

New Plant Growth Regulators of Benzimidazole Series [†]

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Abstract

Benzimidazole derivatives have a wide range of biological activity, including growth-regulating activity in relation to plants. The study of the structure-activity relationship (SAR) in a number of benzimidazole derivatives is important for the development of new effective plant growth regulators. The design of new effective plant growth regulators based on these derivatives is an important scientific direction, as it is relevant for the development of agriculture, taking into account modern stringent environmental requirements.

Keywords: benzimidazole derivatives; growth-regulating activity

1. Introduction

Benzimidazole derivatives exhibit a broad spectrum of biological activity [1], including growth-regulating effects on plants [2]. The study of the structure-activity relationship (SAR) within the series of benzimidazole derivatives is crucial for the development of new effective plant growth regulators [3].

Understanding the unique natural instrument of the plant organism—the system of hormonal control, namely phytohormones—enables the regulation of not only the productivity of agricultural crops and the preservation of the harvest but also a range of various metabolic aspects, leading to results unattainable through other agricultural practices and methods [4]. The effectiveness of phytohormone application is confirmed not only in laboratory conditions but also in practice [4]. The use of growth regulators represents a phytotechnique for directly influencing plants to correct the errors of natural and artificial selection [5].

The search for synthetic plant growth regulators is conducted among various classes of organic compounds. Growth-regulating activity has been noted for synthetic plant growth and development stimulants, such as thiazolidine compounds—5-substituted 2-thiothiazolidin-4-ones—on sunflower and wheat plants, assessed by seedling length [6]. It was found that substance activity depended on the nature of the radical introduced at the 5-position: electron-donating radicals increased the growth-regulating properties of the compound, and the more pronounced the electron-donating properties of the radical,

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the more pronounced the growth-regulating effect. Conversely, if the radical had an electron-withdrawing nature, an inhibitory effect on plant seedlings was observed.

Substances exhibiting growth-regulating activity are also known among urea derivatives. For instance, thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) demonstrates a unique combination of properties characteristic of auxins and cytokinins [7]. Pyrrolidones and carboxylic acid amides have also been noted as physiologically active substances of interest [8].

One of the indicators suggesting the growth-regulating activity of a particular class of compounds is its structural similarity to natural phytohormones. We have previously synthesized a series of benzo-[2,3-b]-1,4-diaza- and benzo-[2,3-b]-1-aza-4-oxa-bicyclo[3.3.0]octane-8-ones and shown their high growth-regulating activity on *Triticum aestivum* L [3]. The derivatives of these compounds, which also contain benzimidazole fragments, were therefore selected for a further study of their growth-regulating activity. This choice is also based on the known fact [9] that benzimidazole fragments are spatial analogs of the purine fragments found in natural phytohormones with cytokinin activity, such as zeatin, N⁶-(Δ^2 -isopentenyl)adenine, and 6-(3-methylbut-2-enylamino)purine.

Despite the existence of several methods for the synthesis of benzimidazole derivatives, the development of new approaches to their modification remains a relevant task. Traditional modification methods often lead to unwanted by-products and require the use of toxic reagents, complicating the purification process and reducing the target product yield. The use of mild conditions and novel methods of molecule activation in the developed synthetic approach can improve reaction efficiency, making the method more accessible and flexible for introducing various structural modifications to the benzimidazole core [10].

2. Materials and Methods

2.1. Physical Measurements

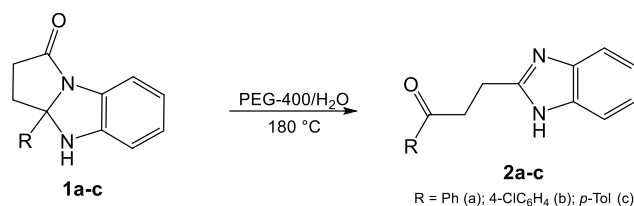
The reaction was monitored and the identification of the obtained compounds was determined by thin-layer chromatography (TLC), infrared spectroscopy (IR spectroscopy) and X-ray diffraction (XRD). TLC analysis was performed on ALUGRAM® SIL G UV254 plates (MACHEREY-NAGEL GmbH & Co. KG, Germany), eluents: hexane-ethyl acetate-acetone (2:2:1), hexane-ethyl acetate-chloroform (2:2:1), developer — UV lamp. FTIR spectra were recorded in KBr tablets on Nicolet 6700 IR-Fourier spectrometer (Thermo Scientific, USA) and FSM 1201 (Infraspek, Russia) in the range of 4000–400 cm⁻¹ with a spectral resolution of 4 cm⁻¹. The ¹H,¹³C NMR HMBC, HSQC, COSY spectra were obtained using a Varian 400 spectrometer at a temperature of 25 °C (400 MHz, 100 MHz). The internal standard is tetramethylsilane for DMSO-*d*₆ and CDCl₃. Melting points were determined on a StuartTM SMP10 device (Cole-Parmer, Great Britain) in open capillaries.

