

Biological Potential of Novel Methoxy and Hydroxy Substituted Heteroaromatic Amides Designed as Promising Antioxidative Agents: Synthesis, 3D-QSAR Analysis, and Biological Activity

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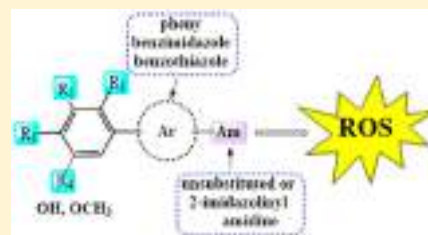
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Supporting Information

ABSTRACT: This paper discusses antioxidative and biological activities of 25 novel amidino substituted benzamides with a variety of heteroaromatic nuclei attached to the benzamide moiety and with a variable number of methoxy or hydroxy substituents. Targeted compounds, bearing either amidino or 2-imidazolynyl substituent, were obtained in the Pinner reaction from cyano precursors. 3D-QSAR models were generated to predict antioxidative activity of the 25 novel aromatic and heteroaromatic benzamide derivatives. The compounds were tested for antioxidative activity using *in vitro* spectrophotometric assays. Direct validation of 3D-QSAR approach for predicting activities of novel benzamide derivatives was carried out by comparing experimental and computationally predicted antioxidative activity. Experimentally determined activities for all novel compounds were found to be within a standard deviation of error of the models. Following this, structure–activity relationships among the synthesized compounds are discussed. Furthermore, antiproliferative activity *in vitro* against HeLa cells as well as antibacterial and antifungal activity was tested to confirm the other biological activities of the prepared compounds.



1. INTRODUCTION

Significant biological importance of nitrogen heterocycles, particularly benzimidazoles and benzothiazoles, makes them very useful building blocks in organic and medicinal chemistry.^{1,2} They are essential structural parts in numerous natural and synthetic bioactive compounds³ possessing a wide range of biological characteristics including antibacterial,^{4,5} anticancer,^{6,7} antifungal,⁸ antiviral,⁹ antioxidative,^{10,11} etc. In the past decade, particular attention and focus were given to their antioxidative potency. It is widely accepted that reactive oxygen species (ROS) could cause the oxidative damage of important biomolecules including nucleic acids, proteins, or lipids. These reactive species could be generated in numerous pathophysiological and biochemical processes in the human body.¹² ROS include nonfree radical species like ozone (O₃), singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), and free radicals including peroxy radicals (ROO•), hydroperoxyl radicals (HOO•), hydroxyl radicals (OH•), and superoxide anion radicals (O₂•⁻). Consequently, it could lead to the development of some chronic diseases like cancer, diabetes, inflammation, neurodegenerative syndrome, or aging.^{13,14} Proper usage of antioxidants could regulate the rate of reactive

oxygen species production; at even at low concentration, they prevent oxidation of easily oxidizable substrates.¹⁵ Thus, there is a constant growing interest for the study of antioxidative capacity of numerous natural and synthetic organic molecules with the aim of prevention of the above-mentioned diseases.

Recently, our scientific research focused on the antiproliferative activity of versatile benzimidazole and benzothiazole derivatives.^{16–18} A large number of active derivatives are designed to have cationic amidino substituents at the termini of the molecule, which have allowed additional interactions with the biological targets. Additional biological activity studies of tetracyclic benzimidazole derivatives have been confirmed their strong interaction with DNA as intercalators, while acyclic, either benzimidazole or benzothiazole, derivatives have been proven to be selective DNA minor groove binders.^{16,17,19} Recent literature data suggest that tumor cells are accompanied by a measurable emission of ROS, which might be regulated by appropriate usage of antioxidant agents.²⁰ Several publications described antioxidative activity and potential of benzimidazole

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Table 1. Statistical Properties of 3D-QSAR Models

model	nO	LV	R^2	SDEC	Q^2_{LOO}	$SDEP_{\text{LOO}}$	Q^2_{LMO}	$SDEP_{\text{LMO}}$	$SDEP_{\text{EXT}}$	nOE
1	59	4	0.73	0.25	0.66	0.28	0.65	0.28	0.41	12
2	32	3	0.71	0.12	0.54	0.16	0.53	0.16	0.18	7

^aNumber of objects used to build the model (training set). ^bNumber of latent variables. ^cSDEC, standard deviation of error of calculation. ^d Q^2 is the cross validated predictive performance, LOO stands for leave one out cross validation procedure, LMO stands for leave many out (i.e., random groups) cross validation procedure. ^eSDEP, standard deviation of error of prediction obtained by cross validation. ^f $SDEP_{\text{EXT}}$ stands for standard deviation of error of prediction obtained by external validation. ^gNumber of objects used for external prediction validation (test set).

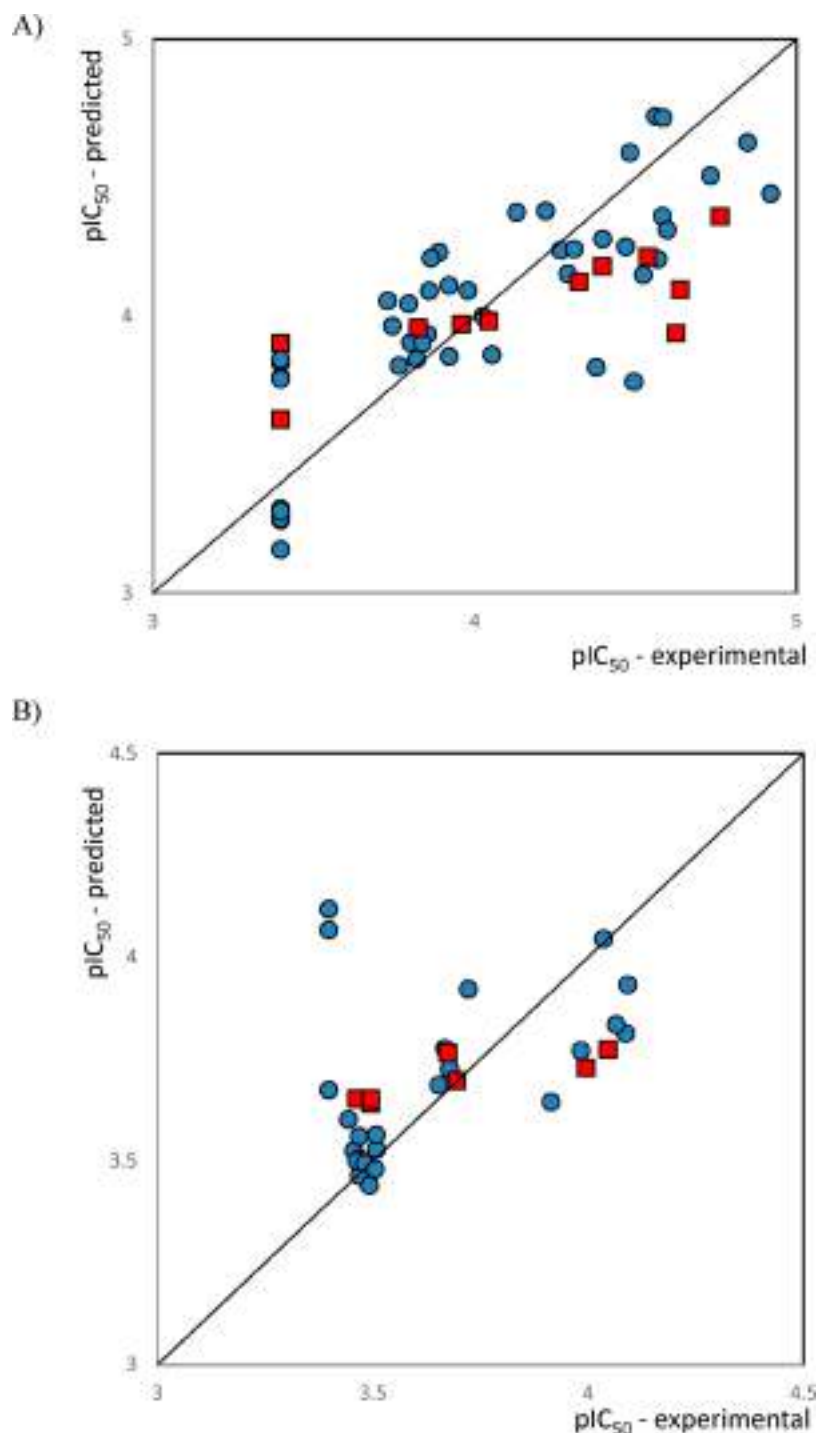


Figure 1. Predicted versus experimental antioxidant activity expressed as pIC₅₀, negative logarithmic value of concentration that causes 50% of antioxidative activity measured: (A) against DPPH stable radical (model 1) and (B) using superoxide test (model 2). Blue circles present training set compounds; red squares present test set compounds.

