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Novel Biologically Active *N*-Substituted Benzimidazole Derived Schiff Bases: Design, Synthesis, and Biological Evaluation

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Abstract: Herein, we present the design and synthesis of novel *N*-substituted benzimidazole-derived Schiff bases, and the evaluation of their antiviral, antibacterial, and antiproliferative activity. The impact on the biological activity of substituents placed at the N atom of the benzimidazole nuclei and the type of substituents attached at the phenyl ring were examined. All of the synthesized Schiff bases were evaluated in vitro for their antiviral activity against different viruses, antibacterial activity against a panel of bacterial strains, and antiproliferative activity on several human cancer cell lines, thus enabling the study of the structure−activity relationships. Some mild antiviral effects were noted, although at higher concentrations in comparison with the included reference drugs. Additionally, some derivatives showed a moderate antibacterial activity, with precursor **23** being broadly active against most of the tested bacterial strains. Lastly, Schiff base **40,** a 4-*N*,*N*-diethylamino-2-hydroxy-substituted derivative bearing a phenyl ring at the N atom on the benzimidazole nuclei, displayed a strong antiproliferative activity against several cancer cell lines (IC_{50} 1.1–4.4 μM). The strongest antitumoral effect was observed towards acute myeloid leukemia (HL-60).

Keywords: antiproliferative activity; antiviral activity; benzimidazoles; cytotoxicity; Schiff bases

Citation: Beč, A.; Cindrić, M.; Persoons, L.; Banjanac, M.; Radovanović, V.; Daelemans, D.; Hranjec, M. Novel Biologically Active *N*-Substituted Benzimidazole Derived Schiff Bases: Design, Synthesis, and Biological Evaluation. *Molecules* **2023**, *28*, 3720. https://doi.org/10.3390/molecules28093720

Academic Editors: Edward Krzyżak, Piotr Świątek and Dominika Szkatuła

Received: 3 March 2023
Revised: 19 April 2023
Accepted: 21 April 2023
Published: 25 April 2023

1. Introduction

Schiff bases comprise a very important class of organic compounds [1], widely used in organic and medicinal chemistry as biologically important structural motifs in many synthetic and semisynthetic organic compounds [2–5]. Some naturally occurring Schiff bases play important roles in several physiological processes, for example rhodopsin, a photoreceptor present in the rod cells of the retina, which is essential in vision processing [6]. Schiff bases can be easily synthesized by a condensation reaction of different amino substituted compounds with versatile aldehydes or ketones [7]. Besides the fact that Schiff bases are important building blocks in medicinal chemistry due to their broad spectrum of biological activity [8–12], they have also been used in coordination chemistry [13–15], in analytical chemistry [16], as dyes [17], as optical chemosensors [18–20], as polymers [21], in catalysis [22], in metallurgy and refining of metals, and as fungicidal and agrochemical compounds. Additionally, the interest of numerous scientists is focused on evaluating Schiff bases as ligands for transition metals displaying diverse biological activities, such as anticancer [23,24], antimicrobial [25,26], and antifungal activities [27].

There is growing interest in the synthesis of benzimidazole-derived Schiff bases, not only because of their significant biological activities, but also because they can easily form complexes with different metals, giving rise to a variety of complexes with interesting electronic [28,29] and biological properties [30].

Nawrocka et al. synthesized novel 2-benzimidazolyl-substituted Schiff bases that were screened for their antiproliferative activity on several cancer cell lines [31]. Another group designed and synthesized benzimidazole and 3-oxopyrimido [1,2-*a*]benzimidazole-derived Schiff bases being evaluated as inhibitors of lipoxygenase (LOX) and of lipid peroxidation (LPO), showing antioxidative and cytotoxic activity (Figure 1a) [32]. In another study, a series of Schiff bases bearing benzimidazole nuclei were evaluated for their antimalarial and antitrypanosomal activity [33]. Furthermore, a group of authors explored the antimicrobial activity of a series of benzimidazole-derived Schiff bases against *Staphylococcus aureus* and *Escherichia coli* (Figure 1b) [34]. The metal–Schiff-base complexes could bind ct-DNA through intercalation and were found to exhibit more potent cytotoxic effects than the widely used drug cisplatin [35]. Another group of authors developed metal complexes that were evaluated for their cytotoxic, antiparasitic, and antibacterial activity, as well as their interaction with DNA [36]. Other authors studied the DNA interaction and antiproliferative activity of two Cu(II) complexes with Schiff bases of benzimidazole [37].

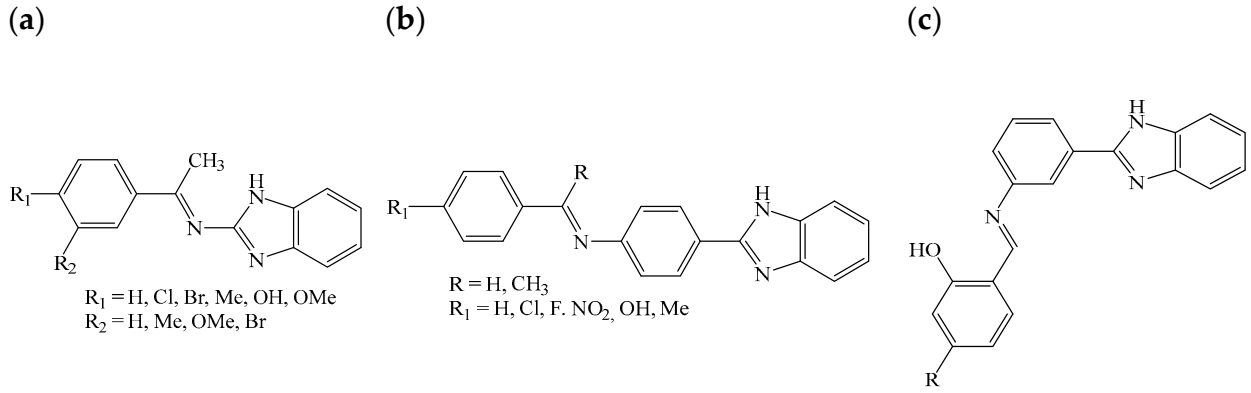


Figure 1. Examples of previously synthesized Schiff bases. (**a**) benzimidazole Schiff bases; (**b**) phenyl-benzimidazol-4-yl Schiff bases and (**c**) phenyl-benzimidazol-3-yl Schiff bases.

Recently, we synthesized a series of novel benzimidazole-substituted Schiff bases (Figure 2a) that were tested in vitro for their antiproliferative activity. They exerted a broad spectrum antiproliferative activity on different cancer cell lines, although only at higher concentrations [38]. Compounds substituted with 4-*N*,*N*-diethylamino-2-hydroxyphenyl bearing either a methyl or a cyano group at the 5(6)-position on the benzimidazole nuclei displayed the strongest antiproliferative effect on all of the tested cell lines, with a significant concentration-dependent effect on HeLa and MCF-7 cell lines.

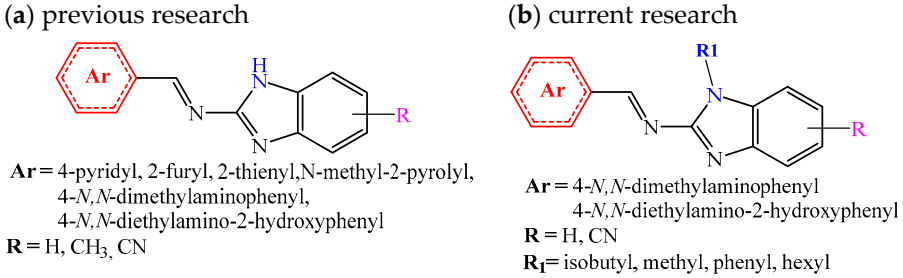


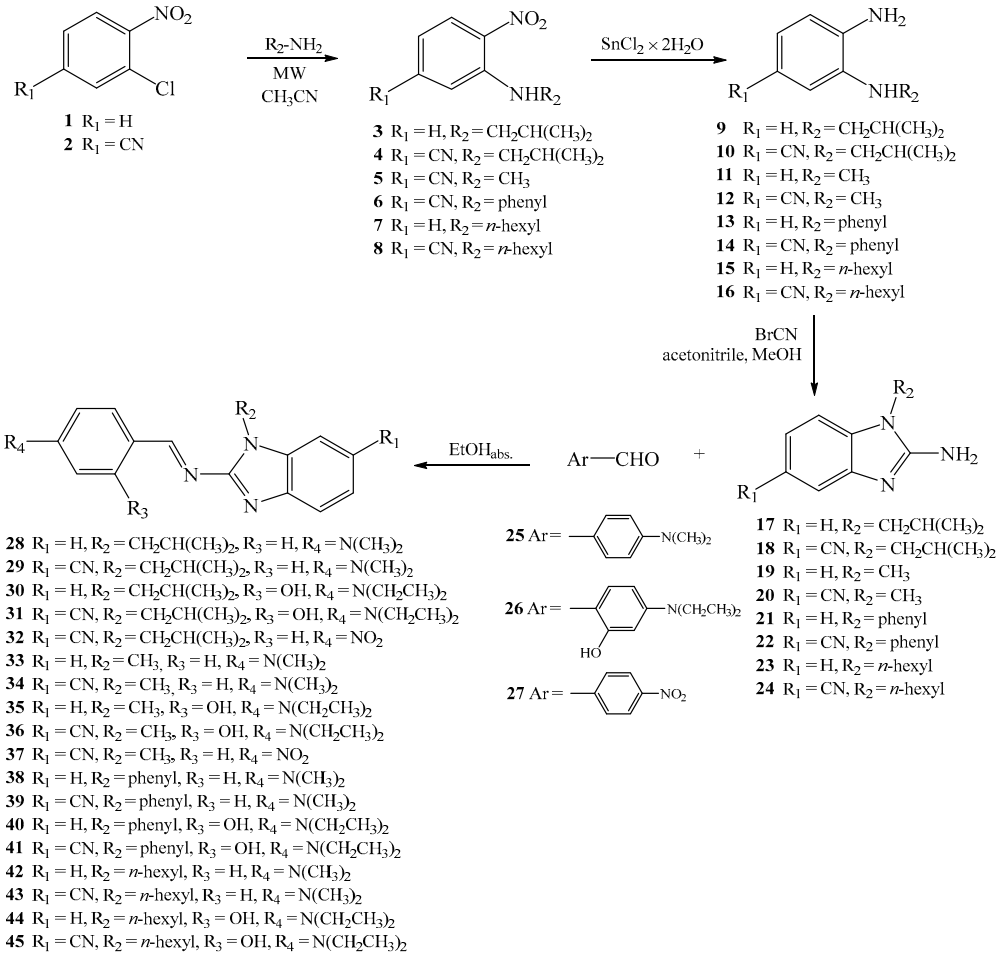
Figure 2. Synthesized benzimidazole-derived Schiff bases.

