

Research Article

## Box-Wilson Optimization of N-(5-nitro-1H-benzimidazol-2-yl)propanamide Synthesis and Its Agrochemical Activity

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### ABSTRACT

Benzimidazole derivatives are considered to be compounds with potential biological activity. It is important to synthesize their derivatives with high yields, select biologically active compounds from the synthesized compounds, and optimize the synthesis process of the selected compounds. The aim of this study was to obtain a new benzimidazole derivative, N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide, as a promising biologically active compound, by nitration of N-(1H-benzo[d]imidazol-2-yl)propionamide and to optimize the synthesis conditions. To increase the yield of the target product, the reaction conditions were optimized using a mathematical design of experiments approach (Box-Wilson method). The main technological parameters such as optimal temperature (0°C), reaction time (5 hours), molar ratio of reagents (1:6:9), and yield (85.3%) were determined; a regression model was constructed and optimal synthesis conditions were created. The structure of the synthesized compound was characterized by <sup>1</sup>H, <sup>13</sup>C NMR, IR spectral data. In addition, N-(5-nitro-1H-benzo[d]imidazol-2-yl) propionamide showed significant activity against the phytopathogenic fungi *Fusarium oxysporum* (33.3%) and *Aspergillus niger* (26.6%). It showed 100% inhibition of herbicidal activity in wheat and cucumber seedlings. In this study, for the first time, optimized conditions were identified for the synthesis of N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide in high yield. The obtained data demonstrate significant biological activity of the compound and highlight its potential as a promising lead for the development of new agrochemicals.

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## 1. INTRODUCTION

Benzimidazole-based heterocyclic compounds are extensively employed in both agriculture and medicine owing to their significant biological activities. Consequently, efficient production technologies for numerous benzimidazole-containing compounds have been developed and successfully commercialised (Chung et al., 2023). Due to their wide spectrum of biological activity, nitrogen-containing heterocyclic compounds occupy a leading position in production volume among other organic compounds. In this regard, derivatives of 2-aminobenzimidazole containing acetyl and nitro groups occupy a special place (Saidov, 2021). Considering this necessity, scientific studies aimed at obtaining new products based on these compounds, identifying biologically active substances among the synthesized compounds, and developing industrial methods for their production have acquired particular importance. The goal is to obtain compounds with high physiological activity and practical significance in large quantities, as well as to improve existing technologies (Bunyatyan et al., 2020). In this direction, it is important to synthesize new products based on 2-aminobenzimidazole, determine the biological activity of the newly obtained substances, develop pharmaceutical preparations based on them for use in veterinary medicine and agriculture, and design and implement their industrial production technologies (Tishkov et al., 2023).

Numerous studies have established that nearly 30% of crop losses are associated with weed infestation. In many developed countries, including the Republic of Uzbekistan, the use of herbicides is widespread due to their high efficiency and economic benefits and has practically replaced mechanical weed control methods with herbicides. Effective chemical methods for weed control reduce labor costs and lower the production cost of agricultural crops. Currently, herbicides containing nitro groups, such as oryzalin (Gupta et al., 2025), acclonifen (Caraba et al., 2023), and butralin (Kou et al., 2024), are used in agriculture to control weeds. Nitro compounds are one of the main classes of heterocyclic organic compounds (Abdurazakov et al., 2021; Vadim et al., 2021), which are usually obtained by nitration reactions that are equally effective in aliphatic and aromatic series (Šarlauskas et al., 2023; Yang et al., 2022). In particular, the nitro group is an important synthon from the point of view of organic chemistry, as the reduction of the nitro group leads to the formation of amino compounds. The amino group is chemically active and allows the introduction of various pharmacophoric groups into the molecule. N-acyl derivatives were synthesized by acylation of 2-aminobenzimidazoles in the presence of carboxylic acids (Kubayev et al., 2025). According to the literature, 2-acetyl- and (aroyl)-aminobenzimidazoles are obtained by acylation of 2-aminobenzimidazole with acid anhydrides or acid chlorides (Jiyaul et al., 2023; Abouelhaoul et al., 2023).

In this study, based on the above data, the main factors affecting the course of the reaction in the synthesis of N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide with herbicidal activity are studied, including the effect of temperature, time duration, and the ratio of substances on the product yield. Based on the results obtained in laboratory conditions, optimal conditions for the synthesis of N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide in large quantities are found and a new one-pot synthesis method is proposed, which allows obtaining it in quantitative yield.