2.2. Synthesis of 3-Aryl-2,3,3a,4-Tetrahydro-1H-Benzo[d]pyrrolo[1,2-a]imidazole-1-Ones (1)

The 3-aryl-2,3,3a,4-tetrahydro-1H-benzo[d]pyrrolo[1,2-a]imidazole-1-ones (**1a–c**) were synthesized using known method [2].

2.3. Synthesis of Aryl-Substituted 3-(1H-Benzo[d]imidazole-2-Yl)-1-Arylpropane-1-Ones (2)

The series of 3-(1H-benzo[d]imidazole-2-yl)-1-arylpropane-1-ones (**2**) was obtained using a sealed vessel reactor (Monowave 50, Anton Paar) (Scheme 1) in good yields.



Scheme 1. Synthesis of 3-(1H-benzo[d]imidazole-2-yl)-1-arylpropane-1-ones (**2a-c**).

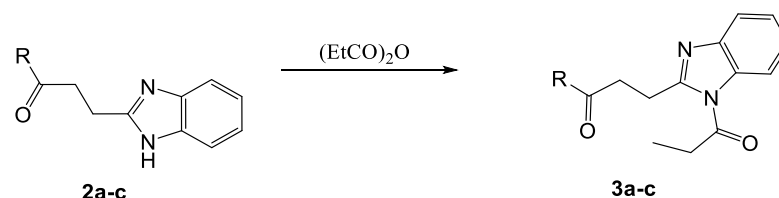
The 0.10 g sample of corresponding 3-aryl-2,3,3a,4-tetrahydro-1H-benzo[d]pyrrolo[1,2-a]imidazole-1-ones (**1a-c**) (5 mmol) is placed in a borosilicate glass vial. The vial is closed by a silicone cap with a PTFE septa. The reaction is carried out in a reactor of sealed vessels at a temperature of 180 °C in a solvent medium consisted of polyethylene glycol-400 (PEG-400) with water in a ratio of 2:1 for 20 min. The resulting precipitate is decanted, washed and dried in air.

3-(1H-benzo[d]imidazole-2-yl)-1-phenylpropane-1-one (**2a**): brown solid; yield 80%. m.p. 200–204 °C. ¹H NMR, δ, ppm: 3.17 (t, *J* = 7.2 Hz, 2H, H₂C-Het); 3.63 (t, *J* = 7.1 Hz, 2H, H₂C-CO); 7.08 (s., 1H, Ph + 1H, benzim); 7.39–7.45 (m., 2H, benzim); 7.53 (t, *J* = 7.2 Hz, 2H, Ph); 7.64 (t, *J* = 7.4 Hz, 2H, benzim); 8.01 (d., *J* = 7.6 Hz, 2H, Ph); 12.19 (s., 1H, NH).

3-(1H-benzo[d]imidazole-2-yl)-1-(4-chlorophenyl)propane-1-one (**2b**): brown solid; yield 82%. m.p. 196–199 °C. ¹H NMR, δ, ppm: 3.49 (t, *J* = 7.6 Hz, 2H, H₂C-benzim); 3.712 (t, *J* = 7.6 Hz, 2H, H₂C-CO); 7.46 (d., *J* = 8.3 Hz, 2H, 4Cl-C₆H₄); 7.62–7.70 (m., 3H, H₄ClAr); 7.83 (s., 2H, -CH₂-benzim); 7.91 (d., *J* = 8.3 Hz, 2H, benzim); 8.00 (d., *J* = 8.3 Hz, 2H, benzim); 8.10 (d., *J* = 8.3 Hz, 2H, 4Cl-C₆H₄).

