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Biological assessments of novel ultrasound-synthesized 2-arylbenzimidazole derivatives: antiproliferative and antibacterial effects†

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This paper describes ultrasound synthesis, structural characterization and biological activity of new derivatives of 2-arylbenzimidazole **12–27** and 1,2,3-triazole derivatives of 2-arylbenzimidazole **28–33**. The tautomeric structures of the prepared target compounds were confirmed by ^{1}H- and ^{13}C-NMR spectroscopy as well as by two-dimensional NOESY, HSQC and HMBC methods. The synthesized compounds underwent *in vitro* antiproliferative assays, revealing that compound **23** exhibited the highest potency against chronic myeloid leukemia cells (K-562, IC_{50} = 2.0 μM) and non-Hodgkin's lymphoma cells (Z-138, IC_{50} = 2.0 μM). Compound **23** was further evaluated for cytotoxicity on normal peripheral blood mononuclear cells (PBMC), and its mechanism of action was investigated. The antibacterial properties of the synthesized compounds were assessed against both Gram-positive and Gram-negative bacterial strains. Derivatives **15–17** exhibited significant selective antibacterial activity against the Gram-positive bacterium *Enterococcus faecalis* (MIC = 0.25–1 μg mL^{-1}). Additionally, among the 1,2,3-triazole derivatives of 2-arylbenzimidazole, compounds **28** and **30** demonstrated strong selective activity against *Enterococcus faecalis* (MIC = 0.25 μg mL^{-1}).

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, characterized by the uncontrolled proliferation of abnormal cells. This malignancy often invades neighboring tissues and spreads through metastasis.[1] According to the International Agency for Research on Cancer (IARC), 19.3 million new cancer cases were diagnosed globally in 2020, resulting in 10 million deaths.[2] These statistics underscore the urgent need for developing novel and effective anticancer agents. Beyond cancer, bacterial infections and antimicrobial resistance (AMR) have emerged as critical global health challenges.[3] Excessive exposure to bacteria, along with the improper selection and dosing of antibiotics, has significantly contributed to the rising resistance of microorganisms. A comprehensive study from 2019 identified over 250 000 deaths associated with AMR pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*-collectively known as ESKAPE pathogens, multidrug-resistant bacteria.[4,5] More recently, a 2024 study reported 1.14 million deaths attributable to bacterial AMR.[6] Alarmingly, projections indicate that by 2050, the incidence of antibiotic resistance could increase tenfold annually.[7] The relentless evolution of resistance mechanisms among these pathogens highlights the pressing need for innovative antibacterial therapies. Benzimidazole, a fused benzene-imidazole bicyclic heterocycle, has long held prominence in medicinal

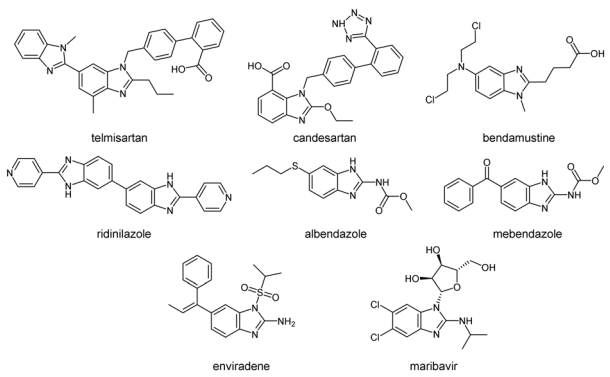

Fig. 1 Therapeutic agents featuring benzimidazole skeleton.

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chemistry.[8,9] First proposed by Woolley in 1944 due to its purine-like structure,[10] the bioactivity of benzimidazole was later reinforced by Brink's discovery of *N*-ribosyl-dimethylbenzimidazole in vitamin B_{12},[11,12] highlighting its therapeutic significance.[13] The structural resemblance of benzimidazole to nucleotides facilitates interactions with proteins, enzymes, and receptors, giving rise to a wide range of biological activities.[14] Benzimidazole derivatives have since been reported to exhibit antimicrobial,[15–17] antiproliferative,[18–20] antioxidative,[21–24] anti-inflammatory,[25,26] and antidiabetic[27–29] properties, earning their designation as a privileged structure. Currently, numerous drugs featuring a benzimidazole pharmacophore core in their structure (Fig. 1) are utilized to treat a range of conditions, including hypertension (telmisartan, candesartan),[26] chronic myeloid leukemia (bendamustine),[30] bacterial infections (ridinilazole),[31] parasitic infections (albendazole, mebendazole),[32] and viral illnesses (enviradene, maribavir).[33,34]

In addition to the listed commercially available drugs, benzimidazole derivatives with strong antiproliferative and antibacterial effects can also be found in the literature.[35,36] It was found that the biological properties of benzimidazole derivatives were influenced by substitutions at the N-1, C-2, and C-5/6 positions.

Substituting the benzimidazole core with a halogen atom at C-5 enhances anticancer activity, whereas the introduction of electron-withdrawing groups at C-4 and C-5 decreases activity. Additionally, substitution of the phenyl ring at position C-2 of benzimidazole increases anticancer activity, especially when combined with cyclic or aliphatic amines rather than aromatic amine groups. Furthermore, introducing an aliphatic chain at the N-1 position of the benzimidazole ring may improve activity.[37,38] Thus, benzimidazole derivatives **I** and **II** (Fig. 2) substituted at the C-2 position with a phenyl moiety and at the C-5 position with carbonyl substituents, showed enhanced activity against lung cancer cells (A549) and breast cancer cells (MCF7).[39–41] Furthermore, the 2-arylbenzimidazole derivative **III** exhibited significant anticancer activity against lung cancer cells (A549), while the introduction of a cycloaminoalkyl group in derivative **IV** increased anticancer activity against both lung (A549) and breast cancer cells (MCF7).[42,43]

Benzimidazole derivatives substituted at the N-1 and C-2 positions **V** also exhibited excellent antitumor activity against lung cancer cells (NCI-H460) and colon carcinoma cells (HCT-116).[44,45] Similarly, the 1,2,3-triazole scaffold has garnered significant attention in drug design due to its chemical versatility and pharmacological potential.[46] As a robust bioisostere for amide bonds, aromatic rings, double bonds, and imidazole rings, 1,2,3-triazole can engage biological targets *via* hydrogen bonding and dipole interactions.[47] Its synthesis was revolutionized by the Huisgen Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC), which enables the efficient production of 1,4-disubstituted 1,2,3-triazoles.[48,49] The strategic combination of benzimidazole and 1,2,3-triazole scaffolds has shown synergistic potential in addressing antiproliferative and antimicrobial activities.[16,50,51] Thus, benzimidazole derivatives substituted with 1,2,3-triazole moiety **VI** exhibit potent antiproliferative activity against lung cancer cells (A549).[52,53] These dual-functional hybrids not only demonstrate robust anticancer effects but also exhibit promising antibacterial activity, suggesting their potential to address both cancer and AMR-related challenges.