As a continuation, we designed and synthesized novel *N*-substituted benzimidazole-derived Schiff bases (Figure 2b) to explore the influence of the substituents attached at the N atom on the benzimidazole nuclei, both for their antiproliferative and antiviral activity. Our main goal was to see how the different lengths and types of aliphatic chain and the aromatic moiety attached to the nitrogen atom on the benzimidazole nuclei would influence the biological activity.

2. Results and Discussion

2.1. Chemistry

The synthesis of the novel *N*-substituted benzimidazole-derived Schiff bases **28–45** is presented in reaction Scheme 1. For the synthesis of the targeted Schiff bases, as main precursors, corresponding *N*-substituted 2-aminobenzimidazoles **17–24** were prepared according to the modified reaction procedures previously described by our research group. Starting from 2-chloronitrobenzene **1** or 2-chloro-4-cyanonitrobenzene **2**, in the reaction of uncatalyzed microwave-assisted amination with an excess of amine, *N*-methyl **5**, *N*-phenyl **6**, *N*-isobutyl **3–4,** and *N*-hexyl **7–8** substituted nitro precursors obtained in moderate yields. After reduction with $SnCl_2 \times 2H_2O$ in acidic media, corresponding 1,2-diamino substituted benzenes **9–16** were obtained. Because of the cyclocondensation with cyanogen bromide in methanol, substituted 2-aminobenzimidazoles with isobuthyl **17** and **21**, methyl **18** and **22**, phenyl **19** and **23,** or *n*-hexyl **20** and **24** side chains at the N atom or cyano group placed at the 5(6)-position on the benzimidazole nuclei were prepared in moderate reaction yields.



Scheme 1. Synthesis of *N*-substituted benzimidazole-derived Schiff bases **28–45**.

Benzimidazole-derived Schiff bases **28–45** were synthesized from *N*-substituted 2-aminobenzimidazoles **17–24** with chosen substituted benzaldehydes, namely 4-*N*,*N*-dimethylamino-**25**, 2-hydroxy-4-*N*,*N*-dimethylamino-**26,** and 4-nitrobenzaldehyde **27,** according to the previously published experimental procedure [38]. All of the Schiff bases were additionally purified either by recrystallization or by using column chromatography on silica gel with dichloromethane/methanol as an eluent to yield the targeted compounds in low to moderate reaction yields (6–68%).

The structures of all newly prepared Schiff bases **28–45** were confirmed by means of ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. Structural analysis was performed

based on the chemical shifts in both the proton spectra and ^{13}C NMR spectra, and on the values of H–H coupling constants in the proton spectra. The amination of reactants **1–2** caused the appearance of signals related to the amino group (8.12–9.90 ppm), as well as the signals for protons from isobutyl, methyl, and *n*-hexyl sidechains (0.87–3.41 ppm) in the structure of compounds **3–8**. Reduction of the nitro group was confirmed within the singlet related to the protons from the amino group (4.44 to 5.25 ppm). The formation of the benzimidazole ring was confirmed by the disappearance of signals for the amino groups in 1,2-diamino-substituted benzenes **9–16,** as well as the appearance of a singlet related to the proton of the amino group placed at position 2 on the benzimidazole nuclei (**17**–**24**), which was downshifted in comparison with the singlet of the amino group in the diamino-substituted benzenes **9**–**16**. The synthesis of targeted Schiff bases **28**–**45** was established by the observation of a singlet related to the proton of the imino group at 7.98–9.67 ppm in the proton spectra, as well as a signal for the C atom of the imino group in the carbon spectra.

2.2. Biological Activity

2.2.1. Cytotoxicity and Antiviral Activity

In Table 1, the summarized results of the antiviral evaluation are depicted. For a better overview, only the derivatives showing antiviral activity against this panel of viruses are included.

The following viruses were used for evaluating the antiviral activity: different strains of human coronavirus, three influenza virus subtypes, RSV, HSV-1, yellow fever virus, Zika virus, and Sindbis virus. The results are expressed as CC_{50} (50% cytotoxic concentration) and EC_{50} (50% effective concentration) values. Overall, the *N*-substituted 2-aminobenzimidazoles **17**–**24** displayed a poor antiviral activity. Substituted 2-aminobenzimidazoles bearing methyl **18** and **22** were both able to inhibit Zika virus replication in Huh-7 cells with EC_{50} values of 43.1 μM and 46.4 μM, respectively. Among the *N*-substituted benzimidazole-derived Schiff bases **28**–**45,** some derivatives showed an antiviral activity, although it was weak when compared with the standard antiviral drugs included (remdesivir, ribavirin, zanamivir, rimantadine, and BVDU). The 4-*N*,*N*-diethylamino-2-hydroxy-substituted derivative bearing a cyano and *N*-isobutyl side chain on the benzimidazole nuclei **31** showed a moderate activity against HCoV-NL63 in Huh-7 cells (EC_{50} 32 μM). Compound **42**, substituted with a *N*,*N*-dimethylamino and a *N*-hexyl side chain showed a moderate activity against HCoV-229E in HEL299 cells (EC_{50} 34.7 μM). In summary, no outstanding antiviral effects were noted for the 4-*N*,*N*-diethylamino-2-hydroxyphenyl ring nor for the 4-*N*,*N*-dimethylaminophenyl and 4-nitrophenyl rings.

2.2.2. Antibacterial Activity

The in vitro antibacterial activity of the synthesized Schiff bases was evaluated against a panel of eight different bacterial strains. Gram-positive bacterial strains comprised *S. aureus*, S. *pneumoniae* and *E. faecalis* and the panel of Gram-negative bacteria consisted of *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. As reference drugs, the antibiotics ampicillin, ceftazidime, ciprofloxacin, and meropenem were included.

As presented in Table 2, the majority of derivatives lacked an antibacterial activity, while some Schiff bases showed a moderate activity against certain bacterial strains.

Table 1. Cytotoxicity and antiviral activity of the synthesized Schiff bases.

Cpd	Cytotoxicity (CC_{50}/μM)			Antiviral Activity (EC_{50}/μM)										
	HEL 229	**Huh-7**	**MDCK**	**HCoV 229E HEL 299**	**HCoV OC43 HEL 299**	**HCoV NL63 Huh-7**	**Influenza H1N1 MDCK**	**Influenza H3N2 MDCK**	**Influenza B MDCK**	**RSV A Long HEL 299**	**HSV-1 KOS HEL 299**	**YFV 17D Huh-7**	**Zika Mr776 Huh-7**	**Sindbis Huh-7**
17	>100	>100	>100	78.9	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	43.1	>100
22	>100	>100	>100	>100	>100	>100	>100	>100	>100	78.7	>100	>100	46.4	>100
29	>100	>100	>100	>100	>100	79.8	94.1	>100	>100	>100	>100	>100	>100	>100
31	>100	85.7	1.3	>100	>100	32	>100	>100	>100	>100	>100	>100	>100	>100
32	>100	90.1	>100	38.5	68.7	>100	>100	>100	>100	>100	>100	>100	>100	>100
34	>100	>100	>100	>100	>100	82.8	>100	>100	83.5	>100	>100	>100	>100	>100
36	>100	>100	>100	88.4	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
38	>100	>100	>100	>100	94.8	>100	>100	>100	>100	>100	>100	>100	>100	>100
41	>100	>100	71.6	>100	89.5	>100	>100	>100	>100	>100	>100	>100	>100	>100
42	>100	2.7	34.6	34.7	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Remdesivir	>10	>10	-	0.06	0.06	0.03	-	-	-	0.03	-	6.2	0.7	>10
Ribavirin	>250	8.9	67.0	82.6	170.1	>250	10.5	4.0	2.8	10.8	-	>250	>250	148.1
Zanamivir	-	-	>100	-	-	-	0.1	16.8	0.05	-	-	-	-	-
Rimantadine	-	-	>100	-	-	-	5.0	0.05	>100	-	-	-	-	-
BVDU	>100	-	-	-	-	-	-	-	-	-	0.05	-	-	-

Table 2. Antibacterial activity of the synthesized Schiff bases.

Cpd	*S. aureus ATCC 29213*	*E. faecalis ATCC 29212*	*S. pneumoniae ATCC 49619*	*E. coli ATCC 25922*	*E. coli efflux del*	*P. aeruginosa ATCC 27853*	*K. pneumoniae ATCC 700603*	*A. baumannii ATCC 17978*
23	16	32	32	64	32	>64	>64	64
37	32	64	64	>64	>64	>64	>64	>64
38	>64	32	>64	>64	>64	>64	>64	>64
41	>64	32	64	>64	>64	>64	>64	>64
42	32	32	64	>64	64	>64	>64	>64
Ampicillin	0.5	2	2	<0.125	0.5	>64	>64	>64
Ceftazidime	8	<0.125	0.25	0.5	>64	1	64	32
Ciprofloxacin	0.125	<0.125	<0.125	0.5	1	0.25	0.25	4
Meropenem	<0.125	<0.125	<0.125	<0.125	8	1	0.25	<0.125

The *N*,*N*-dimethylamino **38** and 4-*N*,*N*-diethylamnino-2-hydroxy **41** derivatives, both substituted with a phenyl ring at the N atom on the benzimidazole nuclei, displayed activity against *E. faecalis* (MIC 32 μM). *p*-Nitro and cyano-substituted Schiff base **37** with a methyl group at the N atom showed activity against *S. aureus* (32 μM). *N*,*N*-dimethylamino derivative **42** with a hexyl side chain placed at the N atom demonstrated a moderate activity against *S. aureus* and *E. faecalis* (32 μM). Overall, precursor *N*-hexyl-2-aminobenzimidazole **23** showed the most pronounced broad spectrum of antibiotic activity against *E. faecalis*, *E. coli efflux dell*, and *K. pneumoniae,* with MIC values of 32 μM, and against *S. aureus* with an MIC value of 16 μM.

2.2.3. Antiproliferative Activity

All of the prepared *N*-substituted 2-aminobenzimidazoles **9**–**16** and benzimidazole-derived Schiff bases **28**–**45** were explored for their in vitro antiproliferative activity against several human cancer cell lines. The results are presented in Table 3 as IC_{50} values (50% inhibitory concentration). The following human cancer cell lines were used for the evaluation of the antiproliferative activity: LN-229, glioblastoma; Capan-1, pancreatic adenocarcinoma; HCT-116, colorectal carcinoma; NCI-H460, lung carcinoma; DND-41, acute lymphoblastic leukemia; HL-60, acute myeloid leukemia; K-562, chronic myeloid leukemia; and Z-138, non-Hodgkin lymphoma cancer cells. All of the obtained results were compared to *etoposide* (ETP) and *nocodazole* (NZO) as the standard chemotherapeutic agents.

Table 3. Antiproliferative activity of the synthesized Schiff bases against a broad panel of cancer cell lines.