3-(1H-benzo[d]imidazole-2-yl)-1-(p-tolyl)propane-1-one (**2c**): brown solid; yield 85%. m.p. 198–200 °C. ¹H NMR, δ, ppm: 2.39 (s., 3H, -CH₃); 3.37 (t, *J* = 6.3 Hz, 2H, -CH₂-benzim); 3.57 (t, *J* = 6.5 Hz, 2H, -CH₂-C=O); 7.19–7.24 (m., 2H, p-Tol + 2H, benzim); 7.53–7.55 (m., 2H, benzim); 7.85 (d, *J* = 7.8 Hz, 2H, p-Tol).

2.4. Synthesis of 1-Aryl-3-(1-Propionyl-1H-Benzo[d]imidazole-2-Yl)propane-1-Ones (**3**)



Scheme 2. Synthesis of 1-aryl-3-(1-propionyl-1H-benzo[d]imidazole-2-yl)propane-1-one (**3a-c**).

The 0.13 g (0.52 mmol) sample of corresponding 3-(1H-benzo[d]imidazole-2-yl)-1-arylpropane-1-one **2a-c** is placed in a 50 mL flat-bottomed flask equipped with an air condenser. The reaction is carried out on a magnetic stirrer when heated (70 °C) in 10 mL of propionic anhydride for 2 h. The reaction mixture is then poured into a saturated NaHCO₃ solution. An oil containing the reaction product formed on the surface of the solution. The extraction of the reaction product emulsified in the organic phase in water is carried out using ultrasound for 35 min. The resulting precipitate is filtered, washed with water and dried in air, then in a vacuum desiccator.

1-phenyl-3-(1-propionyl-1H-benzo[d]imidazole-2-yl)propane-1-one (**3a**): light brown solid; yield 92%. m.p. 91–94 °C; ¹H NMR, δ, ppm: 1.22 (t., *J* = 6.9 Hz, 3H, -CH₃-propionic); 3.21 (q, *J* = 7.1 Hz, 2H, -CH₂-propionic); 3.50 (t., *J* = 6.7 Hz, 2H, -CH₂-benzim); 3.65 (t, *J* = 6.8 Hz, 6H, H₂C-C=O); 7.30 (dq., *J* = 14.3, 7.6 Hz, 1H, Ph + 1H benzim); 7.55 (t. + d., *J* = 7.2 Hz, 2H, Ph + 1H, benzim); 7.65 (t., *J* = 7.4 Hz, 1H, benzim); 7.91 (d., *J* = 7.9 Hz, 2H, benzim); 8.02 (d., *J* = 7.7 Hz, 2H, Ph).

1-(4-chlorophenyl)-3-(1-propionyl-1H-benzo[d]imidazole-2-yl)propane-1-one (**3b**): light brown solid; yield 83%. m.p. 88–91 °C; ¹H NMR, δ, ppm: 1.40 (t., *J* = 6.9 Hz, 3H, -CH₃-propionic); 3.14–3.16 (q., *J* = 7.2 Hz, 2H, -CH₂-propionic); 3.64 (br.s., 4H, H₂C-CH₂); 7.28–7.35 (m., 2H, benzim); 7.45 (d., *J* = 8.3 Hz, 2H, 4Cl-C₆H₄); 7.65–7.71 (m., 2H, benzim); 7.99 (d., *J* = 8.3 Hz, 2H, 4Cl-C₆H₄).

3-(1-propionyl-1H-benzo[d]imidazole-2-yl)-1-(*p*-tolyl)propane-1-one (**3c**): light brown solid; yield 83%. m.p. 95–97 °C; ¹H NMR, δ, ppm: 1.41 (t., *J* = 7.2 Hz, 3H, -CH₃); 2.42 (s., 3H, -CH₃-*p*-Tol); 3.17 (q., *J* = 7.2 Hz, 2H, -CH₂-propionic); 3.60–3.74 (m., 4H, H₂C-CH₂); 7.27 (d., *J* = 7.8 Hz, 2H, *p*-Tol); 7.34–7.38 (m., 2H, benzim); 7.73 (q., *J* = 5.0 Hz, 2H, benzim); 7.94 (d., *J* = 7.8 Hz, 2H, *p*-Tol).