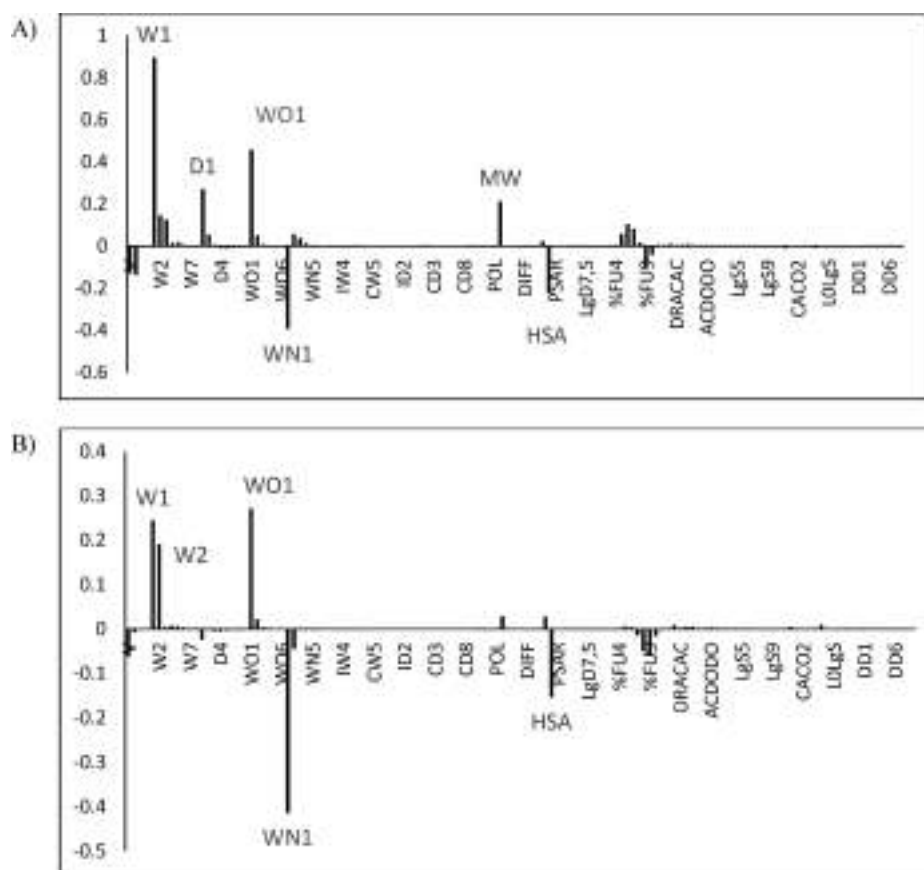


Figure 2. Products of the descriptor's average value calculated for the data set used to derive the model and the associated PLS coefficient of 3D-QSAR model for (A) model 1 and (B) model 2.

and benzothiazole derivatives, as well as a broad range of salicylanilide and benzamide derivatives, as antioxidative agents.^{21–23}

To explore the antioxidative potency of aromatic and heteroaromatic benzamide derivatives bearing benzazole nuclei, we developed 3D-QSAR models and used them for computational prediction of antioxidative activity of the presented compounds.

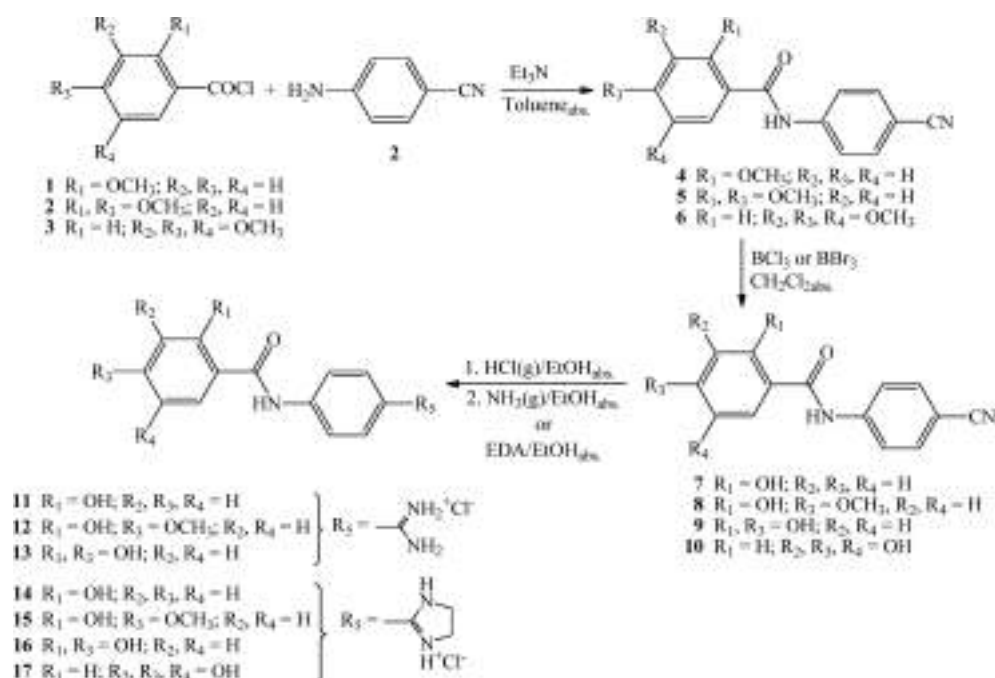
Application of 3D-QSAR modeling for prediction of activity of novel compounds and identification of molecular properties with the highest influence on the studied activity were already proven as useful methods numerous times.^{24–29} In addition, antioxidative activity of different classes of compounds has been studied using different approaches of QSAR modeling.^{30–33} The main advantage of Volsurf+^{34,35} based approach is usage of molecular descriptors that are independent of molecular alignment and have a clear chemical or physical meaning. In this paper, the Volsurf+ approach for generating 3D-QSAR models was experimentally validated on prediction of antioxidative activity of the 25 novel compounds. Combining the demonstrated biological potential of benzamides and amidino substituted benzimidazoles and benzothiazoles, we have designed and synthesized novel derivatives bearing a variable number of the methoxy and hydroxy groups substituted with different amidino substituents. Prepared derivatives were explored for their antioxidative, antimicrobial, and antiproliferative activities. The obtained experimental results were compared to the predicted results, and a very good alignment was observed.

2. RESULTS AND DISCUSSION

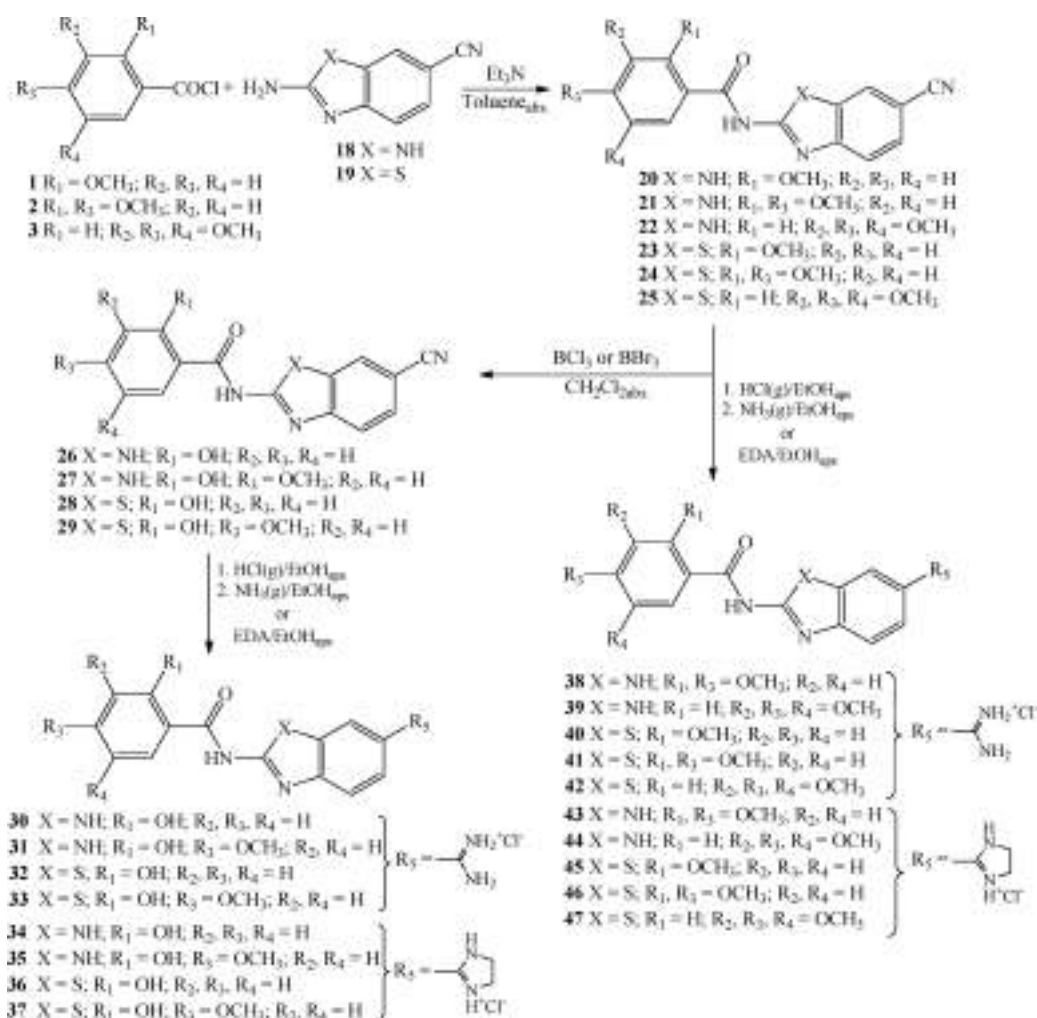
2.1. 3D-QSAR Models. Using a library of experimental data for antioxidative activity^{36–38} of already published compounds, 3D-QSAR models were built. Models with the highest predictive ability, namely model 1 and model 2, were chosen for the prediction of antioxidative activities of novel compounds. Model 1 was generated using the results obtained from the activity testing against DPPH stable radical, while model 2 has been generated using activity from the superoxide test. Quality of the models was validated by internal cross validation procedure, using two procedures, leave one out (LOO) and random groups (LMO), as well as by the external prediction (Table 1, Figure 1). Both models show similar predictive abilities (Q^2_{LOO} , Q^2_{LMO} , $SDEP_{EXT}$ in Table 1). Statistical properties of the models are shown in Table 1. Compounds for which activity was expressed only as “ $\geq 400 \mu\text{M}$ ” were not included in the training set of the model 2 due to the possible largely decreased quality of the model.

2.1.1. 3D-QSAR Analysis. Principal component analysis (PCA) was performed on the overall data set of 59 compounds. The first two principal components explained 95% of the variance (the first three PCs explained 98% of variance) of the descriptors (X-matrix). Molecular descriptors including hydrophilic (W1–W3) and hydrophobic regions (D1) related to the polarizability and dispersion forces, molecular volume (V), and possibility of accepting (WN1) and donating H-bonds (WO1) were identified from the PCA loadings plot (Figure S63 in the Supporting Information), as

Scheme 1



Scheme 2



the ones with the highest variation among the data set compounds.

These results indicated the mentioned descriptors as the ones with the highest variation between the data set compounds, which might be related to the variation in antioxidative activities of the data set compounds. The PLS analysis could estimate the quantitative impact of each descriptor on the studied activities.

The influence of each molecular descriptor to the compound's antioxidative activity could be evaluated from the analyses of the PLS coefficients of the obtained 3D-QSAR. Since both models were generated using raw data, the real impact of a molecular descriptor on the antioxidative activity was given as the product of the descriptor's value and its PLS coefficient.