Cpd	IC_{50} (μM)							
	LN-229	**Capan-1**	**HCT-116**	**NCI-H460**	**DND-41**	**HL-60**	**K-562**	**Z-138**
19	>100	>100	>100	>100	98.3	>100	>100	>100
21	>100	>100	>100	69.2	>100	>100	>100	>100
23	45.2	46.5	57.4	60.2	47.7	44.9	46.7	44.7
28	>100	>100	15.2	>100	>100	>100	>100	>100
29	>100	>100	>100	>100	>100	42.8	>100	>100
30	>100	>100	>100	>100	>100	70.4	>100	>100
31	>100	32.8	>100	>100	34.4	47.6	>100	>100
32	>100	>100	>100	>100	>100	>100	>100	>100
33	>100	>100	>100	>100	>100	>100	>100	>100
34	>100	>100	>100	>100	>100	>100	>100	>100
35	>100	>100	>100	>100	>100	>100	>100	>100
36	>100	>100	>100	>100	41.4	>100	>100	>100
37	>100	>100	>100	>100	>100	>100	>100	>100
38	>100	>100	>100	>100	>100	>100	>100	>100
39	>100	>100	>100	>100	>100	>100	>100	>100
40	21.5	2.4	9.6	43.7	2.2	1.1	12.9	4.4
41	>100	69.5	>100	>100	76.3	49.6	>100	35.2
42	>100	>100	>100	64.4	>100	73.2	90.8	>100
43	>100	>100	>100	91.6	>100	33.5	>100	>100
44	>100	>100	>100	>100	>100	83.9	>100	>100
45	>100	>100	>100	>100	>100	>100	>100	>100
ETP	2.40	0.43	1.45	3.65	2.80	0.42	1.77	0.85
NZO	0.29	0.10	0.13	0.25	0.47	0.10	0.07	0.31

The majority of tested compounds showed low or no activity towards the selection of cancer cell lines (for clarity, some inactive 2-aminobenzimidazoles are excluded from Table 3). Among all of the tested *N*-substituted 2-aminobenzimidazoles, the best activity was demonstrated by *n*-hexyl-substituted derivative **23,** which showed a moderate but broad antiproliferative activity against all of the tested cancer types.

N-phenyl-substituted 2-aminobenzimidazole **21** showed a mild but selective activity against lung carcinoma. Regarding the benzimidazole-derived Schiff bases, the most

potent one was the 4-*N*,*N*-diethylamino-2-hydroxy-substituted Schiff base bearing a phenyl ring at the N atom on benzimidazole nuclei **40**. This derivative displayed a pronounced antitumoral activity against Capan-1, DND-41, HL-60, and Z-138 cancer cells in the low micromolar range, with some selectivity towards the HL-60 (acute myeloid leukemia) cancer cell line (IC_{50} 1.1 μM). In addition, derivative **40** showed moderate activity against all of the other cancer cells lines. Furthermore, the 4-*N*,*N*-dimethylamino-substituted Schiff base bearing an isobutyl side chain at the N atom **28** showed a selective antiproliferative activity against colorectal carcinoma (HCT-116). Derivative **31** substituted with 2-hydroxy and 4-*N*,*N*-diethylamino groups at the phenyl ring, as well as with an isobutyl sidechain on the N atom and a cyano group on the benzimidazole nuclei, displayed a moderate activity against three cancer cell types (IC_{50} = 32.8–47.6 mM). The 4-*N*,*N*-diethylamino-2-hydroxy-substituted Schiff base **41** bearing a phenyl ring at the N atom and a cyano group on the benzimidazole nuclei also proved to be moderately active against several of the tested cancer cell lines. Among the *N*-hexyl-substituted Schiff bases **42**–**45**, a moderate activity was observed for compound **42** substituted with a 4-*N*,*N*-dimethylamino group at the phenyl ring, as well as for compound **43,** which also bears a cyano group at the benzimidazole nuclei.

The 4-*N*,*N*-diethylamino-2-hydroxy-substituted derivatives **44**–**45** were less active in comparison with the 4-*N*,*N*-dimethylamino substituted analogues. Regarding the *N*-methyl-substituted Schiff bases, only the 4-*N*,*N*-diethylamino-2-hydroxy-substituted derivative bearing a cyano group showed a moderate but selective activity against DND-41 cells. When comparing all of the *N*-isobutyl substituted Schiff bases **28**–**31**, we can conclude that the derivative bearing both a 4-*N*,*N*-diethylamino-2-hydroxyphenyl and cyano group **31** was the most active one.

To conclude, we observed that the most significant impact on the antiproliferative activity was seen for the 4-*N*,*N*-diethylamino-2-hydroxyphenyl ring in comparison with the 4-*N*,*N*-dimethylaminophenyl and 4-nitrophenyl rings. A cyano group placed at the 5(6) position on the benzimidazole nuclei increased the antiproliferative activity, but not for the *N*-phenyl-substituted derivatives. The 4-*N*,*N*-diethylamino-2-hydroxy-substituted Schiff base bearing a phenyl ring at the N atom on benzimidazole nuclei **40**, which showed the most promising activity among all of the tested derivatives, was chosen as a lead compound for further optimization in order to obtain more selective and potent antiproliferative agents (Figure 3).

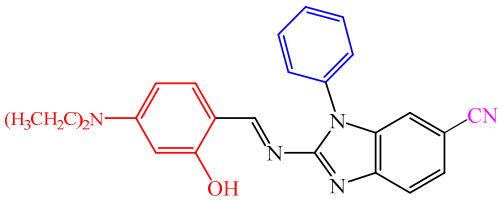


Figure 3. Selected Schiff base **40** as a lead compound for further optimization.

3. Conclusions

Herein, we present the design and synthesis of novel, *N*-substituted benzimidazole-derived Schiff bases, bearing isobutyl, methyl, and *n*-hexyl sidechains or a phenyl group at the N atom on the benzimidazole nuclei as well as a 4-*N*,*N*-dimethylamino- or 4-*N*,*N*-diethylamino-2-hydroxyphenyl ring attached directly to the imino bond. Within this research, our main focus was to study the impact on the biological activity of the substituents placed on the phenyl ring and on the N atom of the benzimidazole nuclei. All Schiff bases were evaluated for their in vitro antiviral activity against a broad selection of viruses, antibiotic activity against Gram-positive and Gram-negative bacterial strains, and antiproliferative activity on a diverse panel of human cancer cell lines.

The series of newly synthesized derivatives lacked a pronounced antiviral activity against the panel of selected viruses. Schiff base **31** substituted with a 4-*N*,*N*-diethylamino-

2-hydroxyphenyl and bearing a cyano and *N*-isobutyl side chain on the benzimidazole nuclei showed a moderate activity against HCoV-NL63 virus on Huh-7 cells, although with a low selectivity (EC_{50} 32 μM and CC_{50} 85.7 μM).

The majority of the tested compounds were inactive against the Gram-positive and Gram-negative bacterial strains used. A selection of Schiff bases showed a moderate activity against one and/or two bacterial strains, while the most active compound was *N*-hexyl-2-aminobenzimidazole **23** with a moderate but broad activity against *E. faecalis*, *E. coli* (*efflux dell*), and *K. pneumoniae* (MIC 32 μM), as well as against *S. aureus* (MIC 16 μM).

Additionally, all of the prepared 2-aminobenzimidazoles **17**–**24** and Schiff bases **28**–**45** were tested for their antiproliferative activity against several cancer cell lines. The obtained results revealed that Schiff base **31** substituted with a 4-*N*,*N*-diethylamino-2-hydroxyphenyl and with an isobutyl side chain on the N atom and a cyano group on the benzimidazole nuclei displayed a moderate activity against three cancer types (IC_{50} 32.8–47.6 μM). In addition, among the tested *N*-substituted-2-aminobenzimidazoles, *N*-hexyl-substituted derivative **23** showed a broad but modest activity against all of the tested cancer cell lines. Furthermore, the 4-*N*,*N*-diethylamino-2-hydroxyphenyl ring as well as the phenyl ring attached to the N atom on the benzimidazole nuclei had a pronounced impact on the activity. Schiff base **40** bearing the above-mentioned substituents showed the most promising selective antiproliferative activity against Capan-1, DND-41, HL-60, and Z-138 cancer cell lines (IC_{50} = 1.1–4.4 μM), and a moderate activity against all of the other tested cell lines.

In conclusion, we have shown that out of the prepared *N*-substituted benzimidazole-derived Schiff bases, derivative **40** showed the most interesting biological potential, with a promising antiproliferative activity, making it a promising candidate for further design and optimization.

4. Materials and Methods

4.1. General Methods

All of the chemicals were purchased from commercial suppliers. Melting points were recorded on Büchi 535 melting apparatus (Büchi, Sankt Gallen, Switzerland). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Bruker Advance III HD 400 MHz/54 mm Ascend instrument (Bruker, Billerica, MA, USA). All of the NMR spectra were measured in DMSO-d6 solutions using TMS as an internal standard. All of the compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates and the spots were detected under UV light. Microwave-assisted synthesis was performed in a Milestone start S microwave (Milestone Srl, Sorisole, Italy) oven using quartz cuvettes under a pressure of 40 bar. Elemental analyses for carbon, hydrogen, and nitrogen were performed on a PerkineElmer 2400 elemental analyzer (Perkin-Elmer, Waltham, MA, USA, SAD). Where analyses are indicated only as symbols of elements, the analytical results obtained were within 0.4% of the theoretical value. NMR spectra of synthesized compounds are given in Supplementary Materials.

4.2. Synthesis

4.2.1. General Method for Preparation of Compounds **7**–**8**

Compounds **7**–**8** were prepared using microwave irradiation, at an optimized reaction time at 170 °C with power 800 W and 40 bar pressure, from 1 or 2 in acetonitrile (10 mL) with an excess of added corresponding amine. After cooling, the resulting product was purified by column chromatography on SiO_2 using dichlormethane/methanol as the eluent.

N-Hexyl-2-nitroaniline **7**

7 was prepared from **1** (0.50 g, 3.2 mmol) and hexylamine (2.90 mL, 22.2 mmol) after 2 h of irradiation to yield 0.60 g (94%) of orange oil. ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 8.12 (t, 1H, J = 5.07 Hz, NH), 8.06 (dd, 1H, J_1 = 1.58 Hz, J_2 = 8.57 Hz, H_{arom}), 7.56–7.51 (m, 1H, H_{arom}), 7.05 (d, 1H, J = 7.99 Hz, H_{arom}), 6.70–7.65 (m, 1H, H_{arom}), 3.37–3.33 (m, 2H,

CH_2), 1.67–1.58 (m, 2H, CH_2), 1.41–1.27 (m, 6H, CH_2), 0.87 (t, 3H, J = 7.06 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 145.7, 137.1, 131.3, 126.7, 115.5, 115.0, 42.7, 31.4, 28.7, 26.5, 22.5, 14.4; Anal. Calcd. for $C_{12}H_{18}N_2O_2$: C, 64.84; H, 8.16; N, 12.60; O, 14.39. Found: C, 64.89; H, 8.23; N, 12.51; O, 14.29%.