Due to the significant pharmacological potential of benzimidazoles, numerous studies have been conducted on the synthesis of the benzimidazole core over the years. The most common and widely used method for synthesizing benzimidazole derivatives with different substituents at positions C-2 and C-5/6 involves the condensation of *o*-phenylenediamine (OPD) with carboxylic acids or their derivatives, such as acid chlorides, orthoesters, and nitriles, or with carbonyl compounds, including aliphatic or aromatic aldehydes, in the presence of an appropriate catalyst.[37,38] Among these methods, the most accepted approach is the condensation of substituted OPD with aldehydes using various alkali catalysts, leading to the formation of mono- and disubstituted benzimidazoles.[9] The condensation reaction between OPD and aldehydes has garnered considerable interest, prompting the development of several novel synthetic methods.[54] Furthermore, recent advancements have introduced innovative methodologies that utilize diverse substrates with high efficiency or employ environmentally friendly procedures, including solvent-free conditions, metal-free catalysts, and photocatalytic systems.[55,56] However, to the best of our knowledge, only a few studies have reported the ultrasound-promoted synthesis of 1,2-disubstituted benzimidazole derivatives using SiTCA or synthesis of 2-substituted benzimidazole derivatives through the reaction of OPD with various substituted benzoyl chlorides in the presence of natural feedstock BPAE or ionic liquids.[57–59]

Based on findings that the most important positions for biological activity is the substitution on benzimidazole core at position C-2, C-5/6 and N-1, we designed and prepared by

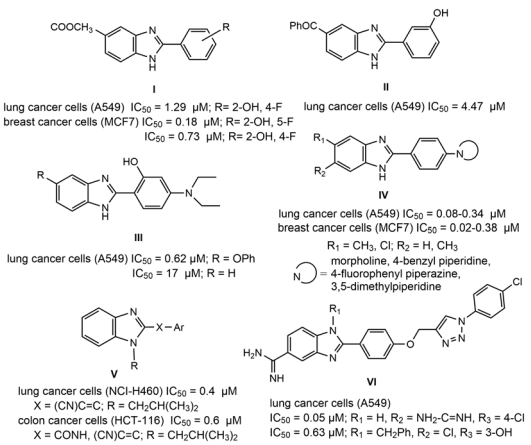


Fig. 2 2-Arylbenzimidazole derivatives with potent *in vitro* antiproliferative activity.

ultrasound-assisted synthesis novel 2-arylbenzimidazole derivatives bearing different aminoalkyl substituents attached directly to the phenyl ring or bridged by 1,2,3-triazole pharmacophoric moiety and chlorine or fluorine atom at position C-6 of benzimidazole (Fig. 3).

In order to examine the influence of the substituents of the newly synthesized benzimidazole derivatives on the biological activity, antiproliferative activity towards eight human cancer cell lines as well as antibacterial activity against Gram-positive and Gram-negative bacteria were performed.

2. Results and discussion

2.1. Chemistry

The *O*-alkylated derivatives of 2-arylbenzimidazole **12–27** (Fig. 3) were synthesized through a two-step process. First, the corresponding *O*-alkylated benzaldehydes **1–6** were prepared as precursors, followed by ultrasound promoted cyclization with the appropriate *o*-phenylenediamine. Similarly, the 1,2,3-triazole derivatives **28–33** were obtained through a three-step procedure, involving *O*-propargylation, a click reaction, and a final ultrasound-assisted condensation step that facilitated benzimidazole ring formation. The *O*-alkylated benzaldehyde derivatives **1–6** were achieved by an *O*-alkylation reaction of the corresponding 4-hydroxybenzaldehydes with appropriate chloroalkylating reagent using K_2CO_3 as a base (Scheme 1).

For the preparation of the target 1,2,3-triazole derivatives, the corresponding terminal alkynes **7** and **8** and morpholine azide **9** were prepared (Scheme 2). The *O*-propargylated benzaldehyde derivatives **7** and **8** were obtained by reacting the corresponding 4-hydroxybenzaldehyde with propargyl bromide using K_2CO_3 as a base, while reaction of *N*-chloroethyl morpholine with sodium azide yielded morpholine azide **9**. Using a copper-catalyzed click reaction of the *O*-propargylated benzaldehydes **7** and **8** with *N*-azidoethylmorpholine **9**, 1,2,3-triazole derivatives of benzaldehyde were synthesized as precursors for the condensation reaction with *o*-phenylenediamine.

2-Arylbenzimidazole derivatives **12–27** were synthesized by ultrasound-assisted cyclocondensation reaction of prepared *O*-alkylated benzaldehydes **1–6** with differently substituted *o*-phenylenediamine in the presence of $Na_2S_2O_5$ as an oxidizing agent in yields of 17.5–84.1% (Scheme 3). The use of an oxidative reagent is necessary to prevent the formation of 1,2-disubstituted benzimidazole derivatives.[60]

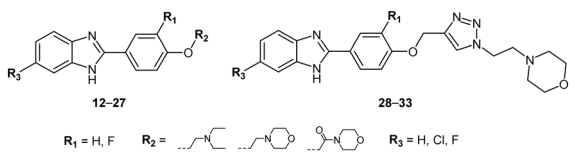
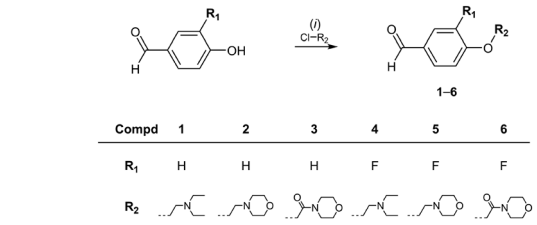
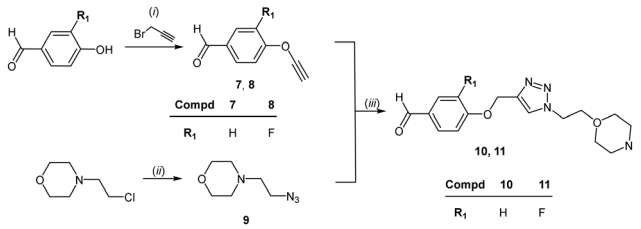


Fig. 3 The designed and synthesized 2-arylbenzimidazole derivatives **12–33**.

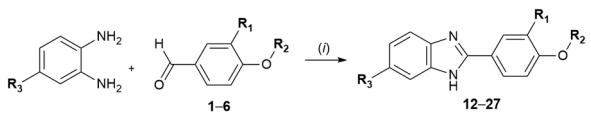


Scheme 1 Reagents and conditions: (i) CH_3CN, K_2CO_3, appropriate chloroalkylating reagent, reflux 6 h, overnight r.t.



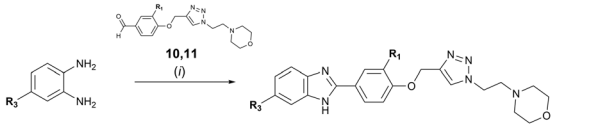
Scheme 2 Reagents and conditions: (i) CH_3CN, K_2CO_3, propargyl bromide, reflux 6 h, overnight r.t., (ii) NaN_3, H_2O, 80 °C, 16 h, (iii) $Cu(OAc)_2$, NaN_3, MeOH, overnight r.t.