Such QSAR analysis performed for both models (Figure 2) enabled the finding of molecular properties with the highest positive or negative impact on antioxidative activity.

Descriptors with the highest positive influence on antioxidative activity for both models have proven to be hydrophilic volume related to polarizability (W1) and possibility of a compound to donate H-bond (WO1). Consequently, their increase should lead to an increase of activity measured by both tests. The possibility of a compound to accept H-bond (WN1) and sum of hydrophobic surface areas (HSA) are both negatively correlated for both models, and their decrease should cause the increase of antioxidative activity in both tests.

2.2. Chemistry. All chosen amide derivatives were synthesized following two synthetic procedures shown in Schemes 1 and 2.³⁹ Cyano substituted benzamides 4–6 and 2-benzimidazolyl/2-benzothiazolyl substituted benzamides 20–25 were obtained from the methoxy substituted acyl-halogenides 1–3 and *p*-cyanoaniline 2 or cyano substituted 2-aminobenzimidazole 18 or 2-aminobenzothiazole 19 prepared by previously published synthetic procedures.⁴⁰

To obtain corresponding hydroxy substituted benzamides 7–10 and 26–29 bearing cyano substituent, the removal of methoxy protecting groups was accomplished by using either boron trichloride or boron tribromide in absolute dichloromethane at $-75\text{ }^{\circ}\text{C}$. Reaction yields of all compounds were moderate.

By using boron trichloride, only one methoxy group was removed from the compounds 5, 21, and 24, which allowed the synthesis of compounds 8, 27, and 29 with one methoxy and one hydroxy group. 2D NOESY NMR spectra confirmed that the methoxy group at the position 2 (R_1) was successfully deprotected. Target compounds with variable number of hydroxy 11–17 and 30–37 or methoxy groups 38–47 bearing amidino or 2-imidazolyl substituent were prepared in acidic two step Pinner reaction from corresponding cyano precursors due to reaction with $\text{NH}_3(\text{g})$ or ethylenediamine (EDA).

The progress of the reaction was monitored by IR spectroscopy. To ensure their better water solubility, all amidino substituted derivatives were prepared as hydrochloride salts. Because of some synthetic issues in the Pinner reaction, monomethoxy substituted analogues of amidino compounds 38–47 have not been successfully synthesized.

2.3. Antioxidative Activity. Results obtained from the DPPH test revealed that all tested compounds displayed improved or similar activity in comparison to the used standard, ascorbic acid (Table 2).

Table 2. Antioxidative Activity of Tested Amidino Substituted Compounds

compound	DPPH (μM)	superoxide test (μM)
11	59 ± 4	137 ± 4
12	66 ± 5	158 ± 8
13	39 ± 3	123 ± 4
14	89 ± 6	170 ± 8
15	70 ± 6	156 ± 7
16	51 ± 6	138 ± 5
17	42 ± 5	133 ± 7
30	42 ± 3	135 ± 4
31	38 ± 3	130 ± 5
32	49 ± 4	139 ± 5
33	46 ± 7	141 ± 6
34	56 ± 5	148 ± 6
35	65 ± 6	163 ± 4
36	80 ± 3	166 ± 3
37	58 ± 3	154 ± 5
38	59 ± 6	142 ± 8
39	52 ± 3	145 ± 4
40	66 ± 7	149 ± 7
41	73 ± 4	171 ± 7
42	57 ± 2	151 ± 6
43	71 ± 5	163 ± 7
44	85 ± 4	180 ± 9
45	82 ± 4	169 ± 5
46	94 ± 2	195 ± 9
47	73 ± 4	172 ± 8
ascorbic acid	79 ± 3	95 ± 2

Interestingly, a regular behavior was noticed in the pairs of tested bioisosteric compounds. The radical scavenging test for the compounds with amidino moiety always resulted in enhanced activity when compared to a compound having imidazole moiety in the same position (pairs are as follows: 11 and 14, 30 and 34, 32 and 36, 40 and 45, 12 and 15, 31 and 35, 33 and 37, 38 and 43, 41 and 46, 13 and 16, 39 and 44, 42 and 47).

According to the presented results, the compound with the highest antioxidative potential has proven to be 13, with activity almost 50% better in comparison to the control compound (ascorbic acid). The pair of compounds including 13 and 16 is the only one having two hydroxy groups in positions 2 and 4 and thus has a highest potential for radical transfer to stable DPPH radical and still maintain his stability by resonance stabilization. Compared to compound 13, its bioisosteric compound 11, having hydroxy group on first phenyl ring in position 2, and compound 12, with hydroxy and methoxy groups placed in positions 2 and 4, respectively, showed significantly lower activity, although still better than the control compound. From this result, we can conclude that hydroxy groups are important to promote radical scavenging activity. This is in accordance with QSAR analysis showing that the values of descriptors WO1 and W1 are higher for compounds 11–13 comparing to ascorbic acid, while the opposite is true for the descriptors WN1 and HSA (Figure 3).

In the second performed antioxidative test, which included measuring capability of compounds for superoxide anion radical scavenging, all compounds showed lower activity compared to ascorbic acid. Nevertheless, compound 13 is the most active in this test as well.

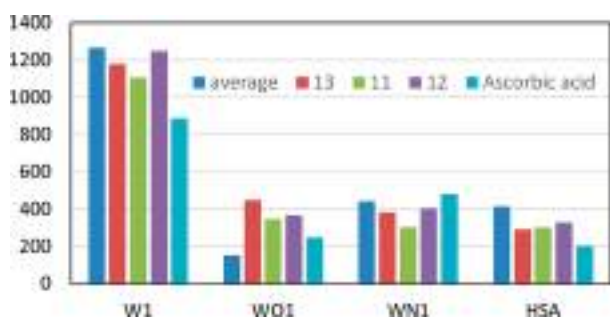


Figure 3. Comparison of values of the descriptors with the highest influence on activity for the compounds 11–13, average value, and ascorbic acid.

2.4. Comparison of Predicted and Experimental Antioxidative Activities. Internal validation using cross validation procedure and validation by external prediction are the most often used methods for validation of QSAR models.

However, the experimental measurements of the activities of novel compounds whose activities were previously predicted by the QSAR model should be the most direct and reliable method for validation of prediction ability of built QSAR model. The antioxidative activities of 25 novel compounds (11–17 and 30–47) were predicted by using model 1 and model 2. Therefore, experimental measurements of their antioxidative activity enabled direct validation of the obtained 3D-QSAR models (Table 3 and Figure 4). Antioxidative activities of all 25 newly synthesized compounds are correctly predicted by both models, that is, experimentally measured activities for all novel compounds are within standard deviation

Table 3. Antiproliferative Activity *in Vitro* against HeLa Cells

comp.	IC ₅₀ (μM) MTT test on HeLa
11	>200
12	>200
13	172.9 ± 15.44
14	>200
15	>200
16	195.1 ± 6.99
17	78.0 ± 2.22
30	167.2 ± 10.93
31	133.6 ± 12.25
32	192.0 ± 7.99
33	41.4 ± 5.15
34	194.6 ± 7.65
35	>200
36	23.0 ± 0.40
37	>200
38	129.2 ± 5.61
39	175.6 ± 1.28
40	20.1 ± 5.71
41	>200
42	32.8 ± 0.21
43	40.9 ± 7.57
44	117.4 ± 10.90
45	13.6 ± 1.24
46	>200
47	55.4 ± 0.54
cisplatin	2.5 ± 0.24

of error of prediction (SDEP) of the models estimated by cross validation procedure (Figure 3). Correlation coefficients (R^2) between experimental and predicted values of antioxidative activities were 0.82 and 0.78 for the models 1 and 2, respectively. Furthermore, comparison of experimental and predicted activities shows that for a majority of the novel compounds (18 out of 25), predicted antioxidative activity is within experimental error (Figure 4). For seven compounds for which discrepancy of predicted and experimental activity is larger than standard deviation of experimental measurements, predicted values are smaller than experimental, showing that models slightly underestimated antioxidative activity of these compounds.

2.5. Antiproliferative, Antibacterial, and Antifungal Activity. Additionally, to explore and confirm the biological potential of newly prepared compounds, antiproliferative activity *in vitro*, antibacterial, and antifungal activities were evaluated. As the antiproliferative potency of suchlike compounds was previously confirmed in our published papers, the synthesized compounds were tested against HeLa cancer cells. Obtained results are depicted in the Table 3 while cisplatin was used as a standard anticancer drug. Obtained results indicated that the majority of tested compounds showed moderate to low activity at micromolar inhibition concentrations.

In general, it could be concluded that benzothiazole derivatives showed stronger antiproliferative activity in comparison to their benzimidazole analogues with the exception of 2,4-dimethoxy substituted benzimidazole 43 bearing 2-imidazolyl group. Thus, the strongest antiproliferative activity against HeLa cells displayed 2-hydroxy 36 (IC₅₀ = 23.0 μM) and 2,4-dimethoxy 45 (IC₅₀ = 13.6 μM) substituted benzothiazoles bearing 2-imidazolyl group and 2-methoxy 40 (IC₅₀ = 20.1 μM) and 3,4,5-trimethoxy 42 (IC₅₀ = 32.8 μM) substituted benzothiazoles bearing amidino substituent. All tested amidino substituted benzamides 11–17 showed very low antiproliferative activity.

Furthermore, the *in vitro* antibacterial activity of the prepared compounds was evaluated against eight different bacterial strains fully sensitive to antibiotics. Gram-positive bacterial strains included key representatives of human pathogens including *S. aureus*, *M. luteus*, *B. subtilis*, and *C. sporogenes*, while the panel of Gram-negative bacteria included *E. coli*, *P. hauseri*, *P. aeruginosa*, and *K. pneumonia*. Obtained results were displayed in Table 4 as the minimal inhibitory concentrations (MICs) and are compared to antibiotic amikacin used as a standard. Table 4 shows only results for compounds, which showed some activity.