3-*N*-(Hexylamino)-4-nitrobenzonitrile **8**

8 was prepared from **2** (0.50 g, 2.7 mmol) and hexylamine (1.80 mL, 13.7 mmol) after 2 h of irradiation to yield 0.50 g (97%) of yellow oil. ^1H NMR (400 MHz, DMSO-d_6) (δ/ppm): 8.58 (t, 1H, J = 5.38 Hz, NH), 8.50 (d, 1H, J = 2.00 Hz, H_{arom}), 7.81 (dd, 1H, J_1 = 1.60 Hz, J_2 = 9.08 Hz, H_{arom}), 7.18 (d, 1H, J = 9.10 Hz, H_{arom}), 3.41 (q, 2H, J = 6.72 Hz, CH_2), 1.65–1.55 (m, 2H, CH_2), 1.38–1.23 (m, 6H, CH_2), 0.87 (t, 3H J = 6.70 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm = 146.8, 130.5, 118.2, 96.0, 42.3, 30.8, 27.9, 25.8, 21.9; Anal. Calcd. for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99; O, 12.94. Found: C, 63.20; H, 6.88; N, 16.91; O, 12.89%.

4.2.2. General Method for Preparation of Compounds **15**–**16**

Derivative **7** or benzonitrile derivative **8** and a solution of $SnCl_2 \times 2H_2O$ in MeOH and concentrated HCl were refluxed for 0.5 h. The resulting solution was treated with 20% NaOH to pH = 14. The resulting precipitate was filtered off, washed with hot ethanol, and filtered. The filtrate was evaporated at a reduced pressure and was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and concentrated at a reduced pressure.

N^1-Hexylbenzene-1,2-diamine **15**

Compound **15** was prepared from **7** (3.52 g, 15.8 mmol), $SnCl_2 \times 2H_2O$ (29.70 g, 131.5 mmol), $HCl_{conc.}$ (49 mL), and MeOH (49 mL) to yield 2.13 g (70%) of red oil. ^1H NMR (600 MHz, DMSO-d_6) (δ/ppm): 6.52 (dd, 1H, J_1 = 1.46 Hz, J_2 = 7.78 Hz, H_{arom}), 6.48 (td, 1H, J_1 = 1.48 Hz, J_2 = 7.56 Hz, H_{arom}), 6.40–6.37 (m 2H, H_{arom}), 4.44 (bs, 2H, NH_2), 4.28 (t, 1H, J = 5.18 Hz, NH), 2.99 (q, 2H, J = 6.88 Hz, CH_2), 1.61–1.55 (m, 2H CH_2), 1.41–1.35 (m, 2H, CH_2), 1.32–1.28 (m, 4H, CH_2), 0.88 (t, 3H, J = 6.98 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 136.1, 135.0, 117.5, 116.5, 114.0, 109.6, 43.4, 31.2, 28.8, 26.5, 22.1, 13.9; Anal. Calcd. for $C_{12}H_{20}N_2$: C, 74.95; H, 10.48; N, 14.57. Found: C, 74.88; H, 10.54; N, 14.66%.

3-Amino-4-*N*-(hexylamino)benzonitrile **16**

Compound **16** was prepared from **8** (2.71 g, 10.9 mmol), $SnCl_2 \times 2H_2O$ (14.85 g, 68.8 mmol), $HCl_{conc.}$ (29 mL), and MeOH (29 mL) to yield 1.65 g (69%) of pink powder. m.p. 157–161 °C; ^1H NMR (600 MHz, DMSO-d_6) (δ/ppm): 6.91 (dd, 1H, J_1 = 1.90 Hz, J_2 = 8.18 Hz, H_{arom}), 6.76 (d, 1H, J = 2.00 Hz, H_{arom}), 6.44 (d, 1H, J = 8.16 Hz, H_{arom}), 5.32 (t, 1H, J = 5.00 Hz, NH), 4.96 (s, 2H, NH_2), 3.08 (q, 2H, J = 6.42 Hz, CH_2), 1.64–1.51 (m, 2H, CH_2), 1.41–1.25 (m, 6H, CH_2), 0.88 (t, 3H, J = 6.58 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm = 140.5, 135.5, 123.4, 121.6, 115.2, 108.8, 96.7, 43.2, 31.6, 28.8, 26.8, 22.6, 14.4; Anal. Calcd. for $C_{13}H_{19}N_3$: C, 71.85; H, 8.81; N, 19.34. Found: C, 71.94; H, 8.87; N, 19.41%.

4.2.3. General Method for Preparation of Compounds **23** and **24**

BrCN was added dropwise to a solution of *o*-phenylenediamine or in 20 mL H_2O and 5 mL acetonitrile. The reaction mixture was refluxed for 2 h and NH_4OH was added to adjust to pH = 9. After cooling, the resulting precipitate was filtered off.

2-Amino-1-hexylbenzimidazole **23**

Compound **23** was prepared from **15** (1.59 g, 8.3 mmol) and BrCN (0.88 g, 8.3 mmol) to to yield 1.45 g (81%) of brown powder. m.p. 245–250 °C; ^1H NMR (600 MHz, DMSO-d_6) (δ/ppm): 7.96 (bs, 2H, NH2), 7.43–7.41 (m, 1H, Harom), 7.32–7.30 (m, 1H, Harom), 7.18–7.14 (m, 2H, Harom), 4.08 (t, 2H, J = 7.37 Hz, CH2), 1.68–1.63 (m, 2H, CH2), 1.33–1.24 (m, 6H, CH2), 0.84 (t, 3H, J = 7.07 Hz, CH3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 151.6, 131.9,

122.9, 121.9, 112.9, 109.9, 42.4, 31.3, 28.2, 26.0, 22.5, 14.3; Anal. Calcd. for $C_{13}H_{19}N_3$: C, 71.85; H, 8.81; N, 19.34. Found: C, 71.87; H, 8.79; N, 19.29%.

2-Amino-6-cyano-1-hexylbenzimidazole **24**

Compound **24** was prepared from **16** (0.03 g, 1.4 mmol) and BrCN (0.14 g, 1.4 mmol) to to yield 0.28 g (83%) of grey powder. m.p. 215–220 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 7.49 (s, 1H, H_{arom}), 7.33–7,28 (m, 2H, H_{arom}), 6.84 (s, 1H, H_{arom}), 4.01 (t, 2H, J = 7.18 Hz, CH_2), 1.63–1.58 (m, 2H, CH_2), 1.27–1.22 (m, 6H, CH_2), 0.83 (t, 3H, J = 6.68 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 157.1, 143.2, 138.3, 121.2, 102.5, 42.1, 31.4, 28.7, 26.1, 22.5; Anal. Calcd. for $C_{14}H_{18}N_4$: C, 69.39; H, 7.49; N, 23.12. Found: C, 69.45; H, 7.40; N, 23.07%.

4.2.4. General Method for Preparation of Schiff Bases **28**–**45**

Solutions of the equimolar amounts of the corresponding *N*-substituted-2-aminobenzimidazole and aromatic aldehyde in absolute ethanol were refluxed for 24–48 h. After cooling, the obtained products were filtered off and recrystallized from ethanol. If necessary, the products were purified by column chromatography on SiO_2 using a gradient elution of dichloromethane/methanol/TEA. Basic TEA was used to prevent the decomposition of the Schiff base conjugates in the silica gel column.

(*E*)-4-(((1-Isobutyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)-*N*,*N*-dimethylaniline **28**

Compound **28** was prepared from 2-amino-1-isobutylbenzimidazole **17** (0.10 g, 0.5 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.08 g, 0.5 mmol) in absolute ethanol (3 mL) after refluxing for 48 h to obtain 0.02 g (14%) of yellow powder. m.p. 277–279 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 9.26 (s, 1H, H_{arom}), 7.91 (d, 2H, J = 8.79 Hz, H_{arom}), 7.55–7.50 (m, 2H, H_{arom}), 7.19–7.14 (m, 2H, H_{arom}), 6.85 (d, 2H, J = 8.88 Hz, H_{arom}), 4.17 (d, 2H, J = 7.27 Hz, CH_2), 3.07 (s, 6H, CH_3), 2.24–2.16 (m, 1H, CH), 0.89 (t, 6H, J = 6.66 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 150.4, 131.2 (2C), 129.1 (2C), 124.0, 123.4, 112.0 (2C), 111.2 (3C), 49.1, 27.7, 19.7 (2C); Anal. Calcd. for $C_{20}H_{24}N_4$: C, 74.97; H, 7.55; N, 17.48. Found: C, 74.95; H, 7.59; N, 17.41%.

(*E*)-2-((4-(Dimethylamino)benzylidene)amino)-1-isobutyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **29**

Compound **29** was prepared from 2-amino-6-cyano-1-isobutylbenzimidazole **21** (0.10 g, 0.5 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.07 g, 0.5 mmol) in absolute ethanol (4 mL) after refluxing for 24 h to obtain 0.03 g (22%) of yellow powder in the form of a mixture of *E*- and *Z*-isomers at a ratio of **29a**/**29b** = 5:3. m.p. 283–285 °C; **29a**: ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 9.28 (s, 1H, H_{arom}), 8.03 (d, 1H, J = 1.01 Hz, H_{arom}), 7.94 (d, 2H, J = 8.78 Hz, H_{arom}), 7.74 (d, 1H, J = 8.37 Hz, H_{arom}), 7.69 (d, 1H, J = 9.02 Hz, H_{arom}), 7.57 (dd, 1H, J_1 = 8.31, J_2 = 1.38 Hz, H_{arom}), 6.85 (d, 2H, J = 9.03 Hz, H_{arom}), 4.21 (d, 2H, J = 7.27 Hz, CH_2), 3.08 (s, 6H, CH_3), 2.23–2.16 (m, 1H, CH), 0.89 (d, 6H, J = 6.66 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 166.2, 159.0, 154.3, 141.6, 138.7, 132.6, 125.2, 122.7, 120.6, 112.1, 104.1, 49.8, 29.3, 20.3; **29b**: ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 9.67 (s, 1H, H_{arom}), 7.69 (d, 1H, J = 9.01 Hz, H_{arom}), 7.49 (d, 1H, J = 1.09 Hz, H_{arom}), 7.33 (d, 1H, J = 8.07 Hz, H_{arom}), 7.28 (dd, 1H, J_1 = 8.11, J_2 = 1.37 Hz, H_{arom}), 6.84–6.78 (m, 3H, H_{arom}), 3.85 (d, 2H, J = 7.56 Hz, CH_2), 3.05 (s, 6H, CH_3), 2.13–2.04 (m, 1H, CH), 0.86 (d, 6H, J = 6.67 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 190.3, 157.4, 143.2, 132.0, 121.2, 111.9, 111.5, 109.3, 102.4, 48.9, 28.4, 20.0; Anal. Calcd. for $C_{21}H_{23}N_5$: C, 73.02; H, 6.71; N, 20.27. Found: C, 73.10; H, 6.68; N, 20.32%.