## 2. METHODOLOGY

### 2.1. General Experimental Procedures

Freshly distilled solvents: chloroform, hexane, benzene, ethanol, and methanol were used in this work. IR spectra were recorded on an FT-IR/NIR Spectrum 3 spectrometer (Perkin Elmer, Switzerland) using a frustrated total internal reflection (FTIR) system. NMR spectra were acquired on JNM-ECZ600R (600 MHz for  $^1\text{H}$ , JEOL, Japan) and JNMECZ400R (400 MHz for  $^1\text{H}$ , JEOL, Japan) spectrometers in DMSO- $d_6$ , DMSO- $d_6$ +CCl $_4$  and TFA- $d$ . Tetramethylsilane (TMS,  $\delta$  0.00 ppm) was used as an internal standard for  $^1\text{H}$  NMR shifts, while solvent signals (DMSO- $d_6$ , 39.52 ppm; TFA- $d$ , 116.60 ppm vs. TMS) served as references for  $^{13}\text{C}$  NMR shifts. Thin layer chromatography (TLC) was performed on Silufol L/W 20×20 cm UV-254 plates (Sigma-Aldrich). The eluents used for TLC were as follows: acetone:benzene = 3:2,  $R_f$ =0.48. The melting points of the synthesized compounds were determined using BIOBASE BMP-M70 (China) and MEL-TEMP (USA) instruments.

### 2.2. Synthesis and Characterization

Synthesis procedure of N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide: The experiments were carried out in a three-necked flask equipped with a mechanical stirrer and a reflux condenser. 10

g (0.057 mol) of *N*-(1*H*-benzo[d]imidazol-2-yl)propionamide was dissolved in 20 mL of concentrated H<sub>2</sub>SO<sub>4</sub> at room temperature for 1 h. To the solution, with vigorous stirring, a nitrating mixture (containing 15 mL of HNO<sub>3</sub> and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub>) was added dropwise. The reaction mixture was stirred for an additional 3 h, maintaining the temperature not higher than 50°C, and 10 mL of HNO<sub>3</sub> was added dropwise over the course of 1 h. The reaction mixture was left at room temperature for 8 h. The contents of the flask were poured onto ice, the precipitated crystals were filtered, washed with water, and dried. The crude was recrystallized from ethanol with the addition of activated charcoal. 9.3 g of *N*-(5-nitro-1*H*-benzimidazol-2-yl)propionamide was obtained with a 74% yield. Other experiments were carried out in an analogous manner.

***N*-(5-nitro-1*H*-benzo[d]imidazol-2-yl)propionamide.** C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>O<sub>3</sub>. Melting point: 292-294°C. Molecular weight: 234.211. R<sub>f</sub> = 0.48. IR (ν, cm<sup>-1</sup>): 3451, 1935, 1945, 1689, 1632, 1580, 1516. <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>+CCl<sub>4</sub>, δ, ppm, *J*/Hz): 1.16 (3H, t, *J* = 7.5, CH<sub>3</sub>), 2.51 (2H, q, *J* = 7.5, CH<sub>2</sub>), 6.05 (2H, br.s., 2-NH), 7.56 (1H, d, *J* = 8.8, H-4), 8.01 (1H, dd, *J* = 8.8 and 2.3, H-5), 8.30 (1H, d, *J* = 2.3, H-7). <sup>13</sup>C NMR (150 MHz, DMSO-d<sub>6</sub>+CCl<sub>4</sub>, δ, ppm): 148.4 (C-2), 113.6 (C-4), 117.8 (C-5), 142.7 (C-6), 109.9 (C-7), 138.8 (C-8), 131.4 (C-9), 173.3 (C-11), 29.0 (C-12), 8.6 (C-13).

### 2.3. Mathematical Model

Based on the experimental data, a mathematical model of the process was calculated, which has the following form:  $Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$  where  $b_0, b_1, b_2, b_3$  are regression coefficients of the incomplete quadratic equation.  $Y = 77.46 - 2.48x_1 - 0.66x_2 + 2.91x_3$ . The results of statistical analysis showed that the mathematical model is adequate and that  $b_2$  is the significant coefficient.