The obtained results revealed that the compound with the most significant antibacterial activity proved to be 3,4,5-trimethoxy substituted benzimidazole derivative 39 bearing amidino group (MICs value 0.37–1.54 μg/mL) without conspicuous selectivity between Gram-negative and Gram-positive bacteria. The highest activity was observed against *P. hauseri*, *P. aeruginosa*, and *B. subtilis* bacteria with MIC value of 0.37 μg/mL. 3,4,5-Trihydroxy substituted benzamide derivative 17 bearing 2-imidazolyl group showed also promising antibacterial activity with MICs values of 0.89–1.77 μg/mL. Interestingly, compounds 36, 40, 42, and 45, which showed the most pronounced antiproliferative activity, were not active on tested bacterial strains at all. In addition, the antifungal activity of prepared compounds was also tested against tree strains including *C. albicans*, *A. brasiliensis*, and *S. cerevisiae*.

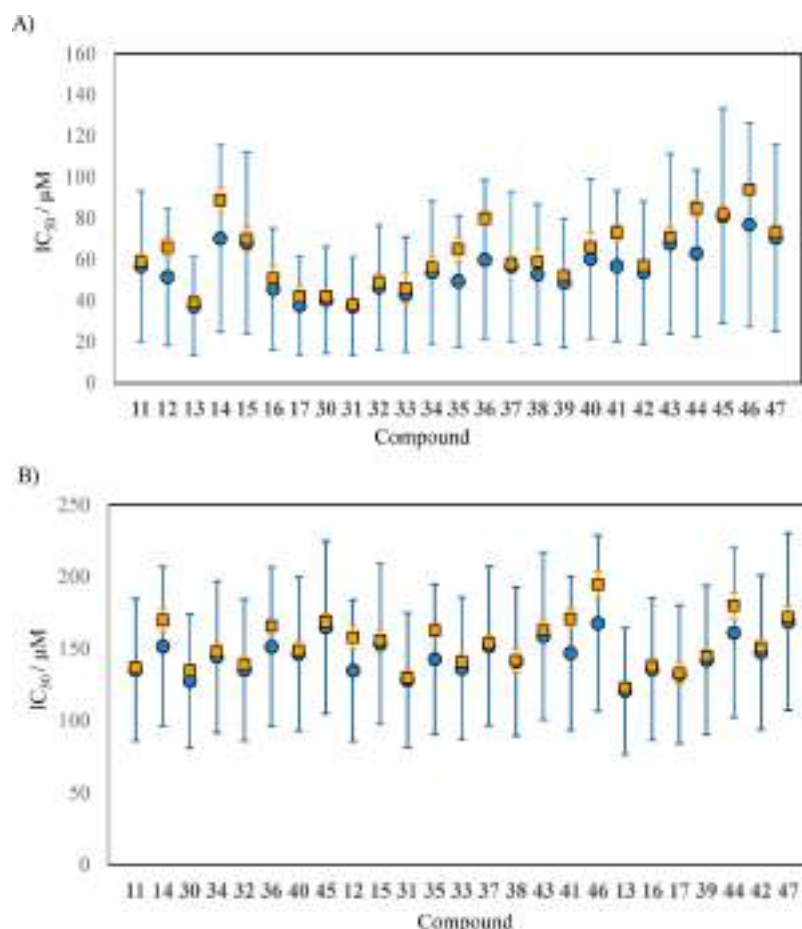


Figure 4. Comparison of experimental (orange squares) and computationally predicted (blue circles) antioxidative activities of the synthesized compounds expressed as IC₅₀. Error bars colored blue on the blue circles present standard deviation of error of prediction (SDEP) of the 3D-QSAR model used to predict activities, (A) model 1 and (B) model 2, while orange error bars on orange squares present standard deviation of experimental measurements.

Table 4. Antibacterial Activity of Tested Compounds

comp.	MIC (mM)							
	<i>P. hauseri</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>M. luteus</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. sporogenes</i>
11	4.23	2.14	2.14	2.14	4.23	2.14	0.13	0.27
13	2.03	2.03	2.03	4.06	4.06	2.03	1.02	2.03
17	0.89	0.89	1.77	0.89	1.77	1.77	0.89	0.89
31	3.46	3.46	3.46	3.46	6.91	6.91	3.46	3.46
32	7.17	7.17	7.17	7.17	7.17	7.17	7.17	7.17
34	6.99	3.50	6.99	6.99	6.99	6.99	6.99	3.50
39	0.37	0.37	0.77	0.77	0.77	0.77	0.37	1.54
43	3.11	6.22	6.22	6.22	6.22	3.11	1.56	0.78
47	1.39	1.39	0.70	1.39	1.39	1.39	0.70	1.39
amikacin	0.012	0.085	0.009	0.003	0.014	0.019	0.072	0.026

The obtained results for compounds which displayed some activity are presented in Table 5 including the nystatin, which was used as a standard antifungal drug.

In general, the obtained results revealed that some tested compounds showed better and improved antifungal activity in comparison to antibacterial activity. The most pronounced and significantly improved antifungal activity of tested compounds was observed against *C. albicans* as well as against *S. cerevisiae* strains compared to the standard drug nystatin. Only 2-hydroxy substituted benzothiazole derivative bearing amidino group 32 showed weaker antifungal activity against *S. cerevisiae*

in comparison to nystatin. The most active compounds with high antifungal potential have proven to be 2-hydroxy substituted benzimidazole 34 and 2,4-dihydroxy substituted benzamide 16 both bearing 2-imidazolynyl group with MIC values of 0.11 and 0.12 μg/mL. Also, among all compounds showing antifungal activity, benzimidazole derivatives displayed more pronounced antifungal activity in comparison to their benzothiazole analogues. In conclusion, it is obvious that this type of amidino substituted benzimidazole and benzothiazole derivatives has a high potential for further optimization and design of very efficient antifungal agents.

Table 5. Antifungal Activity of Tested Compounds

comp.	MIC (mM)		
	<i>C. albicans</i>	<i>A. brasiliensis</i>	<i>S. cerevisiae</i>
11	0.54	1.07	0.54
12	0.25	1.94	0.25
13	0.26	8.12	0.51
15	0.90	1.80	0.45
16	0.12	0.94	0.12
17	0.19	0.94	0.19
31	0.22	1.73	0.11
32	0.90	1.79	1.79
33	0.82	3.30	0.41
34	0.11	1.75	0.11
38	0.21	0.83	0.11
39	0.19	1.54	0.39
40	0.11	3.45	0.43
43	0.20	0.78	0.20
44	0.18	1.44	0.18
45	0.20	3.21	0.10
47	0.69	1.34	0.35
nystatin	2.70	1.35	1.35

3. CONCLUSION

Within this paper, we present the design and synthesis of 25 novel amidino substituted benzamides that differ in the heteroaromatic nuclei attached to the benzamide moiety as well as in the number of methoxy and hydroxy groups. All synthesized benzamide derivatives were also, besides with methoxy and hydroxy groups, substituted with either amidino or 2-imidazolynyl group and were prepared within the acidic Pinner reaction from the corresponding cyano substituted precursors. To ensure better solubility, all amidino derivatives were prepared as their hydrochloride salts.

For prediction of the antioxidative activities of 25 novel compounds, 3D-QSAR models were generated and validated. Comparison of computationally predicted and experimentally obtained activities indicated that the 3D-QSAR method can be successfully applied for design of novel methoxy and hydroxy substituted heteroaromatic amides as well as to successfully predict their antioxidative potential.

The experimental evaluation of antioxidative activity revealed that hydroxy groups are essential and promote radical scavenging activity. The most pronounced antioxidative activity was observed for the compound 13. DPPH test showed that all novel compounds showed improved or similar antioxidative activity compared to standard, ascorbic acid. Furthermore, the majority of tested compounds displayed moderate to low antiproliferative activity at micromolar inhibitory concentrations. In general, it could be concluded that benzothiazole derivatives showed stronger antiproliferative activity in comparison to their benzimidazole analogues. The strongest antiproliferative activity against HeLa cancer cells showed the compound 45. Additionally, to confirm the great biological potential of suchlike derivatives, the antibacterial and antifungal activities were evaluated. The compound with the most significant *in vitro* antibacterial activity proved to be 3,4,5-trimethoxy substituted benzimidazole derivative 39. The antifungal activity of the tested compounds was significantly better than their antibacterial activity, while the majority of tested compounds showed improved activity in comparison to standard.

To conclude, all presented results of biological activities evaluation confirmed the high biological potential of amidino substituted benzimidazole and benzothiazole derivatives, with several compounds that could be chosen as lead compounds for a further structure modification and optimization of suchlike scaffold to developed more efficient biologically active molecules with enhanced antioxidant potential.

4. EXPERIMENTAL SECTION

4.1. 3D-QSAR Modeling. **4.1.1. Data Set.** 3D-QSAR models were generated using the data on antioxidative activities of methoxy and hydroxy substituted heteroaromatic amides found in the literature. For each compound, antioxidative activities were measured using two tests, DPPH stable radical and superoxide tests, and expressed as IC_{50} values, that is, concentration that causes 50% antioxidative activity. For the purpose of generating 3D-QSAR models, pIC_{50} (negative logarithm of IC_{50} value expressed in $mol\ dm^{-3}$) was calculated. Data set for model 1, generated using activities against DPPH stable radical, consisted of 59 compounds from the literature, while data set for model 2, generated using activities obtained with superoxide test, consisted of 32 compounds from the literature.

4.1.2. Molecular Descriptors. Molecular descriptors for each compound were generated with program Volsurf+. Starting from the SMILES codes, 3D structures were generated using VolSurf+ 3D structure generator. Considering high rigidity of the data set compounds, VolSurf+ 3D structure generator was considered to be powerful enough to produce the most relevant conformers, and no additional conformational search was applied. Using the following probes, H₂O (the water molecule), O (sp^2 carbonyl oxygen atom), N1 (neutral NH group (e.g., amide)), and DRY (the hydrophobic probe), molecular interaction fields (MIFs) were calculated. From the MIFs, 128 descriptors with clear chemical and physical meaning were calculated for each compound. The detailed description of all 128 VolSurf+ descriptors is given in the VolSurf+ manual.^{35,36}

4.1.3. Partial Least Square and Principal Component Analysis. Partial least square (PLS) analysis was applied with goal of finding the relationship between the 3D structure-based molecular descriptors and antioxidative activities. The number of significant latent variables (nLV) and quality of the models were determined using the leave-one-out (LOO) cross validation procedures and leave many out (LMO) cross validation procedure with eight random groups. Also, for each model, standard deviation of error of calculation (SDEC) and standard deviation of error of prediction (SDEP) were calculated. Validation of predictive ability of each model was also performed using external validation. For that purpose, the data set was divided into the training set, that was used to build the model, and the test set, that was used to validate predictive ability of the model. Standard deviation of error of prediction (SDEP) for external prediction was calculated for each model.