(*E*)-5-(Diethylamino)-2-(((1-isobutyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)phenol **30**

Compound **30** was prepared from 2-amino-1-isobutylbenzimidazole **17** (0.15 g, 0.8 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.12 g, 0.8 mmol) in absolute ethanol (6 mL) after refluxing for 48 h to obtain 0.10 g (36%) of yellow powder. m.p. 254–258 °C;

^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 12.79 (s, 1H, OH), 9.35 (s, 1H, H_{arom}), 7.55–7.50 (m, 2H, H_{arom}), 7.42 (d, 1H, J = 8.97 Hz, H_{arom}), 7.20–7.15 (m, 2H, H_{arom}), 6.42 (dd, 1H, J_1 = 2.27 Hz, J_2 = 8.87 Hz, H_{arom}), 6.17 (d, 1H, J = 2.09 Hz, H_{arom}), 4.06 (d, 2H, J = 7.18 Hz, CH_2), 3.46 (q, 4H, J = 7.08, CH_2), 2.22–2.12 (m, 1H, CH), 1.12 (t, 6H, J = 7.09, CH_3), 0.91 (d, 6H, J = 6.55 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 163.5, 153.8, 152.9, 135.0, 121.8, 121.3, 117.9, 111.2, 110.0, 108.5, 105.0, 104.4, 96.6, 95.9, 49.4, 44.1 (2C), 28.9, 19.9 (2C), 12.5 (2C); Anal. Calcd. for $C_{22}H_{28}N_4O$: C, 72.50; H, 7.74; N, 15.37; O, 4.39. Found: C, 72.48; H, 7.70; N, 15.32; O, 4.44%.

(*E*)-2-((4-(Diethylamino)-2-hydroxybenzylidene)amino)-1-isobutyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **31**

Compound **31** was prepared from 2-amino-6-cyano-1-isobutylbenzimidazole **21** (0.15 g, 0.7 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.13 g, 0.7 mmol) in absolute ethanol (7 mL) after refluxing for 48 h to obtain 0.11 g (40%) of orange powder. m.p. 205–208 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm = 12.62 (s, 1H, OH), 9.37 (s, 1H, H_{arom}), 8.01 (d, 1H, J = 1.09 Hz, H_{arom}), 7.74 (d, 1H, J = 8.26 Hz, H_{arom}), 7.59 (d, 1H, J = 8.97 Hz, H_{arom}), 7.56 (dd, 1H, J_1 = 1.48 Hz, J_2 = 8.98 Hz, H_{arom}), 6.44 (dd, 1H, J_1 = 2.29 Hz, J_2 = 8.99 Hz, H_{arom}), 6.18 (d, 1H, J = 2.27 Hz, H_{arom}), 4.10 (d, 2H, J = 7.36 Hz, CH_2), 3.45 (q, 4H, J = 6.97 Hz, CH_2), 2.20–2.12 (m, 2H, CH), 1.15 (t, 6H, J = 7.08 Hz, CH_3), 0.90 (d, 6H, J = 6.69 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 164.3, 153.9 (2C), 141.6, 138.7, 125.3, 122.6, 120.6, 111.8 (2C), 109.1, 105.9 (2C), 104.1, 97.0 (2C), 50.0, 44.7, 29.4, 20.3, 13.0; Anal. Calcd. for $C_{23}H_{27}N_5O$: C, 70.92; H, 6.99; N, 17.98; O, 4.11. Found: C, 70.97; H, 6.91; N, 17.93; O, 4.08%.

(*E*)-1-Isobutyl-2-((4-nitrobenzylidene)amino)-1*H*-benzo[*d*]imidazole-6-carbonitrile **32**

Compound **32** was prepared from 2-amino-6-cyano-1-isobutylbenzimidazole **21** (0.10 g, 0.5 mmol) and 4-nitrobenzaldehyde **27** (0.08 g, 0.5 mmol) in absolute ethanol (3 mL) after refluxing for 24 h to obtain 0.02 g (14%) of yellow powder. m.p. 277–279 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 9.66 (s, 1H, H_{arom}), 8.41–8.38 (m, 5H, H_{arom}), 8.19 (d, 1H, J = 0.98 Hz, H_{arom}), 7.88 (d, 1H, J = 8.46 Hz, H_{arom}), 7.67 (dd, 1H, J_1 = 1.47 Hz, J_2 = 8.38 Hz, H_{arom}), 4.31 (d, 2H, J = 7.28 Hz, CH_2), 2.25–2.13 (m, 1H, CH), 0.90 (d, 6H, J = 6.69 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 165.2, 156.2, 149.8, 140.6, 140.2, 138.2, 130.9 (2C), 125.7, 124.2 (2C), 123.9, 119.8, 112.4, 104.6, 49.6, 29.0, 19.8 (2C); Anal. Calcd. for $C_{19}H_{17}N_5O_2$: C, 65.69; H, 4.93; N, 20.16; O, 9.21. Found: C, 65.72; H, 4.87; N, 20.09; O, 9.34%.

(*E*)-*N*,*N*-Dimethyl-4-(((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)aniline **33**

Compound **33** was prepared from 2-amino-1-methylbenzimidazole **18** (0.10 g, 0.7 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.10 g, 0.7 mmol) in absolute ethanol (4 mL) after refluxing for 48 h to obtain 0.01 g (6%) of yellow powder. m.p. 186–188 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm = 7.98–7.94 (m, 2H, H_{arom}), 7.41–7.36 (m, 2H, H_{arom}), 7.32–7.29 (m, 2H, H_{arom}), 7.18–7.15 (m, 3H, H_{arom}), 3.59 (s, 6H, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 190.4, 154.7, 132.0, 125.0, 123.8, 123.1, 112.2, 112.1, 111.6, 111.0, 110.4, 19.8, 9.1 (2C); Anal. Calcd. for $C_{17}H_{18}N_4$: C, 71.27; H, 5.65; N, 23.09. Found: C, 71.30; H, 5.61; N, 23.16%.

(*E*)-2-((4-(Dimethylamino)benzylidene)amino)-1-methyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **34**

Compound **34** was prepared from 2-amino-6-cyano-1-methylbenzimidazole **22** (0.10 g, 0.6 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.09 g, 0.6 mmol) in absolute ethanol (3 mL) after refluxing for 48 h to obtain 0.05 g (27%) of yellow powder. m.p. 222–226 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 9.27 (s, 1H, H_{arom}), 8.01 (d, 1H, J = 1.26 Hz, H_{arom}), 7.95 (d, 2H, J = 8.81 Hz, H_{arom}), 7.68 (d, 1H, J = 8.32 Hz, H_{arom}), 7.58 (dd, 1H, J_1 = 8.28, J_2 = 1.37 Hz, H_{arom}), 6.84 (d, 2H, J = 8.91 Hz, H_{arom}), 3.88 (s, 3H, CH_3), 3.08 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 166.4, 158.9, 154.3, 141.7, 139.0,

132.8, 125.2, 122.9, 122.8, 122.6, 120.7, 118.2, 112.0, 111.6, 108.8, 104.1, 29.5 (2C), 29.1; Anal. Calcd. for $C_{18}H_{17}N_5$: C, 71.27; H, 5.65; N, 23.09. Found: C, 71.30; H, 5.61; N, 23.16%.

(*E*)-5-(Diethylamino)-2-(((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)phenol **35**

Compound **35** was prepared from 2-amino-1-methylbenzimidazole **18** (0.15 g, 1.0 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.19 g, 1.0 mmol) in absolute ethanol (7 mL) after refluxing for 24 h to obtain 0.04 g (13%) of orange powder. m.p. 169–173 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm = 12.61 (s, 1H, OH), 9.36 (s, 1H, H_{arom}), 7.58 (d, 1H, J = 8.87 Hz, H_{arom}), 7.54–7.51 (m, 1H, H_{arom}), 7.50–7.47 (m, 1H, H_{arom}), 7.21–7.15 (m, 2H, H_{arom}), 6.42 (dd, 1H, J_1 = 2.38 Hz, J_2 = 8.99 Hz, H_{arom}), 6.16 (d, 1H, J = 2.26 Hz, H_{arom}), 3.78 (s, 3H, CH_3), 3.45 (q, 4H, J = 7.08 Hz, CH_2), 1.15 (t, 6H, J = 7.07 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 164.5, 163.9, 153.4, 141.9 (2C), 135.8, 122.3, 121.7, 118.3, 110.2, 109.1, 105.5, 97.0, 44.6 (2C), 29.1, 13.0 (2C); Anal. Calcd. for $C_{19}H_{22}N_4O$: C, 70.78; H, 6.88; N, 17.38; O, 4.96. Found: C, 70.71; H, 6.79; N, 17.44; O, 5.03%.

(*E*)-2-((4-(Diethylamino)-2-hydroxybenzylidene)amino)-1-methyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **36**

Compound **36** was prepared from 2-amino-6-cyano-1-methylbenzimidazole **22** (0.20 g, 1.2 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.22 g, 1.2 mmol) in absolute ethanol (10 mL) after refluxing for 48 h to obtain 0.27 g (67%) of yellow powder. m.p. 227–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 12.40 (s, 1H, OH), 9.37 (s, 1H, H_{arom}), 7.99 (d, 1H, J = 1.08 Hz, H_{arom}), 7.68 (d, 1H, J = 8.29 Hz, H_{arom}), 7.61 (d, 1H, J = 8.97 Hz, H_{arom}), 7.57 (dd, 1H, J_1 = 1.45 Hz, J_2 = 8.27 Hz, H_{arom}), 6.43 (dd, 1H, J_1 = 2.36 Hz, J_2 = 9.00 Hz, H_{arom}), 6.15 (d, 1H, J = 2.26 Hz, H_{arom}), 3.80 (s, 3H, CH_3), 3.45 (q, 4H, J = 6.98 Hz, CH_2), 1.15 (t, 6H, J = 6.95 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 165.3, 164.2, 157.6, 153.9, 141.7, 138.9, 125.2, 122.4, 120.7, 111.5, 109.2, 105.9, 104.1, 96.9, 44.7 (2C), 29.5, 13.0 (2C); Anal. Calcd. for $C_{20}H_{21}N_5O$: C, 69.14; H, 6.09; N, 20.16; O, 4.61. Found: C, 69.19; H, 5.98; N, 20.13; O, 4.71%.