3. Results and Discussion

3.1. Spectral Characterization of the Synthesized Benzidazolones (1)

The structure of the products (**2a–c**, **3a–c**) was determined based on a set of ¹H NMR spectroscopy data and using two-dimensional methods COSY, HSQC and HMBC. According to spectral data, they represent a number of benzimidazole derivatives with different steric volumes of the side substituent and the volume of the molecule as a whole (the propionate molecule is sterically more voluminous than the original non-propionate one).

The ¹H NMR spectroscopic analysis provides definitive confirmation of the molecular structures for all six synthesized compounds and allows for a clear comparative discussion of the two series. The general scaffold, featuring a 1,3-disubstituted propanone linker, is consistently identified by the characteristic sets of triplets between 3.1 and 3.8 ppm, corresponding to the diastereotopic methylene groups (-CH₂-Benzim and -CH₂-C(O)-) with coupling constants of *J* ≈ 7 Hz, typical for an ethylenic chain.

The most significant spectral distinction lies between the parent NH-benzimidazole compounds (**2a–c**) and their *N*-propionyl derivatives (**3a–c**). In the spectra of series **2**, a broad singlet resonating between 12.0–12.5 ppm is observed, which is a definitive marker for the proton of the secondary amine (NH) group in the benzimidazole ring. This signal is entirely absent in the spectra of series **3**, providing direct evidence for successful *N*-acetylation. Concomitantly, new signals appear in the spectra of series **3**: a triplet at approximately 1.2–1.4 ppm and a quartet in the region of 3.1–3.2 ppm, integrating for three and two protons respectively, which are assigned to the methyl (-CH₃) and methylene (-CH₂-) groups of the newly incorporated propionyl moiety. Furthermore, *N*-acetylation induces a subtle but consistent upfield shift of the aromatic proton signals belonging to the benzimidazole ring, moving from the 7.4–8.1 ppm region in series **2** to 7.3–7.7 ppm in series **3**, due to the altered electronic environment.

This preliminary signal assignment was unambiguously confirmed and refined using two-dimensional NMR correlation experiments: COSY spectra verified the connectivity within the spin systems of the aliphatic chains and aromatic fragments, HSQC spectra allowed for the direct assignment of all protonated carbon atoms, and key long-range ¹H-¹³C correlations observed in HMBC spectra, particularly between the carbonyl carbon and the adjacent methylene protons, as well as between the heterocycle and its side chain, provided definitive proof of the molecular scaffold and the site of *N*-acetylation, thereby conclusively confirming the proposed structures of the synthesized molecules **2–3a–c**.

3.2. Growth-Regulating Activity

The results of studying the growth-regulating activity of benzoimidazole derivatives **1a–c**, **2a–c** and **3a–c** on common wheat seeds showed that phenyl-substituted tricyclic benzopyrroloimidazolone **1a** demonstrated the greatest effectiveness compared with the control (water) and the reference compound (IAA) (Figure 3). The effect of **3a** surpassed the IAA comparison drug by about 15% in root length and 47% in coleoptile length. Compared with the control (with water), the increase in root length was about 20%, and the length of the coleoptile was almost 77% (Table 1). Other derivatives, such as tricyclic **1c** and non-cyclic **2a**, also showed good results, providing elongation of both roots and seedlings above or at the IAA level.

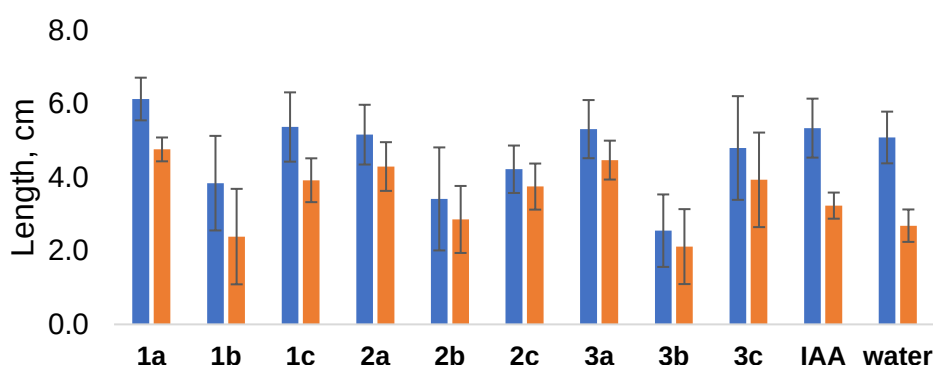


Figure 3. Average lengths of the main root and coleoptile of three-day wheat seedlings (cm) under the action of synthesized compounds **1a–c**, **2a–c** and **3a–c**.