To identify molecular descriptors with the highest positive or negative impact on antioxidative activities of studied compounds, analyses of PLS coefficients of the 3D-QSAR models were performed. Models were built using raw data (without autoscaling pretreatment of descriptors), and the quantitative influence of each descriptor on the antiproliferative activity was given as product of the descriptor's value and its PLS coefficient.

Principal component analysis (PCA) was performed on the molecular descriptors of the complete data set (all 59 compounds). PCA loadings were used for identification of the descriptors with the highest contribution to the overall variance in the X-space.

4.2. Synthesis. **4.2.1. General Methods.** All chemicals and solvents were purchased from commercial available suppliers including Aldrich, Fluka, and Acros. Melting points were recorded on an Original Keller Mikroheitztisch apparatus (Reichert, Wien), SMP11 Bibby, and Büchi 535 apparatus and were not corrected. ¹H and ¹³C NMR spectra were recorded on a Varian Gemini 300 or Varian Gemini 600 spectrophotometer at 300, 600, 150, and 75 MHz, respectively. All NMR spectra were measured in DMSO-*d*₆ solutions

using TMS as an internal standard. Chemical shifts are reported in ppm (δ) relative to TMS. All compounds were routinely checked by thin layer chromatography (TLC) using precoated Merck silica gel 60F-254 plates and the spots were detected under UV light (254 nm). Column chromatography (CC) was performed using silica gel (0.063–0.2 mm) Fluka; glass column was slurry-packed under gravity.

4.2.2. General Method for the Preparation of Carboxamides 20–25. To a solution of mono-, di-, or trimethoxy substituted benzoyl-chloride in dry toluene, corresponding amine was added followed by the addition of Et_3N . The reaction mixture was refluxed for 24 h. After cooling, the resulting product was filtered off and washed with diluted HCl and water to obtain pure solid.

4.2.2.1. 2-Methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]-benzamide 20. Following the general method, from 0.32 g (1.9 mmol) of 2-methoxybenzoylchloride **1**, 0.30 g (1.9 mmol) of 2-amino-5(6)-cyano-1H-benzimidazole **18**, and 0.27 g (2.7 mmol) of Et_3N in dry toluene (15 mL), 0.47 g (86%) of white powder was obtained; mp 234–237 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 11.55 (s, 1H, NH_{amide}), 7.92 (s, 1H, H_{arom}), 7.79 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.6$ Hz, 1H, H_{arom}), 7.63 (t, $J = 7.2$ Hz, 2H, H_{arom}), 7.55 (t, $J = 8.2$ Hz, 1H, H_{arom}), 7.25 (d, $J = 8.4$ Hz, 1H, H_{arom}), 7.13 (t, $J = 7.5$ Hz, 1H, H_{arom}), 3.96 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ /ppm): 165.1, 157.1, 148.6, 133.6, 130.2, 125.1, 121.9, 120.7 (2C), 120.2, 112.3 (2C), 56.2.

4.2.2.2. 2-Methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 23. Following the general method, from 0.29 g (1.7 mmol) of 2-methoxybenzoylchloride **1**, 0.30 g (1.7 mmol) of 2-amino-6-cyanobenzothiazole **19**, and 0.24 g (2.4 mmol) of Et_3N in dry toluene (15 mL), 0.40 g (77%) of white powder was obtained; mp 238–241 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 12.40 (s, 1H, NH_{amide}), 8.62 (d, $J = 1.1$ Hz, 1H, H_{arom}), 7.92 (d, $J = 8.4$ Hz, 1H, H_{arom}), 7.86 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz, 1H, H_{arom}), 7.75 (dd, $J_1 = 7.7$, $J_2 = 1.7$ Hz, 1H, H_{arom}), 7.62 (t, $J = 7.0$ Hz, 1H, H_{arom}), 7.25 (d, $J = 8.3$ Hz, 1H, H_{arom}), 7.13 (t, $J = 7.5$ Hz, 1H, H_{arom}), 3.95 (s, 3H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ /ppm): 165.9, 162.1, 157.8, 152.2, 134.4, 132.8, 130.8, 130.1, 127.6, 121.7, 121.2, 119.6, 112.8, 105.9, 56.6.

4.2.2.3. 2,4-Dimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 24. Following the general method, from 1.94 g (9.7 mmol) of 2,4-dimethoxybenzoylchloride **2**, 1.7 g (9.7 mmol) of 2-amino-6-cyanobenzothiazole **19**, and 1.37 g (13.6 mmol) of Et_3N in dry toluene (30 mL), 2.58 g (78%) of white powder was obtained; mp >280 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 11.89 (s, 1H, NH_{amide}), 8.60 (d, $J = 1.0$ Hz, 1H, H_{arom}), 7.93–7.82 (m, 3H, H_{arom}), 6.78–6.70 (m, 2H, H_{arom}), 4.00 (s, 3H, CH_3), 3.88 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ /ppm): 164.5, 164.1, 161.6, 159.4, 151.7, 132.7, 132.4, 129.6, 127.0, 121.1, 119.2, 112.5, 106.5, 105.3, 98.7, 56.5, 55.8.

4.2.2.4. 3,4,5-Trimethoxy-N-(6-cyanobenzothiazol-2-yl)-benzamide 25. Following the general method, from 2.95 g (13.0 mmol) of 3,4,5-trimethoxybenzoylchloride **3**, 2.24 g (13.0 mmol) of 2-amino-6-cyanobenzothiazole **19**, and 1.88 g (13.0 mmol) of Et_3N in dry toluene (30 mL), 3.30 g (69%) of white powder was obtained; mp 251–253 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ /ppm): 13.16 (brs, 1H, NH_{amide}), 8.62 (s, 1H, H_{arom}), 7.92 (d, $J = 8.3$ Hz, 1H, H_{arom}), 7.87 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.5$ Hz, 1H, H_{arom}), 7.54 (s, 2H, H_{arom}), 3.90 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ /ppm): 165.7, 163.3, 153.3 (2C), 142.0, 130.2, 127.6, 126.5, 121.6, 119.7, 106.5 (2C), 105.9, 60.6, 56.6 (2C).

4.2.3. General Method for Preparation of Hydroxy Substituted Compounds 7–10 and 26–29. Corresponding methoxy substituted compound was dissolved in dry dichloromethane, cooled to –78 °C, and BCl_3 or BBr_3 was added under argon atmosphere. The reaction mixture was stirred at that temperature for several hours and kept refrigerated overnight. The reaction was quenched by the addition of methanol, solvent was evaporated to dryness, and the residue was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$).

4.2.3.1. 2-Hydroxy-N-(4-cyanophenyl)benzamide 7. Following the general method, from 0.37 g (1.5 mmol) of 2-methoxy-N-(4-cyanophenyl)benzamide **4** and 5.0 mL of BCl_3 (2.2 mmol) in absolute

dichloromethane (20 mL) under argon, 0.31 g (85%) of white powder was obtained; mp 177–180 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 11.38 (brs, 1H, OH), 10.65 (s, 1H, NH_{amide}), 7.94 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.88 (dd, $J_1 = 7.9$ Hz, $J_2 = 1.6$ Hz, 1H, H_{arom}), 7.83 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.45 (m, 1H, H_{arom}), 7.02–6.95 (m, 2H, H_{arom}); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ /ppm): 166.5, 157.5, 142.7, 133.7, 133.2 (2C), 129.5, 120.4 (2C), 119.2, 119.0, 118.5, 117.1, 105.6.

4.2.3.2. 2-Hydroxy-4-methoxy-N-(4-cyanophenyl)benzamide 8. Following the general method, from 0.50 g (1.8 mmol) of 2,4-dimethoxy-N-(4-cyanophenyl)benzamide **5** and 10.0 mL of BCl_3 (5.3 mmol) in absolute dichloromethane (30 mL) under argon, 0.21 g (44%) of beige powder was obtained; mp 222–225 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ /ppm): 11.99 (brs, 1H, OH), 10.50 (s, 1H, NH_{amide}), 7.95 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.92 (d, $J = 9.1$ Hz, 1H, H_{arom}), 7.82 (d, $J = 8.8$ Hz, 1H, H_{arom}), 6.58 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.4$ Hz, 1H, H_{arom}), 6.52 (d, $J = 2.4$ Hz, 1H, H_{arom}), 3.80 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ /ppm): 167.0, 164.0, 161.0, 142.7, 133.1 (2C), 130.7, 120.7 (2C), 119.0, 109.5, 106.5, 105.5, 101.3, 55.5.

4.2.3.3. 2,4-Dihydroxy-N-(4-cyanophenyl)benzamide 9. Following the general method, from 0.50 g (1.8 mmol) of 2,4-dimethoxy-N-(4-cyanophenyl)benzamide **5** and 10.0 mL of BBr_3 (2.7 mmol) in absolute dichloromethane (30 mL) under argon, 0.06 g (13%) of beige powder was obtained; mp 250–253 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 11.87 (brs, 1H, OH), 10.44 (s, 1H, NH_{amide}), 10.26 (brs, 1H, OH), 7.91 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.87 (d, $J = 8.7$ Hz, 1H, H_{arom}), 7.81 (d, $J = 8.7$ Hz, 2H, H_{arom}), 6.41 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.3$ Hz, 1H, H_{arom}), 6.38 (d, $J = 2.3$ Hz, 1H, H_{arom}); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ /ppm): 167.5, 163.3, 161.4, 143.3, 133.6 (2C), 131.5, 121.0 (2C), 119.5, 108.7, 108.2, 105.8, 103.3.