### 2.4. Fungicidal Activity

The synthesis of compounds exhibiting phytopathogenic effects against fungi was investigated. The fungicidal activity of the synthesized compounds was determined using the paper disk method (Olimova et al., 2020) against the pathogens *Fusarium oxysporum* and *Aspergillus niger*. The test compounds were dissolved in DMSO and evaluated at different concentrations. In this method, a nutrient medium is first prepared and then poured into Petri dishes. After solidification, the pathogens are inoculated onto the nutrient surface, and subsequently, paper disks impregnated with the test compounds are placed on the infected medium. Measurements were taken after 2–5 days, and the inhibition zones of fungal growth were recorded. An experiment to determine inhibitory activity was conducted on wheat and cucumber seeds. The synthetic compound *N*-(5-nitro-1*H*-benzo[d]imidazol-2-yl)propionamide was tested at concentrations of 0.1%, 0.01%, and 0.001%. Wheat (*Triticum aestivum*, variety 'Thunder', a monocotyledon) and cucumber (*Cucumis sativus*, variety 'Orzu', a dicotyledon) seeds were used as test objects. The seeds were soaked in the test solutions for 16 h (wheat) and 6 h (cucumber). For this experiment, 20 seeds were taken for each variant. The experiment was carried out in quadruplicate. In the control variant, the seeds were soaked in water. Data were analyzed using Student's *t*-test. Differences were considered significant at  $p \leq 0.05$ . The commercial preparation DIRBI EC, registered in the official "Handbook of Pesticides and Agrochemicals" approved for use in the Republic of Uzbekistan, was used as the reference standard.

## 3. RESULTS AND DISCUSSION

### 3.1. Synthesis and Technology of *N*-(5-nitro-1*H*-benzo[d]imidazol-2-yl)propionamide

Initially, methyl (1*H*-benzo[d]imidazol-2-yl)carbamate was reacted with propionic acid to synthesize *N*-(1*H*-benzo[d]imidazol-2-yl)propionamide (Abdurazakov et al., 2021). Subsequent nitration of *N*-(1*H*-benzo[d]imidazol-2-yl)propionamide was performed to introduce a nitro group into the benzene ring. The incorporation of a nitro group, a key pharmacophoric moiety, is known to significantly influence the biological activity of heterocyclic compounds. The overall reaction scheme is presented in Figure 1.

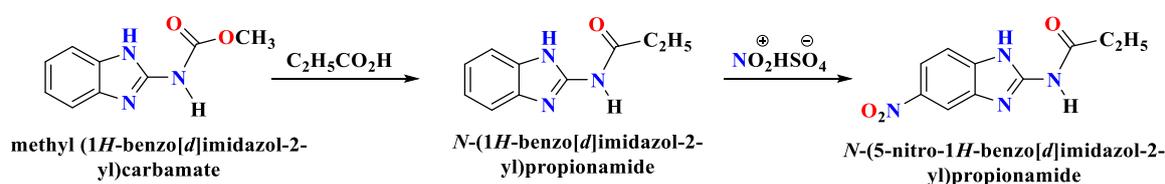


Figure 1. Reaction for the synthesis of *N*-(5-nitro-1*H*-benzo[d]imidazol-2-yl)propionamide

The reaction proceeds through a classical electrophilic aromatic substitution mechanism within the benzene ring. Treatment of *N*-(1H-benzo[d]imidazol-2-yl)propionamide with fuming nitric acid in the presence of concentrated sulfuric acid afforded *N*-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide (Lebedeva et al., 2022). The resulting product is a white to slightly yellowish fine crystalline powder, odorless, sparingly soluble in 96% ethanol, and practically insoluble in water. It has a melting point of 292–294 °C, with an empirical formula of C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>O<sub>3</sub> and a molecular weight of 234.21 g/mol. To evaluate the effects of key reaction parameters, a mathematical model was established using the Box–Wilson method (Takeuchi et al., 2022). This approach enabled determination of optimal conditions for maximizing product yield. The principal factors, along with their levels and variation ranges, are presented in Table 1. The full-factorial experimental design and corresponding results are shown in Table 2. All experiments were performed in duplicate, and the statistical analysis of the matrix data is summarized in Table 3.