(*E*)-1-Methyl-2-((4-nitrobenzylidene)amino)-1*H*-benzo[*d*]imidazole-6-carbonitrile **37**

Compound **37** was prepared from 2-amino-6-cyano-1-methylbenzimidazole **22** (0.10 g, 0.5 mmol) and 4-nitrobenzaldehyde **27** (0.08 g, 0.5 mmol) in absolute ethanol (3 mL) after refluxing for 24 h to obtain 0.02 g (14%) of yellow powder. m.p. 277–279 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 9.67 (s, 1H, H_{arom}), 8.44–8.41 (m, 4H, H_{arom}), 8.20 (d, 1H, J = 0.97 Hz, H_{arom}), 7.83 (d, 1H, J = 8.38 Hz, H_{arom}), 7.69 (dd, 1H, J_1 = 1.49 Hz, J_2 = 8.37 Hz, H_{arom}), 3.99 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 165.9, 156.7, 150.4, 141.2, 140.7, 139.1, 131.5 (2C), 126.2, 124.7 (2C), 124.3, 120.3, 112.6, 105.1, 29.9; Anal. Calcd. for $C_{16}H_{11}N_5O_2$: C, 62.95; H, 3.63; N, 22.94; O, 10.48. Found: C, 62.91; H, 3.69; N, 22.88; O, 10.41%.

(*E*)-*N*,*N*-Dimethyl-4-(((1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)aniline **38**

Compound **38** was prepared from 2-amino-1-phenylbenzimidazole **19** (0.10 g, 0.5 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.07 g, 0.5 mmol) in absolute ethanol (3 mL) after refluxing for 48 h to obtain 0.11 g (68%) of yellow powder. m.p. 204–207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 9.29 (s, 1H, H_{arom}), 7.76 (d, 2H, J = 8.86 Hz, H_{arom}), 1.66–1.57 (m, 5H, H_{arom}), 1.54–1.49 (m, 1H, H_{arom}), 7.30 (d, 1H, J = 7.89 Hz, H_{arom}), 7.28–7.24 (m, 1H, H_{arom}), 7.22–7.17 (m, 1H, H_{arom}), 6.79 (d, 2H, J = 8.87 Hz, H_{arom}), 3.04 (s, 6H, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 165.5, 156.2, 153.9, 142.1, 135.9, 135.6, 132.3, 129.7 (2C), 128.2, 127.4 (2C), 123.1, 122.9, 122.6, 118.9, 112.0, 110.4; Anal. Calcd. for $C_{22}H_{20}N_4$: C, 77.62; H, 5.92; N, 16.46. Found: C, 77.68; H, 5.98; N, 16.39%.

(*E*)-2-((4-(Dimethylamino)benzylidene)amino)-1-phenyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **39**

Compound **39** was prepared from 2-amino-6-cyano-1-phenylbenzimidazole **23** (0.13 g, 0.6 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.09 g, 0.6 mmol) in absolute ethanol (6 mL) after refluxing for 24 h to obtain 0.04 g (17%) of yellow powder. m.p. 247–251 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 9.29 (s, 1H, H_{arom}), 8.14 (d, 1H, J = 1.09 Hz, H_{arom}), 7.75 (d, 2H, J = 8.97 Hz, H_{arom}), 7.66–7.62 (m, 2H, H_{arom}), 7.62–7.59 (m, 2H, H_{arom}), 7.57 (dd, 1H, J_1 = 1.47 Hz, J_2 = 8.28 Hz, H_{arom}), 7.56–7.54 (m, 1H, H_{arom}), 7.41 (d, 1H, J = 8.27 Hz, H_{arom}), 6.78 (d, 2H, J = 8.98 Hz, H_{arom}), 3.05 (s, 6H, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 167.1, 158.6, 154.4, 141.9, 138.6, 135.0, 129.8 (2C), 128.8, 127.5 (2C), 126.1, 123.2, 122.6, 120.4, 112.0, 111.7, 105.0; Anal. Calcd. for $C_{23}H_{19}N_5$: C, 75.59; H, 5.24; N, 19.16. Found: C, 75.65; H, 5.30; N, 19.09%.

(*E*)-5-(Diethylamino)-2-(((1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)phenol **40**

Compound **40** was prepared from 2-amino-1-phenylbenzimidazole **19** (0.10 g, 0.5 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.09 g, 0.5 mmol) in absolute ethanol (7 mL) after refluxing for 48 h to obtain 0.10 g (52%) of yellow powder. m.p. 197–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm = 12.40 (s, 1H, OH), 9.37 (s, 1H, H_{arom}), 7.67–7.62 (m, 3H, H_{arom}), 7.60–7.53 (m, 3H, H_{arom}), 7.50 (d, 1H, J = 8.97 Hz, H_{arom}), 7.29–7.22 (m, 2H, H_{arom}), 7.21–7.17 (m, 1H, H_{arom}), 6.39 (dd, 1H, J_1 = 2.37 Hz, J_2 = 8.99 Hz, H_{arom}), 6.04 (d, 1H, J = 2.27 Hz, H_{arom}), 3.41 (q, 4H, J = 7.54 Hz, CH_2), 1.11 (t, 6H, J = 6.88 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) (δ/ppm): 164.4, 163.5, 153.0, 141.6, 135.6, 135.1, 135.0, 129.7 (2C), 128.2, 126.9 (2C), 122.6, 122.1, 118.3, 109.7, 108.5, 105.0, 96.5, 44.1, 12.5; Anal. Calcd. for $C_{24}H_{24}N_4O$: C, 74.97; H, 6.29; N, 14.57; O, 4.16. Found: C, 74.89; H, 6.19; N, 14.51; O, 4.20%.

(*E*)-2-((4-(Diethylamino)-2-hydroxybenzylidene)amino)-1-phenyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **41**

Compound **41** was prepared from 2-amino-6-cyano-1-phenylbenzimidazole **23** (0.20 g, 0.9 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.16 g, 0.9 mmol) in absolute ethanol (10 mL) after refluxing for 48 h to obtain 0.23 g (66%) of yellow powder. m.p. 111–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 12.28 (bs, 1H, OH), 9.37 (s, 1H, H_{arom}), 8.13–8.11 (m, 1H, H_{arom}), 7.68–7.64 (m, 2H, H_{arom}), 7.61–7.59 (m, 3H, H_{arom}), 7.56 (dd, 1H, J_1 = 8.28 Hz, J_2 = 1.56 Hz, H_{arom}), 7.51 (d, 1H, J = 9.09 Hz, H_{arom}), 7.35 (d, 1H, J = 8.37 Hz, H_{arom}), 6.40 (dd, 1H, J_1 = 9.08 Hz, J_2 = 2.40 Hz, H_{arom}), 6.04 (d, 1H, J = 2.28 Hz, H_{arom}), 3.44–3.39 (m, 1H, CH_2), 1.11 (t, 6H, J = 7.01 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 165.8, 164.4, 156.8, 154.0, 142.0, 138.6, 136.4, 134.7, 130.3, 129.3, 127.8, 126.2, 122.9, 120.4, 111.5, 109.1, 105.9, 105.0, 96.9, 44.7 (2C), 13.0 (2C); Anal. Calcd. for $C_{25}H_{23}N_5O$: C, 73.33; H, 5.66; N, 17.10; O, 3.91. Found: C, 73.36; H, 5.69; N, 17.15; O, 4.02%.

(*E*)-4-(((1-Hexyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)-*N*,*N*-dimethylaniline **42**

Compound **42** was prepared from 2-amino-1-hexylbenzimidazole **20** (0.20 g, 0.9 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.13 g, 0.9 mmol) in absolute ethanol (5 mL) after refluxing for 24 h to obtain 0.09 g (31%) of yellow powder in the form of a mixture of *E*- and *Z*-isomers at a ratio of **42a**/**42b** = 3:1. m.p. 231–234 °C; **42a**: ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 9.26 (s, 1H, H_{arom}), 7.91 (d, 2H, J = 8.88 Hz, H_{arom}), 7.55–7.51 (m, 1H, H_{arom}), 7.51–7.47 (m, 1H, H_{arom}), 7.19–7.14 (m, 2H, H_{arom}), 6.84 (d, 2H, J = 8.99 Hz, H_{arom}), 4.35 (t, 2H, J = 6.88 Hz, CH_2), 3.07 (s, 6H, CH_3), 1.80–1.75 (m, 2H, CH_2), 1.29–1.17 (m, 6H, CH_2), 0.79 (t, 3H, J = 7.27 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 165.8, 153.8, 137.8, 124.7, 122.4 (2C), 122.4, 122.2, 120.1, 117.7, 111.5 (2C), 111.1 (2C), 108.4, 103.6, 41.9, 30.5, 28.9, 25.5, 21.9, 13.8; **42b**: ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 9.67 (s, 1H, H_{arom}), 7.69 (d, 2H, J = 8.97 Hz, H_{arom}), 7.35 (d, 1H, J = 8.36 Hz, H_{arom}), 7.26 (dd, 1H, J_1 = 1.98 Hz, J_2 = 6.67 Hz, H_{arom}), 7.13–7.08 (m, 2H, H_{arom}), 6.79 (d, 2H, J = 8.86 Hz, H_{arom}), 4.03 (t, 2H, J = 7.29 Hz, CH_2), 3.05 (s, 6H, CH_3), 1.67–1.62 (m, 2H, CH_2), 1.29–1.17 (m, 6H, CH_2), 0.84 (t, 3H, J = 6.97 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 156.6, 142.8, 141.2 (2C),

132.1, 123.0, 128.1, 122.4 (2C), 120.7, 117.7 (2C), 111.1, 111.0, 102.0, 41.6, 30.9, 28.2, 25.6, 22.0, 13.8; Anal. Calcd. for $C_{22}H_{28}N_4$: C, 75.82; H, 8.10; N, 16.08. Found: C, 75.78; H, 8.16; N, 16.14%.

(*E*)-2-((4-(Dimethylamino)benzylidene)amino)-1-hexyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **43**

Compound **43** was prepared from 2-amino-6-cyano-1-hexylbenzimidazole **24** (0.15 g, 0.6 mmol) and 4-*N*,*N*-dimethylamino-benzaldehyde **25** (0.09 g, 0.6 mmol) in absolute ethanol (5 mL) after refluxing for 48 h to obtain 0.09 g (43%) of yellow powder. m.p. 165–170 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 9.27 (s, 1H, H_{arom}), 8.02 (d, 1H, J = 0.88 Hz, H_{arom}), 7.95 (s, 1H, H_{arom}), 7.92 (s, 1H, H_{arom}), 7.72 (d, 1H, J = 8.37 Hz, H_{arom}), 7.56 (dd, 1H, J_1 = 1.39 Hz, J_2 = 8.28 Hz, H_{arom}), 7.30 (d, 1H, J = 1.38 Hz, H_{arom}), 6.86–6.82 (m, 2H, H_{arom}), 4.39 (t, 2H, J = 6.79 Hz, CH_2), 3.08 (s, 6H, CH_3), 1.83–1.72 (m, 2H, CH_2), 1.27–1.20 (m, 6H, CH_2), 0.77 (t, 3H, J = 6.97 Hz, CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 165.77, 158.29, 156.63, 153.81, 142.75, 141.22, 137.80, 137.78, 132.15, 129.99, 128.12, 124.68, 122.38, 122.35, 122.20, 120.65, 120.12, 117.68, 111.64, 111.54, 111.14, 111.03, 108.41, 103.57, 101.96, 41.96, 41.60, 30.85, 30.50, 28.90, 28.23, 25.57, 25.50, 21.96, 21.90, 13.80, 13.75; Anal. Calcd. for $C_{23}H_{27}N_5$: C, 73.96; H, 7.29; N, 18.75. Found: C, 74.01; H, 7.24; N, 18.70%.