4.2.3.4. 3,4,5-Trihydroxy-N-(4-cyanophenyl)benzamide 10. Following the general method, from 0.50 g (1.6 mmol) of 3,4,5-trimethoxy-N-(4-cyanophenyl)benzamide **6** and 15.0 mL of BBr_3 (15.0 mmol) in absolute dichloromethane (30 mL) under argon, 0.31 g (71%) of white powder was obtained; mp 273–275 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 10.30 (s, 1H, NH_{amide}), 9.22 (s, 2H, OH), 8.91 (s, 1H, OH), 7.97 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.78 (d, $J = 8.7$ Hz, 2H, H_{arom}), 6.97 (s, 2H, H_{arom}); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ /ppm): 166.6, 146.0, 144.4, 137.8, 133.5 (2C), 124.7, 120.4 (2C), 119.7, 107.9 (2C), 105.2.

4.2.3.5. 2-Hydroxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]-benzamide 26. Following the general method, from 0.50 g (1.7 mmol) of 2-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]-benzamide **20** and 10.0 mL of BBr_3 (10.0 mmol) in absolute dichloromethane (25 mL) under argon, 0.35 g (74%) of white powder was obtained; mp >280 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 12.91 (brs, 2H, $\text{NH}_{\text{benzimidazole}}$, OH), 8.02 (dd, $J_1 = 7.7$ Hz, $J_2 = 1.3$ Hz, 1H, H_{arom}), 7.90 (s, 1H, H_{arom}), 7.69–7.57 (m, 2H, H_{arom}), 7.44 (t, $J = 7.0$ Hz, 1H, H_{arom}), 6.94 (m, 2H, H_{arom}); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ /ppm): 200.7, 173.8, 165.9, 164.3, 150.2, 124.2, 116.1, 108.9.

4.2.3.6. 2-Hydroxy-4-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 27. Following the general method, from 0.20 g (0.6 mmol) of 2,4-dimethoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]-benzamide **21** and 3.6 mL of BBr_3 (3.6 mmol) in absolute dichloromethane (15 mL) under argon, 0.14 g (59%) of white powder was obtained; mp >280 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 12.05 (brs, 2H, $\text{NH}_{\text{benzimidazole}}$, NH_{amide}), 8.58 (s, 1H, H_{arom}), 8.01 (d, $J = 8.9$ Hz, 1H, H_{arom}), 7.86 (s, 2H, H_{arom}), 6.63 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.4$ Hz, 1H, H_{arom}), 6.58 (d, $J = 2.3$ Hz, 1H, H_{arom}), 6.47 (s, 1H, H_{arom}), 3.82 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ /ppm): 167.6, 164.2, 131.7 (2C), 129.6, 126.5, 119.6 (2C), 106.5, 104.1, 101.0 (2C), 55.4.

4.2.3.7. 2-Hydroxy-N-(6-cyanobenzothiazol-2-yl)benzamide 28. Following the general method, from 0.50 g (1.6 mmol) of 2-methoxy-N-[5(6)-cyano-1H-benzothiazol-2-yl]benzamide **23** and 10.0 mL of BBr_3 (10.0 mmol) in absolute dichloromethane (25 mL) under argon, 0.37 g (78%) of white powder was obtained; mp >280 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ /ppm): 12.30 (brs, 1H, NH_{amide}), 11.77

(brs, 1H, OH), 8.61 (s, 1H, H_{arom}), 7.99 (dd, $J_1 = 7.9$ Hz, $J_2 = 1.6$ Hz, 1H, H_{arom}), 7.93–7.84 (m, 2H, H_{arom}), 7.53 (t, $J = 7.7$ Hz, 1H, H_{arom}), 7.13–6.99 (m, 2H, H_{arom}); ^{13}C NMR (150 MHz, DMSO- d_6) (δ /ppm): 144.0, 134.9, 130.6, 129.8, 127.2, 119.9, 119.1, 117.6, 117.2, 105.5.

4.2.3.8. 2-Hydroxy-4-methoxy-N-(6-cyanobenzothiazol-2-yl)-benzamide 29. Following the general method, from 0.50 g (1.5 mmol) of 2,4-dimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide **24** and 0.65 g (5.6 mmol) of BCl_3 in dichloromethane (30 mL) under argon, 0.12 g (25%) of yellow powder was obtained; mp $>280^\circ\text{C}$; ^1H NMR (300 MHz, DMSO- d_6) (δ /ppm): 12.21 (brs, 2H, OH, NH_{amide}), 8.60 (s, 1H, H_{arom}), 8.03 (d, $J = 8.8$ Hz, 1H, H_{arom}), 7.88 (s, 2H), 6.65 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.2$ Hz, 1H, H_{arom}), 6.59 (d, $J = 2.1$ Hz, 1H, H_{arom}), 3.84 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6) (δ /ppm): 165.2, 132.7, 130.3, 127.6, 119.6, 107.7, 105.9, 101.8, 56.0.

4.2.4. General Method for Preparation of Amidines. Dry HCl gas was bubbled for 4 h into a cooled suspension (0°C) of nitrile compound in anhydrous ethanol or methoxyethanol. The suspension was stirred at room temperature until the $-\text{CN}$ band was undetectable (IR). After anhydrous diethyl ether was added, the corresponding imidate ester was filtered off and dried *in vacuo*. The product was then suspended in anhydrous ethanol, and the corresponding amine was added. For the synthesis of unsubstituted amidines, the suspension was treated with anhydrous ammonia gas and stirred for 24 h at room temperature. For the synthesis of imidazolyl-substituted amidines, excess of ethylenediamine (3.5 mol equiv) was added to a suspension of imidate ester, and the reaction mixture was stirred at reflux for 24 h. The crude product was then filtered off and washed with diethyl ether to give powder products, which were suspended in anhydrous ethanol and saturated with HCl gas. The reaction mixture was stirred at room temperature for 24 h. The products were filtered off.

4.2.4.1. 2-Hydroxy-N-(4-amidinophenyl)benzamide 11. The compound was prepared following the general method. A suspension of 0.19 g (0.8 mmol) of 2-hydroxy-N-(4-cyanophenyl)benzamide **7** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 5 days. The crude imidate was filtered off, suspended in absolute ethanol (8 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.98 g (87%) of light yellow powder; mp $270\text{--}273^\circ\text{C}$; ^1H NMR (300 MHz, DMSO- d_6) (δ /ppm): 15.69 (brs, 1H, NH_{amide}), 8.92 (brs, 4H, $\text{NH}_{\text{amidine}}$), 7.84 (s, 4H, H_{arom}), 7.2 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.8$ Hz, 1H, H_{arom}), 7.07–7.01 (m, 1H, H_{arom}), 6.49 (d, $J = 8.2$ Hz, 1H, H_{arom}), 6.27 (t, $J = 7.3$ Hz, 1H, H_{arom}), 4.35 (brs, 1H, OH); ^{13}C NMR (150 MHz, DMSO- d_6) (δ /ppm): 169.9, 167.3, 164.8, 145.8, 132.6, 129.6, 129.2 (2C), 121.0, 120.9, 120.3, 118.9 (2C), 118.0, 111.2.

4.2.4.2. 2-Hydroxy-4-methoxy-N-(4-amidinophenyl)benzamide 12. The compound was prepared following the general method. A suspension of 0.12 g (0.4 mmol) of 2-hydroxy-4-methoxy-N-(4-cyanophenyl)benzamide **8** in anhydrous ethanol (10 mL) was saturated with dry HCl gas and stirred for 4 days. The crude imidate was filtered off, suspended in absolute ethanol (10 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.07 g (51%) of light rose powder; mp $256\text{--}259^\circ\text{C}$; ^1H NMR (600 MHz, DMSO- d_6) (δ /ppm): 15.47 (brs, 1H, NH_{amide}), 9.13 (brs, 4H, $\text{NH}_{\text{amidine}}$), 7.84 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.81 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.61 (d, $J = 8.7$ Hz, 1H, H_{arom}), 5.94 (d, $J = 2.1$ Hz, 1H, H_{arom}), 5.87 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.3$ Hz, 1H, H_{arom}), 4.36 (brs, 1H, OH), 3.67 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO- d_6) (δ /ppm): 172.8, 167.3, 164.9, 163.6, 146.6, 130.5, 129.2 (2C), 119.4, 118.6 (2C), 111.9, 103.5, 99.3, 54.4.

4.2.4.3. 2,4-Dihydroxy-N-(4-amidinophenyl)benzamide 13. The compound was prepared following the general method. A suspension of 0.30 g (1.2 mmol) of 2,4-dihydroxy-N-(4-cyanophenyl)benzamide **9** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imidate was filtered off, suspended in absolute ethanol (15 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.06 g (17%) of light brown powder; mp $>280^\circ\text{C}$; ^1H NMR (600 MHz, DMSO- d_6) (δ /ppm): 15.67 (brs, 1H, NH_{amide}), 8.94 (brs, 6H,

$\text{NH}_{\text{amidine}}$, OH), 7.82–7.78 (m, 4H, H_{arom}), 7.53 (d, $J = 8.6$ Hz, 1H, H_{arom}), 5.82 (s, 1H, H_{arom}), 5.78 (d, $J = 8.5$ Hz, 1H, H_{arom}); ^{13}C NMR (150 MHz, DMSO- d_6) (δ /ppm): 172.8, 167.7, 165.2, 162.4, 146.9, 131.2, 129.7 (2C), 120.0, 119.0 (2C), 111.5, 105.8, 101.6.

4.2.4.4. 2-Hydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 14. The compound was prepared following the general method. A suspension of 0.19 g (0.8 mmol) of 2-hydroxy-N-(4-cyanophenyl)benzamide **7** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 5 days. The crude imidate was filtered off and suspended in absolute ethanol. EDA (0.09 mL, 1.4 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.09 g (37%) of beige powder; mp $290\text{--}293^\circ\text{C}$; ^1H NMR (300 MHz, DMSO- d_6) (δ /ppm): 11.52 (brs, 1H, NH_{amide}), 10.79 (s, 1H, OH), 10.64 (brs, 2H, $\text{NH}_{\text{amidine}}$), 8.07 (d, $J = 8.8$ Hz, 2H, H_{arom}), 8.01 (d, $J = 8.7$ Hz, 2H, H_{arom}), 7.93 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.45 (t, $J = 7.6$ Hz, 1H, H_{arom}), 7.07 (d, $J = 8.2$ Hz, 1H, H_{arom}), 6.98 (t, $J = 7.5$ Hz, 1H, H_{arom}), 3.99 (s, 4H, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6) (δ /ppm): 166.4, 164.1, 157.5, 144.0, 133.7, 129.8 (2C), 129.7, 120.0 (2C), 119.2, 118.5, 117.1, 116.7, 44.2 (2C).