**Table 1.** Coded and Natural Levels of the Investigated Factors and Their Variation Ranges

No.	Factor levels	-	0	+
1	X <sub>1</sub>	0	10	20
2	X <sub>2</sub>	3	4	5
3	X <sub>3</sub>	1:4:7	1:5:8	1:6:9

**Table 2.** Planning Matrix of the Full-Factorial Design with Experimental Results

No	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>1,2</sub>	X <sub>2,3</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>mean</sub>
1	+	+	-	+	-	71.4	73.9	71.1
2	-	-	-	+	+	76.3	73.8	75.0
3	+	-	-	-	+	74.2	74.7	74.4
4	-	+	-	-	-	79.5	76.0	77.7
5	+	+	+	+	+	72.5	73.7	73.1
6	-	-	+	+	-	83.2	80.5	81.8
7	+	-	+	-	-	88.8	76.9	81.3
8	-	+	+	-	+	83.0	87.0	85.3

**Table 3.** Statistical Analysis of Experimental Data and Adequacy Assessment of the Regression Model

No	Y <sub>mean</sub>	ΔY	ΔY <sup>2</sup>	S <sup>2</sup>	Y <sub>calc.</sub>	Y <sub>c</sub> -Y <sub>mean</sub>	(Y <sub>c</sub> -Y <sub>mean</sub> ) <sup>2</sup>
1	71.1	-3.10	9.61	19.22	71.41	0.31	0.09
2	75.0	-0.50	0.25	0.50	77.69	2.69	7.23
3	74.4	0.20	0.04	0.08	72.73	-1.67	2.78
4	77.7	-0.70	0.49	0.98	76.37	-1.33	1.76
5	73.1	0.80	0.64	1.28	77.23	24.13	17.05
6	81.8	-1.10	1.21	2.42	83.51	1.71	2.92
7	81.3	-3.80	14.40	28.80	78.55	-2.75	7.56
8	85.3	-3.00	9.00	18.00	82.19	-3.11	9.67

X<sub>1</sub> – process temperature, °C; X<sub>2</sub> – reaction time, h; X<sub>3</sub> – molar ratio of starting materials; X<sub>1,2</sub> – interaction effect of the main factors X<sub>1</sub> and X<sub>2</sub>; X<sub>2,3</sub> – interaction effect of the main factors X<sub>2</sub> and X<sub>3</sub>; Y<sub>1</sub> – optimization parameter: yield of product (first parallel experiment); Y<sub>2</sub> – optimization parameter: yield of product (second parallel experiment); Y<sub>mean</sub> – average optimization parameter (product yield)

The homogeneity of variance was checked using Cochran's criterion:

$$G_{\text{calc.}} = \frac{S_{\text{imax}}^2}{\sum S_i^2} = 0.40$$

The calculated value ( $G_{\text{calc.}} = 0.40$ ) was lower than the tabulated value ( $G_{\text{table}} = 0.6798$ ), indicating that the experimental variances are homogeneous.

$$G_{\text{calc.}} = 0.40 < 0.6798 = G_{\text{table}}$$

The degrees of freedom were calculated using the formula:  $f = N - (K+1) = 8 - (3+1) = 4$

The mean reproducibility variance was calculated as:

$$S^2 \{y_i\} = \frac{\sum_{i=1}^N S_i^2}{N} = 8.92$$

The adequacy variance was calculated as:

$$S_{ad}^2 = \frac{\sum_{i=1}^N (y_c - \bar{y}_{mean})^2}{f} = 24.53$$

The adequacy of the developed mathematical model was verified using Fisher's criterion.

$$F_{\text{calc.}} = \frac{S_{ad}^2}{S_{\{y\}}^2} = 2.75$$

Since the calculated value ( $F_{\text{calc}} = 2.75$ ) is lower than the tabulated value ( $F_{\text{table}} = 3.8$ ), the regression model can be considered adequate within the chosen confidence level.

