negative effect, in all experimental variants except for the maximum concentration, the decrease relative to the control was 2.36-8.32$\frac{\text{optical density units}}{\text{g×min}}$. A very close direct correlation has been established between azomethine concentrations and peroxidase activity (r = 0.920). In the roots, peroxidase activity increased when pea seeds were treated with concentrations of $1\times10^{-4}$%, $1\times10^{-3}$ and $1\times10^{-2}$%, the increase was 6.9, 19.0 and 18.4 $\frac{\text{optical density units}}{\text{g×min}}$ respectively.

## 4 Conclusion

Thus, based on the study, the following conclusions can be drawn. Synthesis of tropylated azomethines exhibiting biological activity without the use of toxic solvents, i.e. replacing them with ethanol speeds up the process from 3 hours to 5-7 minutes. It also allows you to obtain pure compounds without additional purification methods. The synthesized substances have an antifungal effect on wheat seeds infected with the fungus *Mucor sp*. Treatment of seeds with tropylated azomethine had an ambiguous effect on pea seedlings. An azomethine concentration of $1\times10^{-2}$% had a positive effect on the accumulation of above-ground mass, soluble protein content and peroxidase activity in plant sprouts and roots. Treatment of seeds with the test substance at a concentration of $1\times10^{-5}$% led to a decrease in the above-ground

mass of peas, the content of soluble protein in sprouts and peroxidase activity in sprouts and roots of plants.

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