When comparing the growth-regulating activity of benzoimidazole derivatives on common wheat seeds, it was found that the effectiveness of the compounds depends both on the structure of the molecule (cyclic or non-cyclic form) and on the steric volume of the side substituent and the volume of the molecule as a whole (the propionated molecule is sterically more voluminous than the original non-propionated one).

Table 1. Effect of synthesized compounds **1a–c**, **2a–c** and **3a–c** on the lengths of the main root and coleoptile (mean $\pm$ confidence interval) of three-day wheat seedlings, cm and % of the reference compound—IAA.

Cmpd	Root Length	Coleoptile Length	Root Length (% of IAA)	Coleoptile Length (% of IAA)	Root Length (% of Water)	Coleoptile Length (% of Water)
1a	6.1 $\pm$ 0.6	4.8 $\pm$ 0.3	14.8	47.3	20.6	77.3
1b	3.8 $\pm$ 1.3	2.4 $\pm$ 1.3	−28.1	−26.1	−24.5	−11.1
1c	5.4 $\pm$ 0.9	3.9 $\pm$ 0.6	0.6	21.4	5.6	46.0
2a	5.2 $\pm$ 0.8	4.3 $\pm$ 0.7	−3.3	32.9	1.5	59.9
2b	3.4 $\pm$ 1.4	2.9 $\pm$ 0.9	−36.1	−11.6	−32.9	6.3
2c	4.2 $\pm$ 0.6	3.8 $\pm$ 0.6	−21.0	16.0	−17.0	39.6
3a	5.3 $\pm$ 0.8	4.5 $\pm$ 0.5	−0.5	38.3	4.5	66.4
3b	2.6 $\pm$ 0.1	2.1 $\pm$ 1.0	−52.3	−34.5	−49.9	−21.2
3c	4.8 $\pm$ 1.4	3.9 $\pm$ 1.3	−10.1	21.7	−5.6	46.4
IAA	5.3 $\pm$ 0.8	3.2 $\pm$ 0.4	0.0	0.0	5.0	20.3
water	5.1 $\pm$ 0.7	2.7 $\pm$ 0.4	−4.8	−16.9	0.0	0.0

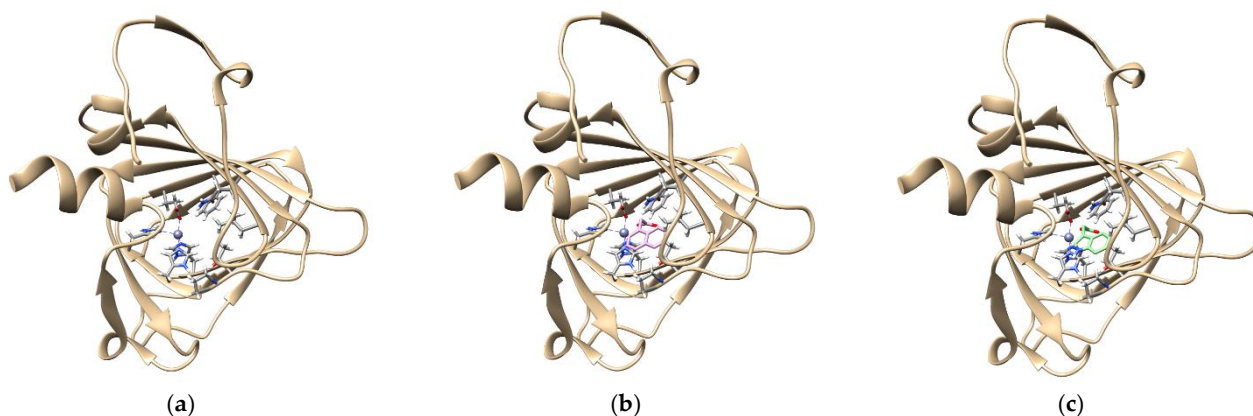
A decrease in activity is noted during the transition from the phenyl to the *p*-tolyl group, whose substituent has a slightly larger steric volume. The tricyclic *p*-tolyl homologue **1c** turned out to be about 12% less effective than phenyl **1a** in relation to the root system and by almost 18% in the length of the coleoptile. Nevertheless, it demonstrates a growth-regulating effect, surpassing the water values by 5% for the root and 46% for the coleoptile.