1,2,3-Triazole derivatives of 2-arylbenzimidazoles **28–33** (Scheme 4) were synthesized by ultrasound-assisted reaction of prepared 1,2,3-triazole derivatives of benzaldehyde **10** and **11** with *o*-phenylenediamine in the presence of $Na_2S_2O_5$ as an oxidizing agent in yields of 39.4–80.4%. Since satisfactory yields of 2-arylbenzimidazoles were obtained through ultrasound-assisted cyclocondensation without the use of a catalyst and without the formation of 1,2-disubstituted derivatives, this approach can be considered applicable to the synthesis of similar 2-arylbenzimidazole derivatives.



Compd	R1	R2	R3	Compd	R1	R2	R3
12	H		H	20	H		F
13	H		H	21	F		H
14	H		H	22	F		H
15	H		Cl	23	F		Cl
16	H		Cl	24	F		Cl
17	H		Cl	25	F		Cl
18	H		F	26	F		F
19	H		F	27	F		F

Scheme 3 Reagents and conditions: (i) $Na_2S_2O_5$, DMF, 1.5 h r.t., 2–7.5 h, 50 °C, ultrasound 1200 W.



Compd	R_3	R_1	Compd	R_3	R_1
28	H	H	31	H	F
29	Cl	H	32	Cl	F
30	F	H	33	F	F

Scheme 4 Reagents and conditions: (i) $Na_2S_2O_5$, DMF, 1.5 h r.t., 3.5 h, 50 °C, ultrasound 1200 W.

2.2. Structural characterization of 2-arylbenzimidazole derivatives

The structures of the prepared 2-arylbenzimidazole derivatives **12–33** were confirmed by mass spectrometry, IR and one-dimensional ^{1}H- and ^{13}C-NMR spectroscopy. The details of the structural characterization are provided in the experimental section and ESI.† The ^{1}H-NMR spectra of derivatives **12–33** (ESI† Fig. S16–S37) exhibit the characteristic signal for the NH proton of the imidazole ring at ~12 ppm, and in the aliphatic region, signals corresponding to the protons of the introduced amine substituents are also observed. Additionally, the spectra of 1,2,3-triazole derivatives **28–33** (ESI† Fig. S32–S37) contain a signal at ~8.26 ppm corresponding to the triazole ring proton. Notably, the ^{1}H-NMR spectra of derivatives with fluorine at position 6 of the benzimidazole moiety show additional splitting of H-4 and H-7 due to coupling with fluorine through two, three, or four bonds. Additionally, the signals for protons H-7 and H-5 are shifted upfield. The APT spectra of 2-arylbenzimidazole derivatives **12–27** (ESI† Fig. S48–S63) show signals for the carbons of the benzimidazole ring in the aromatic region (δ 158.61–97.47 ppm), along with signals in the aliphatic region and additional signals from tautomeric structures. In the APT spectra of fluorine-containing derivatives, doublets with varying coupling constants appear due to splitting through one, two, or three bonds. Two doublets are observed for the tautomeric pairs C-4/C-4·, C-5/C-5·, and C-7/C-7·, all shifted downfield compared to the unsubstituted benzimidazole derivative. A detailed comparison of the influence of C-6 substituents on chemical shifts is provided in the ESI.† To unambiguously determine the chemical shifts of the protons and carbons in the tautomeric structures, heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) spectra of the selected compound **20** were analyzed (Supplementary material). In the HMBC spectrum of compound **20** (Fig. 4), two-bond couplings of H-5 with C-4a/C-4a· and of C-4a· with the NH proton were observed. Three-bond couplings were detected between C-4a/C-4a· and H-7, as well as between C-4a and H-5. The signal of C-7a correlates with H-4, while the signal of C-7a· also shows coupling with proton H-4 and a weak correlation of H-5 and H-7 with C-7/C-7·.

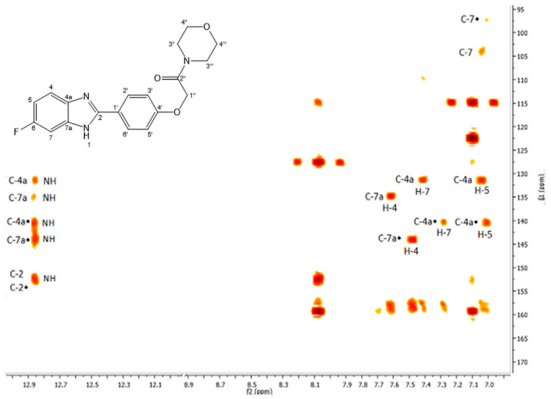


Fig. 4 HMBC spectrum of compound **20**.

2.3. Biological evaluation

2.3.1. Antiproliferative activity of 2-arylbenzimidazole derivatives 12–33. The *in vitro* antiproliferative activity of 2-arylbenzimidazole derivatives **12–33** (Table 1) was evaluated against eight human tumor cell lines: pancreatic adenocarcinoma (CAPAN-1), colon carcinoma (HCT-116), glioblastoma (LN-229), non-small cell lung cancer (NCI-H460), acute lymphoblastic leukemia (DND-41), acute myeloid leukemia (HL-60), chronic myeloid leukemia (K-562) and non-Hodgkin's lymphoma (Z-138). Among the tested compounds, compound **23**, which is substituted with chlorine at the C-6 position of the benzimidazole ring, fluorine at the *meta*-position of the benzene ring, and an *N*,*N*-diethyl group at the *para*-position of the benzene ring, exhibited the most pronounced antiproliferative activity within the concentration range of 2 to 9.4 μM. Specifically, compound **23** demonstrated the highest antiproliferative activity against chronic myeloid leukemia cells (K-562, IC_{50} = 2.0 μM) and non-Hodgkin's lymphoma cells (Z-138, IC_{50} = 2.0 μM). Furthermore, 2-arylbenzimidazole derivatives with an unsubstituted phenyl nucleus at the *meta*-position (compounds **12–20**) generally showed moderate antiproliferative activity across all tested tumor cell lines. Among the derivatives containing an *N*,*N*-diethyl substituent (compounds **12**, **15**, **18** and **23**), compound **15** exhibited notable inhibitory activity against K-562 (IC_{50} = 8.7 μM) and Z-138 (IC_{50} = 9.4 μM) cells. Compounds with a morpholine substituent at the *para*-position of the benzene ring (compounds **13**, **16**, and **19**) displayed moderate to weaker antiproliferative activity (IC_{50} = 37.3–79.8 μM) and did not show inhibitory effects against LN-229, HCT-116, and NCI-H460 cell lines. Derivatives with an unsubstituted benzimidazole nucleus at the C-6 position and a fluorine atom at the *meta*-position of the benzene ring (compounds **21** and **22**) did not inhibit the growth of the tested cancer cell lines, except for derivative **21**, which exhibited a moderate selective effect on the acute lymphoblastic leukemia cell line (DND-41, IC_{50} = 50 μM). Similar antiproliferative activity was observed for benzimidazole derivatives substituted with chlorine at the C-6 position (compounds **24** and **25**), both of which showed weak cytotoxic effects exclusively on the Z-138 cell line (**24**: IC_{50} =