4.2.4.5. 2-Hydroxy-4-methoxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 15. The compound was prepared following the general method. A suspension of 0.20 g (0.8 mmol) of 2-hydroxy-4-methoxy-N-(4-cyanophenyl)benzamide **8** in anhydrous ethanol (35 mL) was saturated with dry HCl gas and stirred for 4 days. The crude imidate was filtered off and suspended in absolute ethanol. EDA (0.07 mL, 1.1 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.06 g (24%) of white powder; mp $292\text{--}295^\circ\text{C}$; ^1H NMR (600 MHz, DMSO- d_6) (δ /ppm): 12.13 (s, 1H, NH_{amide}), 10.72 (s, 3H, $\text{NH}_{\text{amidine}}$, OH), 8.09 (d, $J = 8.7$ Hz, 2H, H_{arom}), 8.06 (d, $J = 8.8$ Hz, 1H, H_{arom}), 8.01 (d, $J = 8.7$ Hz, 2H, H_{arom}), 6.61 (d, $J = 1.9$ Hz, 1H, H_{arom}), 6.58 (dd, $J_1 = 8.9$ Hz, $J_2 = 1.9$ Hz, 1H, H_{arom}), 3.99 (s, 4H, CH_2), 3.81 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO- d_6) (δ /ppm): 167.4, 164.5, 164.4, 161.3, 144.5, 131.5, 130.2 (2C), 120.7 (2C), 117.1, 110.1, 106.9, 101.8, 55.9, 44.7 (2C).

4.2.4.6. 2,4-Dihydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 16. The compound was prepared following the general method. A suspension of 0.14 g (0.6 mmol) of 2,4-dihydroxy-N-(4-cyanophenyl)benzamide **9** in anhydrous ethanol (10 mL) was saturated with dry HCl gas and stirred for 10 days. The crude imidate was filtered off and suspended in absolute ethanol. EDA (0.16 mL, 2.4 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.10 g (53%) of beige powder; mp $>280^\circ\text{C}$; ^1H NMR (300 MHz, DMSO- d_6) (δ /ppm): 11.96 (s, 1H, OH), 10.63–10.62 (m, 3H, $\text{NH}_{\text{amidine}}$, OH), 10.38 (s, 1H, NH_{amide}), 8.06 (d, $J = 8.7$ Hz, 2H, H_{arom}), 7.99 (d, $J = 8.8$ Hz, 2H, H_{arom}), 7.94 (d, $J = 8.6$ Hz, 1H, H_{arom}), 6.45–6.41 (m, 2H, H_{arom}), 3.99 (s, 4H, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6) (δ /ppm): 167.4, 164.6, 163.4, 161.2, 144.6, 131.7, 130.1 (2C), 120.6 (2C), 116.9, 108.7, 108.3, 103.3, 44.7 (2C).

4.2.4.7. 3,4,5-Trihydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 17. The compound was prepared following the general method. A suspension of 0.14 g (0.5 mmol) of 3,4,5-trihydroxy-N-(4-cyanophenyl)benzamide **10** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 10 days. The crude imidate was filtered off and suspended in absolute ethanol. EDA (0.05 mL, 0.8 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.03 g (24%) of yellow powder; mp $>280^\circ\text{C}$; ^1H NMR (300 MHz, DMSO- d_6) (δ /ppm): 10.50 (s, 2H, $\text{NH}_{\text{amidine}}$), 10.38 (s, 1H, NH_{amide}), 9.24 (s, 2H, OH), 9.01 (s, 1H, OH), 8.02 (d, $J = 9.2$ Hz, 2H, H_{arom}), 7.98 (d, $J = 9.2$ Hz, 2H, H_{arom}), 6.99 (s, 2H, H_{arom}), 3.98 (s, 4H, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6) (δ /ppm): 166.6, 164.8, 146.01, 145.8, 138.0, 130.0 (2C), 124.6, 120.0 (2C), 116.3, 107.9 (2C), 44.7 (2C).

4.2.4.8. 2-Hydroxy-N-[5(6)-amidino-1H-benzimidazol-2-yl]benzamide 30. The compound was prepared following the general method. A suspension of 0.14 g (0.5 mmol) of 2-hydroxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide **26** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 9 days. The crude

imide was filtered off, suspended in absolute ethanol (10 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.04 g (58%) of light yellow powder; mp $>280^\circ\text{C}$; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ/ppm): 17.49 (brs, 1H, OH), 12.44 (brs, 1H, NH_{amide}), 9.08 (s, 2H, $\text{NH}_{\text{imidazole}}$), 8.53 (s, 2H, NH_{amide}), 7.87–7.80 (m, 2H, H_{arom}), 7.50 (s, 1H, H_{arom}), 7.45 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.15 (t, $J = 7.5$ Hz, 1H, H_{arom}), 6.63 (d, $J = 8.2$ Hz, 1H, H_{arom}), 6.53 (t, $J = 7.2$ Hz, 1H, H_{arom}); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ/ppm): 170.6, 166.9 (2C), 166.4, 132.8, 129.5 (2C), 120.6, 119.4, 119.1 (2C), 114.4.

4.2.4.9. 2-Hydroxy-4-methoxy-N-[5(6)-amidino-1H-benzimidazol-2-yl]benzamide 31. The compound was prepared following the general method. A suspension of 0.08 g (0.3 mmol) **27** in anhydrous ethanol (10 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off, suspended in absolute ethanol (10 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.05 g (57%) of light brown powder; mp $255\text{--}260^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ/ppm): 17.12 (brs, 1H, OH), 12.23 (brs, 1H, $\text{NH}_{\text{benzimidazole}}$), 8.76 (brs, 4H, NH_{amide}), 7.83 (s, 1H, H_{arom}), 7.70 (s, 1H, H_{arom}), 7.48 (s, 2H, H_{arom}), 6.09 (s, 2H, H_{arom}), 3.70 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ/ppm): 166.1, 165.5, 163.9, 163.1, 154.8, 132.9, 131.4, 130.5, 121.4, 121.1, 119.3, 118.4, 101.2, 55.2.

4.2.4.10. 2-Hydroxy-N-(6-amidinobenzothiazol-2-yl)benzamide 32. The compound was prepared following the general method. A suspension of 0.20 g (0.7 mmol) of **28** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 10 days. The crude imide was filtered off, suspended in absolute ethanol (10 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.03 mg (12%) of beige powder; mp $>280^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ/ppm): 15.75 (brs, 1H, OH), 9.14 (brs, 2H, NH_{amide}), 8.63 (brs, 2H, NH_{amide}), 8.26 (d, $J = 1.3$ Hz, 1H, H_{arom}), 7.88 (dd, $J_1 = 7.7$ Hz, $J_2 = 1.7$ Hz, 1H, H_{arom}), 7.73–7.62 (m, 2H, H_{arom}), 7.25 (t, $J = 8.5$ Hz, 1H, H_{arom}), 6.83–6.69 (m, 2H, H_{arom}); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ/ppm): 171.3, 169.4, 165.8, 162.2, 155.4, 133.6, 132.9, 129.8, 125.4, 122.0, 120.3, 119.9, 119.0, 117.5, 117.4.

4.2.4.11. 2-Hydroxy-4-methoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 33. The compound was prepared following the general method. A suspension of 0.08 g (0.2 mmol) of 2-hydroxy-4-methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide **29** in anhydrous ethanol (10 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off, suspended in absolute ethanol (10 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.04 mg (40%) of white powder; mp $>280^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ/ppm): 13.02 (brs, 2H, OH, NH_{amide}), 9.29 (brs, 2H, NH_{amide}), 8.99 (brs, 2H, NH_{amide}), 8.47–8.43 (m, 1H, H_{arom}), 7.96–7.88 (m, 1H, H_{arom}), 7.85–7.75 (m, 2H, H_{arom}), 6.52 (s, 2H, H_{arom}), 3.80 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ/ppm): 165.5, 164.4, 160.6, 131.9, 129.6, 126.8, 126.0, 122.6, 122.3, 119.3, 109.5, 106.7, 104.9, 102.8, 101.4, 55.4.

4.2.4.12. 2-Hydroxy-N-[5(6)-(imidazolinil-2-yl)-1H-benzimidazol-2-yl]benzamide 34. The compound was prepared following the general method. A suspension of 0.14 g (0.5 mmol) of 2-hydroxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide **26** in anhydrous methoxyethanol (15 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off and suspended in absolute ethanol (10 mL). EDA (0.05 mL, 0.8 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.05 g (68%) of white powder; mp $>280^\circ\text{C}$; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ/ppm): 17.36 (brs, 1H, OH), 12.41 (brs, 1H, $\text{NH}_{\text{benzimidazole}}$), 9.63 (brs, 2H, NH_{amide}), 7.92 (s, 1H, H_{arom}), 7.86 (dd, $J_1 = 7.7$, $J_2 = 1.5$ Hz, 1H, H_{arom}), 7.57 (d, $J = 8.3$ Hz, 1H, H_{arom}), 7.51 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.17 (t, $J = 7.6$ Hz, 1H, H_{arom}), 6.65 (d, $J = 8.2$ Hz, 1H, H_{arom}), 6.56 (t, $J = 7.4$ Hz, 1H, H_{arom}), 3.94 (s, 4H, CH_2); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ/ppm): 165.7, 134.7, 130.7, 123.6, 119.4, 117.7 (2C), 116.4, 113.6, 44.8 (2C).

4.2.4.13. 2-Hydroxy-4-methoxy-N-[5(6)-(imidazolinil-2-yl)-1H-benzimidazol-2-yl]benzamide 35. The compound was prepared following the general method. A suspension of 0.08 mg (0.02 mmol)

of 2-hydroxy-4-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]-benzamide **27** in anhydrous methoxyethanol (10 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off and suspended in absolute ethanol (10 mL). EDA (0.02 mL, 0.4 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.02 g (38%) of white powder; mp $>280^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ/ppm): 10.02 (brs, 2H, NH_{amide}), 7.92 (s, 1H, H_{arom}), 7.74 (d, $J = 8.4$ Hz, 1H, H_{arom}), 7.59–7.49 (m, 2H, H_{arom}), 6.13–6.09 (m, 2H, H_{arom}), 3.95 (s, 4H, CH_2), 3.71 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ/ppm): 169.7, 166.5, 163.9, 155.8, 154.8, 133.7, 130.7, 125.6, 121.9, 121.1, 119.0, 114.6, 113.4, 112.1, 102.4, 55.2, 45.0 (2C).