Derivatives with a *p*-alkyl substituent with a significant steric volume turned out to be the least active. In the non-cyclic propionated form **3b**, they showed a 52% and 34% reduction in root and coleoptile length, respectively, compared with IAA, and were inferior to the control samples (water) by 50% in root length and 21% in coleoptile length. This indicates a significant inhibition of growth in the presence of a bulky *p*-tolyl substituent.

Thus, a decrease in plant growth uniquely correlates with an increase in the steric volume of both the side substituent and the molecule as a whole when the propionated fragment is introduced along the benzimidazole ring. Phenyl analogues, especially in tricyclic form, show the greatest activity, while sterically more bulky 4-chlorophenyl ones show a pronounced lower effect, and sterically loaded *p*-tolyl derivatives, especially in the non-cyclic form, have a weakly pronounced positive or even inhibitory effect. These patterns illustrate the importance of both spatial and electronic factors in the biological activity of benzimidazole derivatives.

3.3. Molecular Docking

In order to confirm the effect of the steric volume of the substituent and the molecule as a whole, we modeled protein-ligand interactions using molecular docking, where auxin-binding protein 1 (auxin-binding protein 1) of corn (PDB code 1LR5, complex with naphthylacetic acid (NAA)—1LRH) was used as a receptor. The choice of a target from corn is due to the lack of data in the PDB for the corresponding wheat protein. The general appearance of the 1LRH target, as well as 1LRH complexes with NAA, IAA, and phenyl-substituted derivatives (**1a**, **2a**, **3a**) are shown in Figure 4. The complex with nucleoxin-binding protein 1 includes a zinc ion in the active center, which promotes coordination through electrostatic interactions with the carboxyl group of the ligand. Docking was performed with a target with or without a zinc ion, and as a result, very close energy values were obtained, and therefore no zinc ion removal was performed to simulate the complexes.



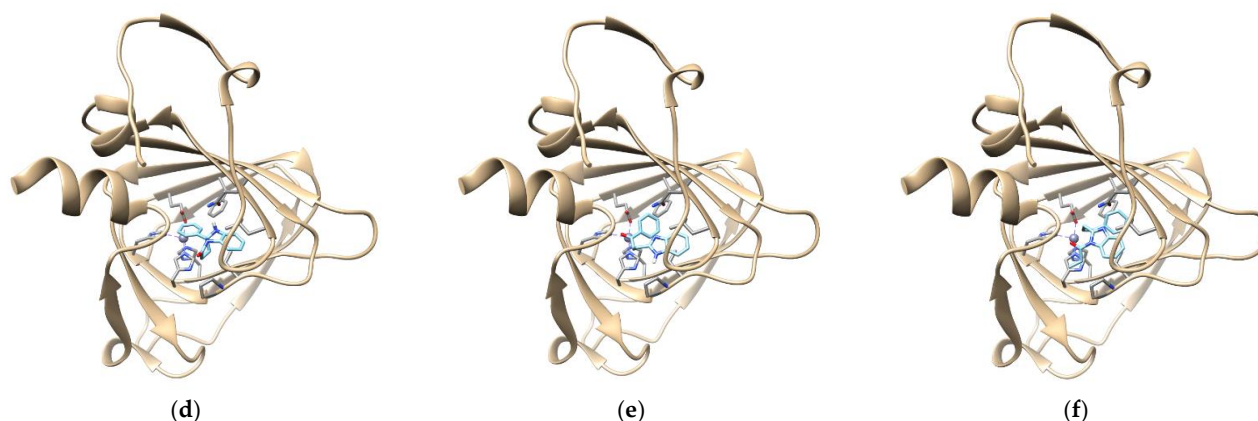


Figure 4. The view of receptor 1LRH (a) and complexes 1LRH with NAA, energy of -7.6 kcal/mol (b), with IAA, energy of -6.7 kcal/mol (c), with **1a**, energy of -4.3 kcal/mol (d), with **2a**, energy of -5.5 kcal/mol (e), with **3a**, energy of -2.9 kcal/mol (f).