Table 1 Antiproliferative activity of 2-arylbenzimidazole derivatives **12–33**

Compd	IC_{50}/μM							
	LN-229	Capan-1	HCT-116	NCI-H460	DND-41	HL-60	K-562	Z-138
12	≥65.1	47.7 ± 3.2	>100	52.4 ± 1.3	47.0 ± 4.6	46.4 ± 1.3	38.2 ± 4.5	46.2 ± 1.8
13	>100	42.1 ± 3.7	>100	>100	42.9 ± 3.2	54.2 ± 4.1	79.8 ± 1.3	48.3 ± 0.1
14	>100	>100	>100	>100	≥50.2	>100	>100	>100
15	44.0 ± 0.8	25.1 ± 3.7	47.2 ± 1.8	41.3 ± 3.7	42.9 ± 5.9	29.2 ± 2.2	8.7 ± 1.1	9.4 ± 1.2
16	>100	45.0 ± 5.1	>100	>100	39.3 ± 3.3	51.6 ± 1.5	37.3 ± 3.9	47.0 ± 4.8
17	>100	28.5 ± 4.4	>100	>100	34.0 ± 4.9	>100	>100	>100
18	44.1 ± 5.3	39.1 ± 2.1	61.1 ± 0.7	37.2 ± 4.3	43.4 ± 3.7	51.9 ± 5.9	27.6 ± 3.3	31.1 ± 3.7
19	>100	≥69.1	>100	>100	52.1 ± 0.4	>100	>100	≥65.7
20	>100	>100	>100	>100	30.3 ± 4.6	>100	>100	>100
21	>100	>100	>100	>100	50.0 ± 5.5	>100	>100	>100
22	>100	>100	>100	>100	>100	>100	>100	>100
23	**5.0 ± 1.3**	**2.2 ± 0.5**	**9.4 ± 1.2**	**2.3 ± 0.8**	**2.6 ± 1.2**	**4.4 ± 0.5**	**2.0 ± 0.7**	**2.0 ± 0.0**
24	>100	>100	>100	>100	>100	>100	>100	≥80.5
25	>100	>100	>100	>100	>100	>100	>100	≥64.8
26	51.5 ± 0.1	35.4 ± 2.7	49.5 ± 0.4	35.9 ± 1.3	30.3 ± 1.3	33.8 ± 3.3	19.1 ± 1.8	11.5 ± 2.1
27	>100	>100	>100	>100	41.2 ± 1.3	>100	>100	>100
28	>100	47.2 ± 0.4	>100	>100	49.1 ± 2.3	≥70.0	≥88.0	45.8 ± 0.6
29	>100	>100	>100	>100	>100	≥98.1	>100	55.7 ± 6.8
30	>100	58.8 ± 1.9	>100	46.9 ± 4.4	49.6 ± 3.2	≥60.6	>100	54.4 ± 5.4
31	>100	40.6 ± 2.5	>100	>100	46.9 ± 3.9	66.1 ± 4.0	73.0 ± 3.0	45.7 ± 0.0
32	>100	54.9 ± 5.1	>100	>100	>100	>100	>100	>100
33	>100	47.0 ± 4.3	>100	>100	41.6 ± 4.0	65.1 ± 2.8	29.6 ± 1.9	65.4 ± 3.4
Etoposide	0.03 ± 0.0	3.4 ± 0.9	3.7 ± 0.3	6.1 ± 1.1	1.0 ± 0.7	0.8 ± 0.2	4.0 ± 1.3	0.7 ± 0.1

80.5 μM, 25: IC_{50} = 64.8 μM). Conversely, the derivative disubstituted with fluorine at the C-6 position and the *meta*-position of the 2-arylbenzimidazole ring (compound **27**) strongly inhibited the growth of acute lymphoblastic leukemia cells (DND-41, IC_{50} = 4.2 μM). Among the evaluated 1,2,3-triazole derivatives of 2-aryl benzimidazole (compounds **28–33**), derivative **33** showed moderate activity against K-562 chronic myeloid leukemia cells (IC_{50} = 29.6 μM), while the other derivatives exhibited moderate to weaker cytotoxic effects (IC_{50} = 40.6–98.1 μM). In order to elucidate the mechanism of action of the benzimidazole derivative **23**, which showed the most pronounced activity against all tested tumor cell lines, an acridine orange displacement assay using fluorescence polarization was performed (Fig. 5). The enhancement in fluorescence polarization of acridine orange when it binds to DNA molecules serves as the foundation for a competitive assay designed to investigate the possible interaction between DNA and various small molecules. Compounds that interact with DNA can inhibit the binding of acridine orange to DNA, thereby preventing the associated increase in fluorescence polarization. Benzimidazole derivatives are known for their ability to intercalate into DNA. The planar structure of the benzimidazole ring system allows them to insert between DNA base pairs, stabilizing the DNA–intercalator complex. The series of 2-arylbenzimidazole derivatives **12–33** has structural features that suggest they could interact with DNA, and this interaction was confirmed through the competitive binding assay with acridine orange (Fig. 5B).

Although compounds **23** and **26**, which also emerged as the most potent inhibitors in the proliferation assays on cancer cell lines, scored best in the binding assay, these biological activities are not correlated for all derivatives. For example, compounds **22** and **27** also exhibit clear intercalating properties, but this does not translate into antiproliferative activity. Therefore, there may be other underlying mechanisms leading to cytotoxicity in the cancer cell lines. Further research is needed to investigate this.

Finally, derivative **23**, which exhibited the highest antiproliferative activity against cancer cells, was also tested on normal cells to determine selectivity. For this purpose, PBMC purified from two healthy donors were used. Only at the highest tested concentration (100 μM), there was an impact on the viability of these normal cells (Fig. 6A), and likewise an increase in the number of dead and apoptotic cells was observed at 100 μM (Fig. 6B). From these results, we

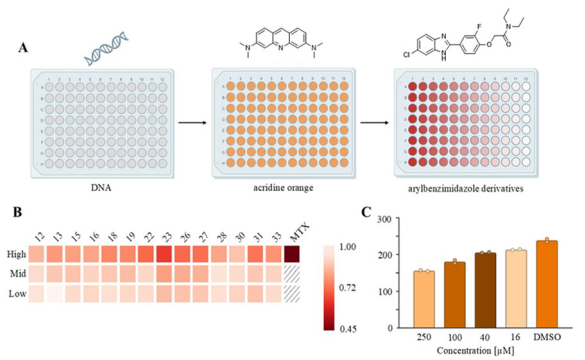


Fig. 5 Fluorescent intercalator displacement (FID) assay. (A) Principle of the FP-assay for the detection of compounds with DNA intercalating properties. (B) Normalized milli P (mP) results relative to the DMSO control for a selection of the 2-arylbenzimidazole derivatives **12–33** tested at three different doses (high = 250 μM, mid = 100 μM, low = 40 μM). Positive control mitoxantrone (MTX) is included at 10 μM. (C) Measured milli P (mP) results for compound **23** at the indicated doses.

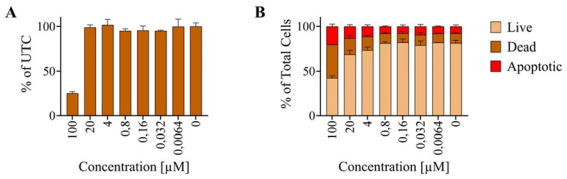


Fig. 6 Analysis of cytotoxicity and apoptosis induced by compound **23** on PBMC. (A) Cell viability was determined by MTS assay and is expressed as the mean ± SD of two independent experiments. (B) Quantification of the percentages of live, dead and apoptotic cells after treatment with the indicated doses of **23**, expressed as mean ± SD.

can conclude that the observed cytotoxicity of compound **23** is selective for cancer cells, making this compound a promising candidate for further optimization as an antitumor agent.