(*E*)-5-(Diethylamino)-2-(((1-hexyl-1*H*-benzo[*d*]imidazol-2-yl)imino)methyl)phenol **44**

Compound **44** was prepared from 2-amino-1-hexylbenzimidazole **20** (0.15 g, 0.7 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.13 g, 0.7 mmol) in absolute ethanol (5 mL) after refluxing for 48 h to obtain 0.03 g (11%) of yellow powder. m.p. 201–204 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 12.68 (s, 1H, OH), 9.36 (s, 1H, H_{arom}), 7.56 (d, 1H, J = 8.96 Hz, H_{arom}), 7.54–7.48 (m, 2H, H_{arom}), 7.21–7.13 (m, 2H, H_{arom}), 6.42 (dd, 1H, J_1 = 2.37 Hz, J_2 = 8.97 Hz, H_{arom}), 6.16 (d, 1H, J = 2.27 Hz, H_{arom}), 4.24 (t, 2H, J = 7.07 Hz, CH_3), 3.44 (q, 4H, J = 7.09 Hz, CH_2), 1.78–1.73 (m, 2H, CH_2), 1.29–1.20 (m, 6H, CH_2), 1.15 (t, 6H, J = 7.08 Hz, CH_3), 0.80 (t, 3H, J = 7.07 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 164.0, 163.9, 154.6, 154.3, 153.4, 141.9, 135.1, 122.2, 121.8, 118.4, 111.7, 110.2, 109.0, 105.5, 104.9, 97.0, 96.4, 44.6, 44.6, 42.6, 31.2, 29.6, 26.4, 22.5, 14.2, 13.0, 12.9; Anal. Calcd. for $C_{24}H_{32}N_4O$: C, 73.43; H, 8.22; N, 14.27; O, 4.08. Found: C, 73.37; H, 8.09; N, 14.15; O, 4.02%.

(*E*)-2-((4-(Diethylamino)-2-hydroxybenzylidene)amino)-1-hexyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **45**

Compound **45** was prepared from 2-amino-6-cyano-1-hexylbenzimidazole **24** (0.15 g, 0.6 mmol) and 4-*N*,*N*-diethylamino-2-hydroxybenzaldehyde **26** (0.11 g, 0.6 mmol) in absolute ethanol (6 mL) after refluxing for 48 h to obtain 0.02 g (8%) of yellow powder. m.p. 233–237 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm = 12.47 (s, 1H, OH), 9.38 (s, 1H, H_{arom}), 8.00 (d, 1H, J = 1.19 Hz, H_{arom}), 7.72 (d, 1H, J = 8.27 Hz, H_{arom}), 7.60 (d, 1H, J = 8.99 Hz, H_{arom}), 7.56 (dd, 1H, J_1 = 1.45 Hz, J_2 = 8.27 Hz, H_{arom}), 6.44 (dd, 1H, J_1 = 2.37 Hz, J_2 = 8.96 Hz, H_{arom}), 6.16 (d, 1H, J = 2.28 Hz, H_{arom}), 4.28 (t, 2H, J = 7.06 Hz, CH_2), 3.45 (q, 4H, J = 6.98 Hz, CH_2), 1.76–1.72 (m, 2H, CH_2), 1.28–1.25 (m, 4H, CH_2), 1.23–1.20 (m, 2H, CH_2), 1.15 (t, 6H, J = 7.09 Hz, CH_3), 0.80 (t, 3H, J = 7.08 Hz, CH_3), ^{13}C NMR (151 MHz, DMSO-d_6): δ/ppm = 164.3, 153.9, 141.7, 138.3, 125.2, 122.6, 120.6, 111.5, 109.1, 105.9 (2C), 104.1, 96.9 (2C), 44.7 (2C), 42.9, 31.1, 29.5, 26.3, 22.4, 14.2, 13.0; Anal. Calcd. for $C_{25}H_{31}N_5O$: C, 71.91; H, 7.48; N, 16.77; O, 3.83. Found: C, 71.94; H, 7.42; N, 16.71; O, 3.88%.

4.3. Biology

4.3.1. Antiviral Activity

HEL 299 (ATCC CCL-137; human lung fibroblast), Huh-7 (CLS—300156; human hepatoblastoma), and MDCK (Madin-Darby canine kidney cells; a kind gift from M. Matrosovich, Marburg, Germany) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco Life Technologies) supplemented with 8% heat-inactivated fetal bovine serum (HyClone, GE Healthcare Life Sciences), 0.075% sodium bicarbonate (Gibco Life

Technologies) and 1mM sodium pyruvate (Gibco Life Technologies), and maintained at 37 °C under 5% CO_2. Antiviral assays towards herpes simplex virus-1 (HSV-1 KOS), human coronavirus (HCoV-229E and -OC43), and respiratory syncytial virus A in HEL 299 cell cultures; sindbis virus, yellow fever virus, Zika virus, and human coronavirus (HCoV-NL63) in Huh-7 cell cultures; and influenza A/H1N1 (A/Ned/378/05), influenza A/H3N2 (A/HK/7/87), and influenza B (B/Ned/537/05) in MDCK cell cultures were performed. On the day of the infection, the growth medium was aspirated and replaced by serial dilutions of the test compounds. The virus was then added to each well and diluted to obtain a viral input of 100 $CCID_{50}$ ($CCID_{50}$ being the virus dose that is able to infect 50% of the cell cultures). Mock-treated cultures receiving solely the test compounds were included in order to determine the cytotoxicity.

After 3 to 7 days of incubation, the virus-induced cytopathogenic effect was measured colorimetrically by the formazan-based MTS cell viability assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay from Promega, Madison, WI), and the antiviral activity was expressed as the 50% effective concentration (EC_{50}). In parallel, the 50% cytotoxic concentration (CC_{50}) was derived from the mock-infected cells. The activities were compared with the activities of the reference antiviral drugs: remdesivir, ribavirin, zanamivir, rimantadine, and brivudine (BVDU).

4.3.2. Antibacterial Activity

Materials

In addition to the synthesized compounds, standard antibiotics ampicillin, ceftazidime, ciprofloxacin, and meropenem from USP were tested. Selected bacterial strains were Gram-negative *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* and Gram-positive *S. aureus*, *S. pneumoniae*, and *E. faecalis*. The synthesized compounds were prepared as 10 mM DMSO solutions and tested in a final concentration range of 0.2–100 μM [39]. Standard antibiotics were prepared as 5 mg/mL DMSO solutions and tested in a final concentration range of 0.125–64 μg/mL.

Methods

Broth microdilution testing was performed according to CLSI (Clinical Laboratory Standards Institute) guidelines. The MIC (minimal inhibitory concentration) value was defined as the last tested concentration of the compound at which there was no visible growth of bacteria. Inoculums for each microorganism were prepared using the direct colony suspension method, where broth solutions that achieved turbidity equivalent to 0.5 McFarland standard were additionally diluted 100× with Ca adjusted MH media (Becton Dickinson, Franklin Lakes, NJ, USA). All of the test plates were incubated for 16–24 h at 37 °C.

MIC values for reference antibiotics against quality control strains were used for confirming the validity of the screen according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically followed M07, 11th edition, 2018, and Clinical and Laboratory Standards Institute (CLSI) guidelines. Performance standards for antimicrobial susceptibility testing followed the M100, 28th edition, 2018.

4.3.3. Cell Culture and Reference Compounds

Human cancer cells used in this manuscript, namely Capan-1, HCT-116, NCI-H460, LN-229, HL-60, K-562, and Z-138, were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA), while the DND-41 cell line was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ Leibniz-Institut, Braunschweig, Germany). Culture media were purchased from Gibco Life Technologies, Merelbeke, Belgium, and supplemented with 10% fetal bovine serum (HyClone, Cytiva, Marlborough, MA, USA). Vincristine and docetaxel, which were used as the reference inhibitors, were purchased from Selleckchem (Munich, Germany). Stock solutions were prepared in DMSO.

4.3.4. Proliferation Assays

Adherent cell lines LN-229, HCT-116, and NCI-H460 and Capan-1 cells were seeded at a density between 500 and 1500 cells per well, in 384-well tissue culture plates (Greiner, Kremsmünster, Austria). After overnight incubation, the cells were treated with seven different concentrations of the test compounds, ranging from 100 to 0.006 μM.

Suspension cell lines HL-60, K-562, Z-138, and DND-41 were seeded at densities ranging from 2500 to 5500 cells per well in 384-well culture plates containing the test compounds at the same concentration points. The cells were incubated for 72 h with the compounds and were then analyzed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) reagent (Promega, Madison, WI, USA) according to the manufacturer's instructions. The absorbance of the samples was measured at 490 nm using a SpectraMax Plus 384 (Molecular Devices, Silicon Valley, CA, USA), and OD values were used to calculate the 50% inhibitory concentration (IC_{50}). The compounds were tested in two independent experiments.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/molecules28093720/s1. Pages S2–S5: 13C NMR data and elemental analysis of prepared compounds. Pages S5–S7: Biological experimental data. Figures S1–S48: NMR spectra of the prepared compounds.

Author Contributions: Synthesis and characterization of targeted compounds A.B. and M.C.; antiproliferative activity in vitro L.P. and D.D.; antibacterial activity in vitro V.R. and M.B.; antiviral activity in vitro L.P. and D.D.; writing—original draft preparation L.P., D.D. and M.H. All authors have read and agreed to the published version of the manuscript.