4.2.4.14. 2-Hydroxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 36. The compound was prepared following the general method. A suspension of 0.20 g (0.7 mmol) of **28** in anhydrous ethanol (15 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off and suspended in absolute ethanol (10 mL). EDA (0.08 mL, 1.2 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.08 g (56%) of beige powder; mp $>280^\circ\text{C}$; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ/ppm): 15.69 (brs, 1H, OH), 9.49 (brs, 2H, NH_{amide}), 8.37 (s, 1H, H_{arom}), 7.90 (d, $J = 6.9$ Hz, 1H, H_{arom}), 7.82 (d, $J = 8.0$ Hz, 1H, H_{arom}), 7.70 (d, $J = 8.5$ Hz, 1H, H_{arom}), 7.28 (t, $J = 6.7$ Hz, 1H, H_{arom}), 6.80–6.76 (m, 2H, H_{arom}), 3.99 (s, 4H, CH_2); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ/ppm): 171.2, 165.6, 161.8, 155.7, 133.7, 133.1, 129.9, 125.7, 122.3, 120.1, 119.4, 118.0, 117.7, 117.5, 114.2, 44.7 (2C).

4.2.4.15. 2-Hydroxy-4-methoxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 37. The compound was prepared following the general method. A suspension of 0.08 g (0.2 mmol) of 2-hydroxy-4-methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide **29** in anhydrous ethanol (10 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off and suspended in absolute ethanol (10 mL). EDA (0.03 mL, 0.5 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.02 g (23%) of white powder; mp $>280^\circ\text{C}$; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ/ppm): 16.21 (brs, 1H, OH), 9.69 (brs, 2H, NH_{amide}), 8.33 (s, 1H, H_{arom}), 7.81–7.77 (m, 2H, H_{arom}), 7.66 (d, $J = 8.4$ Hz, 1H, H_{arom}), 6.33 (d, $J = 8.7$ Hz, 1H, H_{arom}), 6.31 (s, 1H, H_{arom}), 3.98 (s, 4H, CH_2), 3.75 (s, 3H, CH_3).

4.2.4.16. 2-Methoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 40. The compound was prepared following the general method. A suspension of 0.15 g (0.5 mmol) of 2-methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide **23** in anhydrous ethanol (20 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off, suspended in absolute ethanol (10 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.14 g (81%) of white powder; mp $258\text{--}261^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) (δ/ppm): 8.76 (brs, 4H, NH_{amide}), 8.22 (s, 1H, H_{arom}), 7.70 (d, $J = 7.7$ Hz, 1H, H_{arom}), 7.58–7.53 (m, 2H, H_{arom}), 7.32 (t, $J = 7.1$ Hz, 1H, H_{arom}), 7.03 (d, $J = 8.2$ Hz, 1H, H_{arom}), 6.95 (t, $J = 7.3$ Hz, 1H, H_{arom}), 3.79 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) (δ/ppm): 165.7, 157.4, 133.5, 130.0, 125.1, 121.7, 120.3, 118.3, 112.5, 56.0.

4.2.4.17. 2,4-Dimethoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 41. The compound was prepared following the general method. A suspension of 0.23 g (6.6 mmol) of 2,4-dimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide **24** in anhydrous methoxyethanol (20 mL) was saturated with dry HCl gas and stirred for 27 days. The crude imide was filtered off, suspended in absolute ethanol (15 mL), and dry NH_3 was bubbled into the suspension. The mixture was worked up as described to give 0.09 mg (33%) of white powder; mp $261\text{--}264^\circ\text{C}$; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) (δ/ppm): 11.86 (s, 1H, NH_{amide}), 8.58 (s, 1H, H_{arom}), 7.88 (d, $J = 8.3$ Hz, 1H, H_{arom}), 7.85–7.83 (m, 2H, H_{arom}), 6.75 (s, 1H, H_{arom}), 6.72 (d, $J = 8.5$ Hz, 1H, H_{arom}), 3.99 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) (δ/ppm): 164.7, 163.9, 161.6, 159.6, 151.8, 132.8, 132.6, 129.5, 126.8, 121.1, 119.0, 112.5, 106.8, 105.5, 99.0, 56.6, 55.8.

4.2.4.18. 3,4,5-Trimethoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 42. The compound was prepared following the general

method. A suspension of 0.18 g (0.5 mmol) of 3,4,5-trimethoxy-*N*-(6-cyanobenzothiazol-2-yl)benzamide **25** in anhydrous methoxyethanol (20 mL) was saturated with dry HCl gas and stirred for 70 days. The crude imide was filtered off, suspended in absolute ethanol (8 mL), and dry NH₃ was bubbled into the suspension. The mixture was worked up as described to give 0.05 mg (26%) of white powder; mp >280 °C; ¹H NMR (600 MHz, DMSO-*d*₆) (δ/ppm): 8.55 (s, 1H, H_{arom}), 7.89 (s, 2H, H_{arom}), 7.53 (s, 2H, H_{arom}), 7.39 (s, 4H, NH_{amide}), 3.87 (s, 6H, OCH₃), 3.74 (s, 3H, OCH₃).

4.2.4.19. 2-Methoxy-*N*-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide **45.** The compound was prepared following the general method. A suspension of 0.15 g (0.5 mmol) of 2-hydroxy-4-methoxy-*N*-(6-cyanobenzothiazol-2-yl)benzamide **29** in ethanol (20 mL) was saturated with dry HCl gas and stirred for 9 days. The crude imide was filtered off and suspended in absolute ethanol (10 mL). EDA (0.11 mL, 3.4 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.15 g (81%) of beige powder; mp >280 °C; ¹H NMR (600 MHz, DMSO-*d*₆) (δ/ppm): 10.84 (brs, 2H, NH_{amide}), 8.76 (s, 1H, H_{arom}), 8.09 (d, *J* = 8.5 Hz, 1H, H_{arom}), 7.96 (d, *J* = 8.5 Hz, 1H, H_{arom}), 7.77 (d, *J* = 6.3 Hz, 1H, H_{arom}), 7.60 (t, *J* = 7.2 Hz, 1H, H_{arom}), 7.25 (d, *J* = 8.4 Hz, 1H, H_{arom}), 7.12 (t, *J* = 7.5 Hz, 1H, H_{arom}), 4.00 (s, 4H, CH₂), 3.96 (s, 3H, OCH₃); ¹³C NMR (150 MHz, DMSO-*d*₆) (δ/ppm): 165.8, 165.4, 162.1, 158.0, 153.4, 134.5, 132.7, 130.9, 127.0, 123.7, 121.7, 121.4, 121.3, 117.6, 113.0, 56.8, 44.9 (2C).

4.2.4.20. 2,4-Dimethoxy-*N*-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide **46.** The compound was prepared following the general method. A suspension of 0.23 g (6.6 mmol) of 2-hydroxy-4-methoxy-*N*-(6-cyanobenzothiazol-2-yl)benzamide **29** in anhydrous methoxyethanol (20 mL) was saturated with dry HCl gas and stirred for 27 days. The crude imide was filtered off and suspended in absolute ethanol (10 mL). EDA (0.06 mL, 0.9 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.09 g (34%) of light yellow powder; mp 261–264 °C; ¹H NMR (600 MHz, DMSO-*d*₆) (δ/ppm): 10.64 (brs, 2H, NH_{amide}), 8.58 (d, *J* = 1.3 Hz, 1H, H_{arom}), 7.88 (d, *J* = 8.4 Hz, 1H, H_{arom}), 7.85–7.82 (m, 2H, H_{arom}), 6.75 (d, *J* = 2.1 Hz, 1H, H_{arom}), 6.71 (dd, *J*₁ = 8.7, *J*₂ = 2.2 Hz, 1H, H_{arom}), 3.99 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃); ¹³C NMR (150 MHz, DMSO-*d*₆) (δ/ppm): 164.7, 164.0, 161.6, 159.5, 151.8, 132.8, 129.6, 126.9, 121.1, 119.0, 112.5, 106.7, 105.5, 98.9, 56.6, 55.8.

4.2.4.21. 3,4,5-Trimethoxy-*N*-[6-(imidazolin-2-yl)benzothiazol-2-yl]benzamide **47.** The compound was prepared following the general method. A suspension of 0.18 g (0.5 mmol) of 3,4,5-trimethoxy-*N*-(6-cyanobenzothiazol-2-yl)benzamide **25** in anhydrous methoxyethanol (20 mL) was saturated with dry HCl gas and stirred for 70 days. The crude imide was filtered off and suspended in absolute ethanol (8 mL). EDA (0.05 mL, 1.7 mmol) was added, and the mixture was stirred at reflux for 24 h. The mixture was worked up as described to give 0.04 g (45%) of light yellow powder; mp >280 °C; ¹H NMR (600 MHz, DMSO-*d*₆) (δ/ppm): 13.32 (brs, 1H, NH_{amide}), 10.79 (s, 2H, NH_{amide}), 8.79 (s, 1H, H_{arom}), 8.14 (d, *J* = 8.2 Hz, 1H, H_{arom}), 8.01 (d, *J* = 8.6 Hz, 1H, H_{arom}), 7.57 (s, 2H, H_{arom}), 4.04 (s, 3H, OCH₃), 3.91 (s, 4H, CH₂), 3.11 (s, 6H, OCH₃); ¹³C NMR (150 MHz, DMSO-*d*₆) (δ/ppm): 165.7, 153.3, 142.0, 127.7, 124.8, 124.5, 106.5 (2C), 60.6, 56.6 (2C), 43.2 (2C).

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.9b00256.

General methods for synthesis, figures of NMR spectrum, pharmacology methods, antimicrobial activity tests, antioxidative tests, structures and activities of data set compounds used for QSAR models, computationally predicted activities of novel compounds, PCA plots (PDF)

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Notes

The authors declare no competing financial interest.

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