Analysis of the structure and antiproliferative activity of *O*-alkylated derivatives of 2-arylbenzimidazoles **12–27** (Fig. 7) reveals that chlorination at the C-6 position of the benzimidazole ring enhances antiproliferative activity compared to the unsubstituted analogs. Additionally, derivatives with an *N*,*N*-diethyl substituent at the *para*-position of the benzene ring exhibit the highest antiproliferative activity.

2.3.2. *In vitro* antibacterial activity of 2-arylbenzimidazole derivatives 12–33. The synthesized 2-arylbenzimidazole derivatives with various substituents at the *para*-position of the benzene ring **12–27** and 1,2,3-triazole benzimidazole derivatives **28–33** were evaluated *in vitro* for antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*), as well as Gram-negative bacteria *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*) (Table 2). Standard antibiotics ceftazidime (CAZ) and ciprofloxacin (CIP) were used as reference controls.

Among the tested *O*-alkylated derivatives of 2-arylbenzimidazole **12–27**, those substituted with a chlorine atom at the C-6 position of the benzimidazole ring and an unsubstituted benzene ring **15–17** exhibited the most pronounced selective antibacterial activity against the Gram-positive bacterium *Enterococcus faecalis*, with MIC values ranging from 0.25 to 1 μg mL^{-1}, and exhibited better activity compared to the standard antibiotics CAZ and CIP. The electron-withdrawing chlorine atom at the C-6 position appears to enhance interactions with bacterial targets by increasing lipophilicity and improving membrane permeability. Lipophilicity is a well-established factor that facilitates membrane penetration, playing a pivotal role in

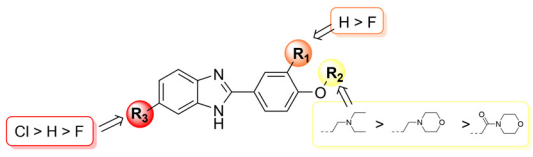


Fig. 7 Structure and antiproliferative activity relationship of *O*-alkylated 2-arylbenzimidazole derivatives **12–27**.

Table 2 *In vitro* antibacterial activity of benzimidazole derivatives **12–33**

	MIC/μg mL^{-1}				
Compd	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*	*E. faecalis*
12–14	>256	>256	>256	>256	>256
15	256	>256	>256	>256	**1**
16	256	>256	>256	64	**0.25**
17	>256	>256	>256	>256	**0.5**
18–22	>256	>256	>256	>256	256
23	256	>256	>256	256	64
24–27	>256	>256	>256	>256	>256
28	256	>256	>256	>256	**0.25**
29	>256	>256	>256	>256	>256
30	>256	>256	>256	>256	**0.25**
31–33	>256	>256	>256	>256	>256
CAZ	0.5	2	256	64	256
CIP	<0.125	0.5	>256	0.5	1

determining antimicrobial activity within biological systems. Additionally, the unsubstituted benzene ring may contribute to molecular simplicity and steric compatibility, optimizing interactions with bacterial enzymes or cell wall components. The observed selective efficacy against *E. faecalis* can be attributed to distinct structural features of Gram-positive bacterial cell envelopes. Unlike Gram-negative bacteria, Gram-positive bacteria lack an outer membrane, leaving their lipid-rich peptidoglycan layer directly exposed to lipophilic agents. This structural configuration minimizes permeability barriers, rendering Gram-positive bacteria more vulnerable to hydrophobic compounds.[61] Derivative **16** exhibited moderate activity against *S. aureus* (MIC = 64 μg mL^{-1}), while derivative **23** demonstrated moderate activity against *E. faecalis* (64 μg mL^{-1}). None of the *O*-alkylated benzimidazole derivatives inhibited growth of Gram-negative bacteria, indicating selective action. Among the tested 1,2,3-triazole derivatives of 2-arylbenzimidazole **28–33**, only derivatives **28** and **30** showed strong selective activity against the Gram-positive bacterium *Enterococcus faecalis* (MIC = 0.25 μg mL^{-1}). The other tested benzimidazole derivatives did not inhibited the growth of either Gram-positive or Gram-negative bacteria.

The structure–activity relationship of *O*-alkylated 2-arylbenzimidazole derivatives **12–27** indicates that antibacterial activity is enhanced by chlorine substitution at the C-6 position of the benzimidazole ring and an unsubstituted *meta*-position on the benzene ring. In contrast, for 1,2,3-triazole derivatives **28–33**, only compounds with an unsubstituted benzimidazole ring exhibit selective activity against the Gram-positive bacterium *Enterococcus faecalis*. Chlorine substitution at the *meta*-position of the benzene ring in these derivatives results in a lack of antibacterial activity.

3. Experimental

3.1. Material and methods

All the solvents and chemicals were purchased from Aldrich and Acros. Melting points of compounds were determined with Kofler hot-stage microscopy (Reichert, Wien, Austria) and uncorrected. The course of the reaction was monitored

by thin layer chromatography (TLC), which was carried out on 60F-254 plates coated with a layer of Merck silica gel in the appropriate solvent system. Column chromatography was performed on Fluka silica gel (0.063–0.2 nm), and the glass columns were filled under the influence of gravity, and the dichloromethane : methanol solvent mixture in the appropriate ratio was used as the eluent. UV light with a wavelength of 254 and 365 nm was used for the detection of isolated components. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300, 400 and 600 MHz spectrometer. All samples were dissolved in DMSO-d_6 and measured at 298 K in NMR tube with a diameter of 5 mm. Chemical shifts (δ/ppm) in ^{1}H-NMR spectra are expressed in units of ppm relative to tetramethylsilane (TMS, δ = 0.0 ppm), and coupling constants (J) in hertz (Hz). Individual resonances were assigned based on their chemical shifts, signal intensities, signal multiplicity and H–H coupling constants. Ultrasound-assisted reactions were performed in a Bandelin-Sonorex digiplus DL 1028 H with a nominal power of 1200 W and a frequency of 35 kHz. Infrared (IR) spectra were carried out on a Bruker Vertex 70 spectrometer in attenuated total reflection (ATR) mode. The average spectrum of 32 measurements in the range from 400 to 4000 cm^{-1} with spectral resolution of 2 cm^{-1} was measured after the samples were placed on the diamond. Mass spectra were recorded on an Agilent Technologies 1290 Infinity II in positive mode.

3.2. Synthesis of compounds

3.2.1. General procedure for the synthesis of *O*-alkylated benzaldehydes 1–8.[62] To a solution of appropriate benzaldehyde in acetonitrile was added potassium carbonate (1.2 eq.). The reaction mixture was stirred for 1 hour at room temperature, then corresponding halide (1 eq.) was added. The reaction mixture was stirred at reflux for 6 hours and additionally at room temperature overnight. After completion of the reaction monitored by TLC, the reaction mixture was filtered, and the solvent was evaporated under reduced pressure. As needed, the compound was additionally purified by column chromatography.