Funding: We greatly appreciate the financial support of the Croatian Science Foundation under project 4379, titled Exploring the antioxidative potential of benzazole scaffold in the design of novel antitumor agents.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: D.D. and L.P. are grateful for the excellent technical assistance of J. Punjwani, N. Van Winkel, N. Willems, and Y. Smolders.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Raczuk, E.; Dmochowska, B.; Samaszko-Fiertek, J.; Madaj, J. Different Schiff Bases-Structure, Importance and Classification. *Molecules* **2022**, *27*, 787. [CrossRef] [PubMed]
2. Tidwell, T.T. Hugo (Ugo) Schiff, Schiff bases, and a century of β-lactam synthesis. *Angew. Chem. Int. Ed.* **2008**, *47*, 1016–1020. [CrossRef] [PubMed]
3. Arulmurugan, S.; Kavitha, H.P.; Venkatraman, B.R. Biological activities of Schiff base and its complexes: A review. *Rasayan J. Chem.* **2010**, *3*, 385–410.
4. Qin, W.L.; Long, S.; Panunzio, M.; Biondi, S. Schiff bases: A short survey on anevergreen chemistry tool. *Molecules* **2013**, *18*, 12264–12289. [CrossRef]
5. Zoubi, W.A. Biological Activities of Schiff Bases and Their Complexes: A Review of Recent Works. *Int. J. Org. Chem.* **2013**, *3*, 73–95. [CrossRef]
6. Lenahan, C.; Sanghavi, R.; Huang, L.; Zhang, J.H. Rhodopsin: A Potential Biomarker for Neurodegenerative Diseases. *Front. Neurosci.* **2020**, *14*, 326. [CrossRef]
7. Carey, F.A. *Organic Chemistry*, 5th ed.; MacGraw-Hill: New York, NY, USA, 2003; p. 724.
8. Anand, P.; Patil, V.M.; Sharma, V.K.; Khosa, R.L.; Masand, N. Schiff bases: A review on biological insights. *Int. J. Drug Design Disc.* **2012**, *3*, 851–865.

9. Şener, N.; Özkinali, S.; Altunoglu, Y.C.; Yerlikaya, S.; Gökçe, H.; Zurnaci, M.; Gür, M.; Baloglu, M.C.; Şener, İ. Antiproliferative properties and structural analysis of newly synthesized Schiff bases bearing pyrazole derivatives and molecular docking studies. *J. Mol. Struct.* **2021**, *1241*, 130520. [CrossRef]
10. Sztanke, K.; Maziarka, A.; Osinka, A.; Sztanke, M. An insight into synthetic Schiff bases revealing antiproliferative activities in vitro. *Bioorg. Med. Chem.* **2013**, *21*, 3648–3666. [CrossRef]
11. Vicini, P.; Geronikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Cabras, C.A.; la Colla, P. Synthesis and biological evaluation of benzo[d]isothiazole, benzothiazole and thiazole Schiff bases. *Bioorg. Med. Chem.* **2003**, *11*, 4785–4789. [CrossRef]
12. Jos, S.; Suja, N.R. Chiral Schiff base ligands of salicylaldehyde: A versatile tool for medical applications and organic synthesis—A review. *Inorg. Chim. Acta* **2023**, *547*, 121323. [CrossRef]
13. Cozzi, P.G. Metal-Salen Schiff base complexes in catalysis: Practical aspects. *Chem. Soc. Rev.* **2004**, *33*, 410–421. [CrossRef] [PubMed]
14. Ashraf, T.; Ali, B.; Qayyum, H.; Haroone, M.S.; Shabbir, G. Pharmacological aspects of schiff base metal complexes: A critical review. *Inorg. Chem. Comm.* **2023**, *150*, 110449. [CrossRef]
15. Saloutin, V.I.; Edilova, Y.O.; Kudyakova, Y.S.; Burgart, Y.V.; Bazhin, D.N. Heterometallic Molecular Architectures Based on Fluorinated β-Diketone Ligands. *Molecules* **2022**, *27*, 7894. [CrossRef] [PubMed]
16. Cimerman, Z.; Snežana, M.; Nives, G. Schiff bases derived from aminopyridines as spectrofluorimetric analytical reagents. *Croat. Chem. Acta* **2000**, *73*, 85–96.
17. Gupta, K.C.; Sutar, A.K. Catalytic activities of Schiff base transition metalcomplexes. *Coord. Chem. Rev.* **2008**, *252*, 1420–1450. [CrossRef]
18. Rauf, A.; Shah, A.; Khan, A.A.; Shah, A.H.; Abbasi, R.; Qureshi, I.Z.; Ali, S. Synthesis, pH dependent photometric and electrochemical investigation, redox mechanism and biological applications of novel Schiff base and its metallic derivatives. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2017**, *176*, 155–167. [CrossRef] [PubMed]
19. Zhang, S.H.; Feng, C. Microwave-assisted synthesis, crystal structure and fluorescence of novel coordination complexes with Schiff base ligands. *J. Mol. Struct.* **2010**, *977*, 62–66. [CrossRef]
20. Horak, E.; Kassal, P.; Hranjec, M.; Murković Steinberg, I. Benzimidazole functionalised Schiff bases: Novel pH sensitivefluorescence turn-on chromoionophores for ion-selective optodes. *Sens. Act. B* **2018**, *258*, 415–423. [CrossRef]
21. Castillo-Martnez, E.; Carretero-Gonzlez, J.; Armand, M. Polymeric Schiff Bases as Low-Voltage Redox Centers for Sodium-Ion Batteries. *Angew. Chem. Int. Ed.* **2014**, *53*, 5341–5345. [CrossRef]
22. Abdel Hameed, R.S. Schiff' Bases as Corrosion Inhibitor for Aluminum Alloy in Hydrochloric Acid Medium. *Tenside Surf. Det.* **2019**, *56*, 3–10. [CrossRef]
23. Gama, S.; Mendes, F.; Marques, F.; Santos, I.C.; Carvalho, M.F.; Correia, I.; Pessoa, J.C.; Santos, I.; Paulo, A. Copper(II) complexes with tridentate pyrazole-based ligands: Synthesis, characterization, DNA cleavage activity and cytotoxicity. *J. Inorg. Biochem.* **2011**, *105*, 637–644. [CrossRef]
24. Katwal, R.; Kaur, H.; Kapur, B.K. Applications of copper—Schiff's base complexes: A review. *Sci. Rev. Chem. Commun.* **2013**, *3*, 1–15.
25. Aiyelabola, T.; Jordaan, J.; Otto, D.; Akinkunmi, E. Syntheses, Characterization, Antimicrobial Activity and Extraction Studies of Tetraaza Macrocyclic/Linear Schiff Bases Derived from Benzene-1,4-Dicarboxaldehyde and Their Coordination Compounds. *Adv. Biolog. Chem.* **2021**, *11*, 79–105. [CrossRef]
26. Fonkui, T.Y.; Ikhile, M.I.; Ndinteh, D.T.; Njobeh, P.B. Microbial activity of some heterocyclic Schiff bases and metal complexes: A review. *Trop. J. Pharm. Res.* **2018**, *17*, 2507–2518. [CrossRef]
27. Ugras, H.I.; Basaran, I.; Kilic, T.; Cakir, U. Synthesis, Complexation and Antifungal, Antibacterial Activity Studies of a New Macrocyclic Schiff Base. *J. Het. Chem.* **2006**, *43*, 1679–1684. [CrossRef]
28. Singh, H.L. Synthesis and characterization of tin (II) complexes of fluorinated Schiff bases derived from amino acids. *Spect. Acta Part A* **2010**, *76*, 253–258. [CrossRef] [PubMed]
29. Halder, S.; Bhattacharjee, A.; Roy, A.; Chatterjee, S.; Roy, P. Chromogenic andfluorescence sensing of pH with a Schiff-base molecule. *RSC Adv.* **2016**, *6*, 39118–39124. [CrossRef]
30. Hameed Haddad, H. A New Schiff Base Derivatives Designed to Bind Metal Ion (Cu, Co): Thermodynamics and Biological Activity Studies. *J. Anal. Chem.* **2016**, *7*, 445–451. [CrossRef]
31. Nawrocka, W.; Sztuba, B.; Kowalska, M.W.; Liszkiewicz, H.; Wietrzyk, J.; Nasulewicz, A.; Pełczynska, M.; Opolski, A. Synthesis and Antiproliferative Activity in vitro of New 2-Aminobenzimidazole Derivatives Part 2 [1]. *Farmaco* **2004**, *59*, 1047–1055. [CrossRef]
32. Neochoritis, C.G.; Zarganes-Tzitzikas, T.; Tsoleridis, C.A.; Stephanidou-Stephanatou, J.; Kontogiorgis, C.A.; Hadjipavlou-Litina, D.J.; Choli-Papadopoulou, T. One-pot microwave assisted synthesis under green chemistry conditions, antioxidant screening, and cytotoxicity assessments of benzimidazole Schiff bases and pyrimido[1,2-*a*]benzimidazol-3(4H)-ones. *Eur. J. Med. Chem.* **2011**, *46*, 297–306. [CrossRef] [PubMed]
33. Fonkui, T.Y.; Ikhile, M.I.; Njobeh, P.B.; Ndinteh, D.T. Benzimidazole Schiff base derivatives: Synthesis, characterization and antimicrobial activity. *BMC Chem.* **2019**, *13*, 127–138. [CrossRef] [PubMed]
34. Alam, S.A.M.F.; Ahmad, T.; Nazmuzzaman, M.; Ray, S.K.; Sharifuzzaman, M.; Karim, M.R.; Alam, M.G.; Ajam, M.M.; Maitra, P.; Mandol, D.; et al. Synthesis of Benzimidazole Derivatives Containing Schiff Base Exhibiting Antimicrobial Activities. *Int. J. Res. Stud. Biosci.* **2017**, *5*, 18–24.

35. Kumaravel, G.; Utthra, P.P.; Raman, N. Exploiting the biological efficacy of benzimidazole based Schiff base complexes with L-Histidine as a co-ligand: Combined molecular docking, DNA interaction, antimicrobial and cytotoxic studies. *Bioorg. Chem.* **2018**, *77*, 269–279. [CrossRef]
36. Aragón-Muriel, A.; Liscano, Y.; Upegui, Y.; Robledo, S.M.; Ramírez-Apan, M.T.; Morales-Morales, D.; Oñate-Garzón, J.; Polo-Cerón, D. In Vitro Evaluation of the Potential Pharmacological Activity and Molecular Targets of New Benzimidazole-Based Schiff Base Metal Complexes. *Antibiotics* **2021**, *10*, 728. [CrossRef]
37. Song, W.-J.; Cheng, J.-P.; Jiang, D.-H.; Guo, L.; Cai, M.-F.; Yang, H.-B.; Lin, Q.-Y. Synthesis, interaction with DNA and antiproliferative activities of twonovel Cu(II) complexes with Schiff base of benzimidazole. *Spect. Acta Part A Mol. Biomol. Spectr.* **2014**, *121*, 70–76. [CrossRef] [PubMed]
38. Hranjec, M.; Starčević, K.; Kraljević Pavelić, S.; Lučin, P.; Pavelić, K.; Karminski Zamola, G. Synthesis, spectroscopic characterization and antiproliferative evaluation in vitro of novel Schiff bases related to benzimidazoles. *Eur. J. Med. Chem.* **2011**, *46*, 2274–2279. [CrossRef]
39. Grgičević, I.; Mikulandra, I.; Bukvić, M.; Banjanac, M.; Radovanović, V.; Habinovec, I.; Bertoša, B.; Novak, P. Discovery of macrozones, new antimicrobial thiosemicarbazone-based azithromycin conjugates: Design, synthesis and in vitro biological evaluation. *Int. J. Antimicrob. Agents* **2020**, *56*, 106147. [CrossRef]