The results of molecular docking generally correlate with the data obtained in vitro. It is worth noting that in some cases, a negative correlation in growth-regulating activity in vitro and the results in silico may be associated with the selected suboptimal concentrations of active substances, since at the initial stage of research, concentrations were not selected, and the results obtained in previous studies were chosen as the working concentration of benzimidazole derivatives.

Modeling of receptor-ligand interactions has generally confirmed the importance of the steric volume of both the lateral aromatic substituent and the molecule as a whole. Thus, compound **1b**, which has a maximum volume, demonstrates the maximum energy of the complex, which indicates the predominance of repulsive forces, which make the complex less durable than its *p*-tolyl-substituted and phenyl-substituted analogues. These differences in steric volume affect the spatial arrangement of ligands relative to the key amino acids of the 1LRH receptor pocket, as well as, probably, their penetration through cell membranes.

The molecular docking data correlate with our initial assumption that open-chain derivatives of benzimidazole **2a–c** should be more active, since they are a product of water addition and can be considered as hydrolysis products occurring during the metabolism of introduced benzopyrroloimidazolones **1a–c**. This is confirmed by the lower energies of the **2a–c** complexes compared to **1a–c** (Table 2). However, in vitro data indicate an inverse relationship with generally close absolute and relative lengths of wheat seedlings and roots, which may be due, as we suggested above, to the choice of a suboptimal concentration of the active substance, which opens up prospects for further study of the biological activity of compounds **1a–c** in this direction.

Table 2. Energies of 1LRH complexes with **1a–c**, **2a–c** and **3a–c** in the presence of in the absence of Zn^{2+} ion.

	1a	1b	1c	2a	2b	2c	3a	3b	3c
with Zn	-4.3	-1.2	-1.7	-5.5	-5.7	-5.8	-2.9	-2.9	-0.8
without Zn	-4.2	-1.2	-1.6	-5.5	-4.8	-5.5	-2.7	-3.1	-1.2

In turn, *p*-tolyl analogues in both tricyclic and non-cyclic forms show moderate activity in vitro, which is probably due to a more voluminous methyl substituent in the para position, which somewhat limits the possibilities of placing the ligand in the receptor pocket, depending on the structure of the main heterocyclic core.

Derivatives with the 4-chlorophenyl group turned out to be the least active, especially in the non-cyclic protonated form, where the root (2.55 cm) and seedling (2.12 cm) lengths were the lowest among all samples. However, the molecular docking data show the promise of studying the 4-chlorophenyl non-ionized derivative of benzimidazole **2b**. If the optimal concentration of **2b** and **2c** is detected, the derivatives may exhibit activity at or even higher than that for phenyl-substituted **2a** and all tricyclic **1a–c** compounds.

Thus, it can be concluded that not only the presence of a tricyclic system, but also the compactness of the lateral aromatic substituent positively affect the growth-regulating activity of benzimidazole derivatives on common wheat seedlings, while not excluding the activity of open-chain **2a–c** derivatives. At the same time, obtaining more lipophilic propionated derivatives is futile from the point of view of increasing growth-regulating activity, however, it can play a positive role in the study of other types of activity of these compounds associated not with plants, for which the water solubility of the compound is significantly more important, but for animals.

4. Conclusions

Based on 3-(benzimidazole-2-yl)-1-arylpropane-1-ones, 3-(1*H*-benzo[*d*]imidazole-2-yl)-1-arylpropane-1-ones were obtained under PEG-400 conditions with water. Heating of 3-(benzimidazole-2-yl)-1-arylpropane-1-ones in propionic anhydride, the nitrogen atom of the benzimidazole fragment is acylated to form 1-aryl-3-(1-propionyl-1*H*-benzo[*d*]imidazole-2-yl)propane-1-ones. The structure of the obtained substances has been studied using spectral methods. From the point of view of growth-regulating activity, the presence of a tricyclic system and the steric compactness of the lateral aromatic substituent of benzimidazole derivatives positively affect the increase in the length of roots and seedlings of common wheat in the early stages of germination.

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