*4-(2-(Diethylamino)ethoxy)benzaldehyde **1**.* Compound **1** was synthesized according to the general procedure 3.2.1. from 4-hydroxybenzaldehyde (3.00 g, 0.025 mol) and 2-chloro-*N*,*N*-diethylethanamine (4.23 g, 0.025 mol). After filtration and purification by column chromatography (CH_2Cl_2 : MeOH = 100 : 1) compound **1** was isolated as a brown oil (2.7 g, 49.1%). ^{1}H-NMR (600 MHz, DMSO-d_6) (δ/ppm) 9.84 (s, 1H, H-1), 7.83 (d, J = 8.7 Hz, 2H, H-3,7), 7.10 (d, J = 8.7 Hz, 2H, H-4,6), 4.11 (t, J = 6.0 Hz, 2H, H-1′), 2.79 (t, J = 5.8 Hz, 2H, H-2′), 2.54 (q, J = 7.0 Hz, 4H, H-3′,3″), 0.95 (t, J = 7.1 Hz, 6H, H-4′,4″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 191.74 (C-1), 164.04 (C-5), 132.27 (C-3,7), 130.03 (C-2), 115.43 (C-4,6), 67.39 (C-1′), 51.62 (C-2′), 47.43 (C-3′), 12.34 (C-4′).

*4-(2-Morpholinoethoxy)benzaldehyde **2**.* Compound **2** was synthesized according to the general procedure 3.2.1. from 4-hydroxybenzaldehyde (3.00 g, 0.025 mol) and 4-(2-chloroethyl)morpholine (4.65 g, 0.025 mol). After filtration compound **2** was isolated as a yellow oil (2.10 g, 35.4%). ^{1}H-NMR (600 MHz, DMSO-d_6) (δ/ppm) 9.87 (s, 1H, H-1), 7.86 (d, J = 6.9 Hz, 2H, H-3,7), 7.14 (d, J = 8.7 Hz, 2H, H-4,6), 4.21 (t, J = 5.7 Hz, 2H, H-1′), 3.58 (t, J = 4.5 Hz, 4H, H-4′,4″), 2.72 (t, J = 5.7 Hz, 2H H-2′), 2.50 (t, J = 4.5 Hz, 4H, H-3′,3′). ^{13}C-NMR (75 MHz, DMSO-d_6) (δ/ppm) 191.77 (C-1), 163.92 (C-5), 132.26 (C-3,7), 130.08 (C-2), 115.46 (C-4,6), 66.61 (C-4′,4″), 66.27 (C-1′), 57.25 (C-2′), 54.03 (C-3′,3″).

*4-(2-Morpholino-2-oxoethoxy)benzaldehyde **3**.* Compound **3** was synthesized according to the general procedure 3.2.1. from 4-hydroxybenzaldehyde (1.00 g, 0.008 mol) and 4-(chloroacetyl)morpholine (1.30 g, 0.008 mol). After filtration compound **3** was isolated as a white powder (1.1 g, 52.0%, m.p. = 189–190 °C). ^{1}H-NMR (300 MHz, DMSO-d_6) (δ/ppm) 9.87 (s, 1H, H-1), 7.85 (d, J = 8.7 Hz, 2H, H-3,7), 7.10 (d, J = 8.7 Hz, 2H, H-4,6), 5.01 (s, 2H, H-1″), 3.54 (m, 8H, H-3′,3″,4′,4″). ^{13}C-NMR (75 MHz, DMSO-d_6) (δ/ppm) 191.76 (C-1), 165.86 (C-2′), 163.57 (C-5), 132.06 (C-3,7), 130.30 (C-2), 115.61 (C-4,6), 66.49 (C-4), 66.42 (C-4″), 66.14 (C-1′), 45.03 (C-3′), 42.05 (C-3″).

*4-(2-(Diethylamino)ethoxy)-3-fluorobenzaldehyde **4**.* Compound **4** was synthesized according to the general procedure 3.2.1. from 3-fluoro-4-hydroxybenzaldehyde (1.00 g, 0.007 mol) and 2-chloro-*N*,*N*-diethylethan-1-amine (1.23 g, 0.007 mol). After filtration compound **4** was isolated as a brown oil (0.85 g, 49.6%). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 9.85 (d, J = 2.1 Hz, 1H, H-1), 7.75 (ddd, J = 8.4, 1.9, 0.9 Hz, 1H, H-7), 7.67 (dd, J = 11.4, 1.9 Hz, 1H, H-3), 7.40 (t, J = 8.3 Hz, 1H, H-6), 4.20 (t, J = 5.9 Hz, 2H, H-1′), 2.81 (t, J = 5.9 Hz, 2H, H-2′), 2.54 (q, J = 7.1 Hz, 4H, H-3′,3″), 0.96 (t, J = 7.1 Hz, 6H, H-4′,4″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 191.24 (C-1), 152.32 (d, $J_{C-F}{}^2$ = 10.7 Hz, C-5), 152.13 (d, $J_{C-F}{}^1$ = 247.0 Hz, C-4), 130.02 (d, $J_{C-F}{}^3$ = 5.0 Hz, C-2), 128.82 (d, $J_{C-F}{}^3$ = 2.2 Hz, C-6), 115.61 (d, $J_{C-F}{}^2$ = 18.2 Hz, C-3), 115.04 (C-7), 68.45 (C-1′), 51.45 (C-2), 47.48 (C-3′,3″), 12.34 (C-4′,4″).

*3-Fluoro-4-(2-morpholinoethoxy)benzaldehyde **5**.* Compound **5** was synthesized according to the general procedure 3.2.1. from 3-fluoro-4-hydroxybenzaldehyde (1.00 g, 0.007 mol) and 4-(2-chloroethyl)morpholine (1.3 g, 0.007 mol). After filtration compound **5** was isolated as a pale orange powder (1.5 g, 83.4%, m.p. = 238–240 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 9.87 (d, J = 2.1 Hz, 1H, H-1), 7.78 (ddd, J = 8.4, 1.9, 0.9 Hz, 1H, H-7), 7.69 (dd, J = 11.4, 1.9 Hz, 1H, H-3), 7.43 (t, J = 8.3 Hz, 1H, H-6), 4.30 (t, J = 5.7 Hz, 2H, H-1′), 3.57 (t, J = 4.5 Hz, 4H, H-4′,4″), 2.75 (t, J = 5.7 Hz, 2H, H-2′), H-3′,3″ overlapped by the DMSO signal. ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 191.31 (C-1), 152.19 (d, $J_{C-F}{}^2$ = 10.5 Hz, C-5), 152.12 (d, $J_{C-F}{}^1$ = 247.1 Hz, C-4), 130.13 (d, $J_{C-F}{}^3$ = 4.8 Hz, C-2), 128.83 (C-6), 115.68 (d, $J_{C-F}{}^2$ = 18.3 Hz, C-3), 115.18 (C-7), 67.46 (C-1′), 66.64 (C-4′), 57.10 (C-2′), 54.02 (C-3′).