$$F_{\text{calc.}} = 2.75 < 3.8 = F_{\text{table}}$$

The model adequately estimated the confidence interval using the formula. First, the variance of the regression coefficients was calculated using the following expression:

$$S_{\{b_i\}}^2 = \frac{S_{\{y\}}^2}{N} = 1.12$$

The standard error of the regression coefficients was then obtained as the square root of this value:

$$S_{\{b_i\}} = \sqrt{1.12} = 1.06$$

For the selected confidence level, the tabulated Student's coefficient was  $t_{\text{table}} = 2.306$ . The confidence interval of the regression coefficients was therefore calculated as:

$$\Delta b_i = t \cdot S_{\{b_i\}} = 2.306 \times 1.06 = 2.44$$

It is worth noting that the factors affecting the reaction were calculated based on the coefficient of the regression equation. According to the results of the analysis, it was found that the factor with the greatest impact on the optimization parameter is the reaction time ( $X_1 - 2.48$ ). The remaining factors have a lesser impact, and their level of influence is arranged in the following order:  $X_1 > X_2 > X_3$ . From the regression coefficients of the equation after calculating the confidence interval ( $b_i = 2.05$ ), it was established that the main factor affecting the process is the reaction time. Statistical analysis ( $F_{\text{exp.}} = 1.2 < F_{\text{table}} = 3.8$ ) showed that the mathematical model is adequate.

### 3.2. Biological Studies

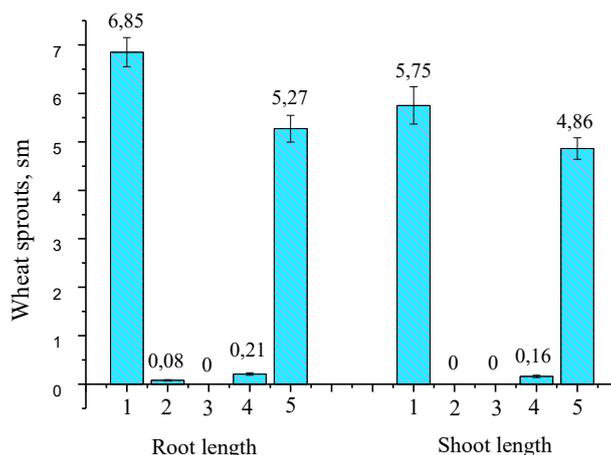
The compound *N*-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide showed inhibitory activity at 0.1%, comparable to the reference standard (Table 4). At this concentration, it completely inhibited the germination of both wheat and cucumber seeds. Inhibitory effects were also observed at lower concentrations, although less pronounced. The reference fungicide Tebuconazole exhibited strong activity at all tested concentrations. At 1%, it produced a 15 mm inhibition zone against *Fusarium oxysporum*, corresponding to 100% inhibition. At 0.1% and 0.01%, inhibition remained high at 93.3% and 80%, respectively. Similar results were obtained against *Aspergillus niger*, confirming its high efficacy even at lower concentrations. In comparison, the test compound demonstrated moderate antifungal activity. At 1%, inhibition zones of 5 mm (*F. oxysporum*) and 4 mm (*A. niger*) were recorded, corresponding to 33.3% and 26.6% inhibition relative to the standard. At 0.01%, inhibition decreased to 23.3%, indicating a concentration-dependent effect.

**Table 4.** Fungicidal activity of *N*-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide

Experimental variant	Concentration %	Zone of pathogen growth inhibition				Standard deviation (±)
		<i>Fusarium oxysporum</i>		<i>Aspergillus niger</i>		
		MM	%	MM	%	
Tebuconazole	1	15	100	15	100	0.9
	0.1	14	93.3	15	100	0.6
	0.01	12	80	14	93.3	0.6
<i>N</i> -(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide	1	5	33.3	4	26.6	0.7
	0.1	4	26.6	4	26.6	0.4
	0.01	3.5	23.3	3.5	23.3	0.2

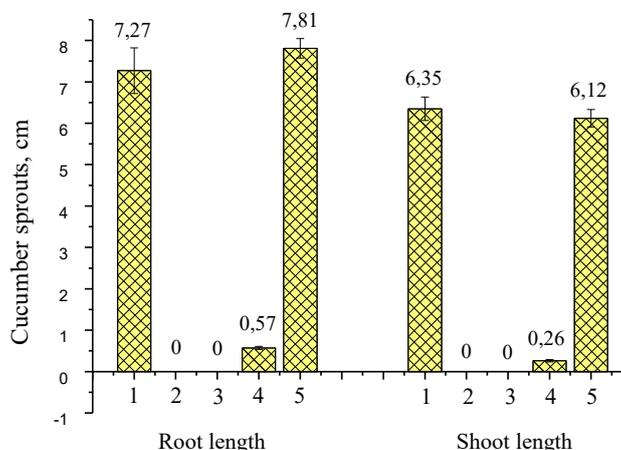
Statistical analysis was performed using Student's t-test, with differences regarded as statistically significant at  $p \leq 0.05$