*3-Fluoro-4-(2-morpholino-2-oxoethoxy)benzaldehyde **6**.* Compound **6** was synthesized according to the general procedure 3.2.1. from 3-fluoro-4-hydroxybenzaldehyde (0.50 g, 0.003 mol) and 4-(chloroacetyl)morpholine (0.60 g, 0.003

mol). After filtration compound **6** was isolated as a white powder (1.90 g, 99.6%, m.p. = 94–95 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 9.86 (d, J = 2.0 Hz, 1H, H-1), 7.72 (m, 2H, H-3,7), 7.28 (t, J = 8.3 Hz, 1H, H-6), 5.13 (s, 2H, H-1′), 3.61 (m, 4H, H-3′,3″), 3.44 (t, J = 4.8 Hz, 4H, H-4′,4″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 191.27 (C-1), 165.45 (C-2′), 152.00 (d, J = 247.0 Hz, C-4), 151.87 (d, J = 10.4 Hz, C-5), 130.33 (d, J = 4.9 Hz, C-2), 128.30 (C-6), 115.89 (d, J = 18.2 Hz, C-3), 115.49 (C-7), 66.55 (C-1′), 66.49 (C-4′), 66.37 (C-4″), 44.96 (C-3′), 42.08 (C-3″).

*4-(Prop-2-yn-1-yloxy)benzaldehyde **7**.* Compound **7** was synthesized according to the general procedure 3.2.1. from 4-hydroxybenzaldehyde (3.00 g, 0.025 mol) and propargyl bromide (1.9 mL, 0.025 mol). After filtration compound **7** was isolated as a white powder (3.80 g, 60.8%, m.p. = 92–94 °C). ^{1}H-NMR (600 MHz, DMSO-d_6) (δ/ppm) 9.87 (s, 1H), 7.88 (d, J = 8.8 Hz, 2H, H-3,7), 7.17 (d, J = 8.7 Hz, 2H, H-4,6), 4.93 (d, J = 2.4 Hz, 2H, H-1′), 3.62 (t, J = 2.4 Hz, 1H, H-3). ^{13}C-NMR (151 MHz, DMSO-d_6) (δ/ppm) 191.31 (C-1), 162.01 (C-5), 131.64 (C-3,7), 130.14 (C-2), 115.28 (C-4,6), 78.79 (C-2′), 78.51 (C-3′), 55.82 (C-1′).

*3-Fluoro-4-(prop-2-yn-1-yloxy)benzaldehyde **8**.* Compound **8** was synthesized according to the general procedure 3.2.1. from 3-fluoro-4-hydroxybenzaldehyde (0.50 g, 0.004 mol) propargyl bromide (0.4 mL, 0.004 mol). After filtration and purification by column chromatography (CH_2Cl_2 : MeOH = 50 : 1) compound **8** was isolated as a pale brown powder (2.4 g, 72.3%, m.p. = 91–93 °C). ^{1}H-NMR (600 MHz, DMSO-d_6) (δ/ppm) 9.88 (s, 1H, H-1), 7.81 (d, J = 8.3 Hz, 1H), 7.74 (d, J = 11.1 Hz, 1H, H-3), 7.45 (t, J = 8.2 Hz, 1H), 5.05 (d, J = 1.7 Hz, 2H, H-1′), 3.73 (s, 1H, H-3′). ^{13}C-NMR (151 MHz, DMSO-d_6) (δ/ppm) 190.81 (C-1), 151.72 (d, J = 247.4 Hz, C-4), 150.13 (d, J = 10.7 Hz, C-5), 130.32 (d, J = 5.0 Hz, C-2), 127.91 (d, J = 2.9 Hz, C-6), 115.49 (d, J = 18.3 Hz, C-3), 115.20 (C-7), 79.43 (C-2′), 78.00 (C-3), 56.74 (C-1′).

*4-(2-Azidoethyl)morpholine **9**.*[63] *N*-(2-Chloroethyl)morpholine hydrochloride (3.00 g, 0.020 mol) was dissolved in water (16.2 mL) followed by the addition of NaN_3 (3.15 g, 0.020 mol). The reaction mixture was heated at 80 °C for 16 h. Upon completion of the reaction, the reaction mixture was cooled to room temperature and basified to pH = 10 with NaOH (20%). The crude mixture was extracted with ether, and the organic layer was dried over $MgSO_4$. The filtrate was evaporated at room temperature and transparent oily compound **9** was obtained (2.10 g, 66.6%).

3.2.2. General procedure for the synthesis of compounds 10 and 11. To a solution of compounds **7** or **8** (700 mg) in dry methanol (5 mL), 4-(2-azidoethyl)morpholine **9** (1 eq.) and copper(II) acetate (0.05 eq.) were added. The reaction mixture was stirred overnight at room temperature. After completion of the reaction monitored by TLC, the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography (CH_2Cl_2 : MeOH = 100 : 1).

*4-((1-(2-Morpholinoethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde **10**.* Compound **10** was synthesized according to general procedure 3.2.2. from **7** (0.70 g, 0.004 mol), **9** (0.62 g, 0.004 mol) and $Cu(OAc)_2$ (0.04 g, 0.22 mmol). A brown oily compound **10** was isolated (1.03 g, 74.7%). ^{1}H-NMR (300 MHz, DMSO-d_6) (δ/ppm) 9.88 (s, 1H, H-1), 8.26 (s, 1H, H-10), 7.88 (d, J = 8.8 Hz, 2H, H-3,7), 7.24 (d, J = 8.7 Hz, 2H, H-4,6), 5.29 (s, 2H, H-8), 4.50 (t, J = 6.3 Hz, 2H, H-1′), 3.51 (m, J = 4.5 Hz, 4H, H-4′,4″), 2.73 (t, J = 6.3 Hz, 2H, H-2′), 2.40 (t, J = 4.5 Hz 4H, H-3′,3″). ^{13}C-NMR (75 MHz, DMSO-d_6) (δ/ppm) 191.79 (C-1), 163.40 (C-5), 142.20 (C-9), 132.23 (C-3,7), 130.31 (C-2), 125.64 (C-10), 115.70 (C-4,6), 66.55 (C-4′,4″), 61.96 (C-8), 57.79 (C-2′), 53.39 (C-3′,3″), 46.98 (C-1′).

*3-Fluoro-4-((1-(2-morpholinoethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde **11**.* Compound **11** was synthesized according to general procedure 3.2.2. from **8** (0.70 g, 0.004 mol), **9** (0.62 g, 0.004 mol) and $Cu(OAc)_2$ (0.04 g, 0.22 mmol). A light yellow powdery compound **11** was isolated (1.23 g, 95.0%, m.p. > 250 °C). ^{1}H-NMR (300 MHz, DMSO-d_6) (δ/ppm) 9.87 (d, J = 2.1 Hz, 1H, H-1), 8.29 (s, 1H, H-10), 7.80 (ddd, J = 8.5, 1.9, 0.9 Hz, 1H, H-7), 7.70 (dd, J = 11.3, 1.9 Hz, 1H, H-3), 7.61 (t, J = 8.3 Hz, 1H, H-6), 5.38 (s, 2H, H-8), 4.50 (t, J = 6.3 Hz, 2H, H-1′), 3.51 (t, J = 4.5 Hz, 4H, H-4′,4″), 2.73 (t, J = 6.3 Hz, 2H, H-2′), 2.40 (t, J = 4.5 Hz, 4H, H-3′,3″). ^{13}C-NMR (75 MHz, DMSO-d_6) (δ/ppm) 191.33 (C-1), 152.16 (d, J = 247.0 Hz, C-4), 151.58 (d, J = 10.9 Hz, C-5), 141.72 (C-9), 130.38 (d, J = 5.1 Hz, C-2), 128.67 (d, J = 3.0 Hz, C-6), 125.95 (C-10), 115.79 (d, J = 18.4 Hz, C-3), 115.56 (C-7), 66.55 (C-4,4″), 62.75 (C-8), 57.76 (C-2′), 53.38 (C-3′,3″), 47.00 (C-1′).