Analysis of the experimental data revealed strong inhibitory effects of *N*-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide on wheat seedlings, affecting both root and shoot development. In the control group, the average root and shoot lengths were 6.85 cm and 5.75 cm, respectively. In the reference treatment, seedling growth was markedly suppressed, with root and shoot lengths reduced to 0.08 cm and 0.16 cm. At 0.1%, the test compound completely inhibited wheat seed germination (100%) relative to the control. At 0.01%, substantial growth inhibition was still observed, with root and shoot lengths reduced to 0.21 cm (96.9% inhibition) and 0.16 cm (97.2% inhibition), respectively. At these concentrations, the biological activity was comparable to the reference product DIRBI EC. At the lowest concentration tested (0.001%), no significant inhibitory effect was detected. The average root and shoot lengths were 5.27 cm and 4.86 cm, respectively, values close to those of the control. These findings indicate high sensitivity of wheat seedlings to the compound and confirm its strong inhibitory effect during early plant development (Figure 2).



**Figure 2.** Herbicidal activity on wheat seedlings: 1-Control; 2-Reference (DIRBI EC); 3-Test compound (0.1%); 4-Test compound (0.01%); 5-Test compound (0.001%)

Laboratory studies also demonstrated the inhibitory activity of the test compound on cucumber seedlings compared to the control variant. In the control, where cucumber seeds were soaked in water, the average root and shoot lengths reached 7.27 cm and 6.12 cm, respectively. This result indicates the absence of a stress effect on the control seedlings. In the reference variant, on the contrary, complete suppression of growth processes was noted: cucumber seed germination was not observed, and the development of both the root system and the aboveground part was completely inhibited by 100%. In the experimental variant, at a concentration of 0.1%, complete growth suppression (100%) was observed, which is comparable to the effect of the reference preparation. At a concentration of 0.01%, the compound retained high phytotoxic activity: root growth was inhibited by 92.1%, while the average root length did not exceed 0.57 cm compared to the control, and shoot development was completely absent (0 cm). The obtained data indicate pronounced phytotoxicity of the compound at the indicated concentrations (Figure 3).



**Figure 3.** Herbicidal activity on cucumber seedlings: 1-Control; 2-Reference (DIRBI EC); 3-Test compound (0.1%); 4-Test compound (0.01%); 5-Test compound (0.001%)

#### 4. CONCLUSION

In this study, a one-pot nitration approach for the synthesis of N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide was developed and optimized using the Box–Wilson experimental design methodology. The influence of temperature, reaction time, and reagent molar ratio on the product yield was systematically evaluated, and a statistically adequate regression model was established. Preliminary biological assays demonstrated that N-(5-nitro-1H-benzo[d]imidazol-2-yl)propionamide exhibits moderate fungicidal activity against *Fusarium oxysporum* and *Aspergillus niger*, as well as pronounced inhibitory effects on the early growth of wheat and cucumber seedlings at higher tested concentrations. These results indicate that the presence of a nitro group in the benzimidazole framework may contribute to phytotoxic and antifungal properties. Although the biological evaluations are exploratory in nature, the obtained data suggest that this compound represents a promising

scaffold for further investigation. Future studies should include modifying this compound to increase selectivity and potency, determining the toxicity profile, and conducting extended biological evaluations, such as studying the modes of action. Such studies will serve to increase the practical value of this compound and its derivatives as potential agrochemical agents.

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#### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

#### AUTHOR CONTRIBUTION

E. Rakhmatov, D. Arabov: Data curation, Methodology, Formal analysis. S. Saidov: Conceptualization, Methodology, Writing original draft, Writing - review & editing. N. Olimov, Kh. Nabieva: Visualization, Investigation, Writing - review & editing. R. Karimov Ch. Elmurodov: Software, Data curation. E. Kurbanova: Conceptualization, Investigation, Data curation, Writing original draft, and editing. A. Abdurazakov, Sh. Sagdullayev: Project administration, Resources.

#### DATA AVAILABILITY

The data that support the findings of this study are available on request.

#### DECLARATION OF GENERATIVE AI

Not applicable.

#### ETHICS

Not applicable.

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