3.2.3. General procedure for the synthesis of compounds 12–33. To a solution of corresponding benzaldehyde **1–6**, **10** and **11** in DMF (7 mL) was added $Na_2S_2O_5$ (1.2 eq.). The reaction mixture was stirred at room temperature for 1.5 hours, then appropriate 1,2-diaminobenzene (1 eq.) was added. The reaction mixture was exposed to an ultrasound power of 1200 W and frequency of 35 kHz for 2–7.5 h in an ultrasonic bath at 50 °C. The course of the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure, and the crude mixture was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH).

*2-(4-(1H-Benzo[d]imidazol-2-yl)phenoxy)-N,N-diethylethan-1-amine **12**.* Compound **12** was synthesized according to the general procedure 3.2.3. from 4-(2-(diethylamino)ethoxy) benzaldehyde **1** (205 mg, 0.92 mmol), $Na_2S_2O_5$ (209 mg, 1.10 mmol) and 1,2-diaminobenzene (100 mg, 0.92 mmol). After 5 hours the reaction was completed, crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1) and compound **12** was isolated as a light yellow powder (157 mg, 57.9% m.p. = 201–203 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.77 (1H, s, NH), 8.12 (d, J = 8.8 Hz, 2H, H-2′,6′), 7.55 (m, 2H, H-4,7), 7.16 (m, 2H, H-5,6), 7.11 (d, J = 8.8 Hz, 2H, H-3′,5′), 4.15 (t, J = 5.5 Hz, 2H H-2″), 2.92 (s, 2H, H-1″), 2.67 (s, 4H, H-3″,3‴), 1.03 (t, J = 7.1 Hz, 6H, H-4″,4‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 160.28 (C-4′), 151.81 (C-2), 144.36 (C-4a), 135.45 (C-7a), 128.49 (C-2′,6′), 123.18 (C-1′), 122.52 (C-6), 121.90 (C-5), 118.95 (C-4), 115.34 (C-3′,5′), 111.50 (C-4), 66.69 (C-1″), 51.66

(C-2″), 47.49 (C-3″,3‴), 12.09 (C-4″,4‴). IR (ν, cm^{-1}) 2969, 2925, 2800, 1612, 1499, 1436, 1256, 1178, 742. EI^+ mode: m/z = 310.0, $[M^+]$ (calcd for $C_{19}H_{23}N_3O$ = 309.4).

4-(2-(4-(1H-Benzo[d]imidazol-2-yl)phenoxy)ethyl)morpholine ***13***. Compound **13** was synthesized according to the general procedure 3.2.3. from 4-(2-morpholinoethoxy)benzaldehyde **2** (216 mg, 0.92 mmol), $Na_2S_2O_5$ (209 mg, 1.10 mmol) and 1,2-diaminobenzene (100 mg, 0.92 mmol). After 3 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **13** was isolated as a yellow powder (241 mg, 80.7%, m.p. = 194–196 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.72 (s, 1H, NH), 8.10 (d, J = 8.9 Hz, 2H, H-2′,6′), 7.55 (m, 2H, H-4,7), 7.17 (dd, J = 6.0, 3.0 Hz, 2H, H-5,6), 7.11 (d, J = 8.9 Hz, 2H, H-3′,5′), 4.17 (t, J = 5.7 Hz, 2H, H-1″), 3.58 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.72 (m, 2H, H-2″), H-3″,3‴ overlapped by the DMSO signal. ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 160.29 (C-4′), 151.81 (C-2), 128.47 (C-2′,6′), 123.19 (C-1′), 122.49 (C-6), 121.95 (C-5), 118.98 (C-4), 115.38 (C-3′,5′) 111.16 (C-7), 66.66 (C-4″,4‴), 65.99 (C-1″), 57.44 (C-2″), 54.10 (C-3″,3‴). IR (ν, cm^{-1}) 2860, 2794, 1611, 1499, 1445, 1250, 1108, 835, 744. EI^+ mode: m/z = 323.9, $[M^+]$ (calcd for $C_{19}H_{21}N_3O_2$ = 323.4).

2-(4-(1H-Benzo[d]imidazol-2-yl)phenoxy)-1-morpholinoethan-1-one ***14***. Compound **14** was synthesized according to the general procedure 3.2.3. from 4-(2-morpholino-2-oxoethoxy) benzaldehyde **3** (276 mg, 0.92 mmol), $Na_2S_2O_5$ (209 mg, 1.10 mmol) and 1,2-diaminobenzene (100 mg, 0.92 mmol). After 4 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **14** was isolated as a white powder (234 mg, 75.2%, m.p. = 156–159 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.73 (s, 1H, NH), 8.09 (d, J = 8.9 Hz, 2H, H-2′,6′), 7.56 (m, 2H, H-4,7), 7.17 (m, 2H, H-5,6), 7.10 (d, J = 8.9 Hz, 2H, H-3′,5′), 4.94 (s, 2H, H-1″), 3.60 (m, 4H, H-3″,3‴), 3.48 (s, 4H, H-4″,4‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 166.29 (C-2″), 159.89 (C-4′), 151.76 (C-2), 144.36 (C-4a), 135.45 (C-7a), 128.33 (C-2′,6′), 123.51 (C-1′), 122.58 (C-6), 121.94 (C-5), 119.00 (C-4), 115.54 (C-3′,5′), 111.52 (C-7), 66.51 (C-4″,4‴), 66.23 (C-1″), 45.18 (C-3″), 42.10 (C-3‴). IR (ν, cm^{-1}) 2917, 2851, 1659, 1637, 1500, 1437, 1274, 1226, 1030, 745. EI^+ mode: m/z = 337.9, $[M^+]$ (calcd for $C_{19}H_{19}N_3O_3$ = 337.3).

2-(4-(6-Chloro-1H-benzo[d]imidazol-2-yl)phenoxy)-N,N-diethylethan-1-amine ***15***. Compound **15** was synthesized according to the general procedure 3.2.3. from 4-(2-(diethylamino)ethoxy)benzaldehyde **1** (154 mg, 0.70 mmol), $Na_2S_2O_5$ (160 mg, 0.84 mmol) and 4-chlorobenzene-1,2-diamine (100 mg, 0.70 mmol). After 6 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **15** was isolated as a yellow solid (157 mg, 65.2%, m.p. = 174–176 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 13.02 (s, 1H, NH), 8.12 (d, J = 8.9 Hz, 2H, H-2′,6′), 7.60 (m, 2H, H-4,7), 7.21 (m, 1H, H-5), 7.13 (d, J = 8.9 Hz, 2H, H-3′,5′), 4.17 (t, J = 5.8 Hz, 2H, H-1″), 2.94 (s, 2H, H-2″), 2.69 (d, J = 6.7 Hz, 4H, H-3″,3‴), 1.04 (t, J = 7.1 Hz, 6H, H-4″,4‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 160.45 (C-4′), 128.69 (C-2′,6′), 122.74 (C-1′), 120.15 (C-5), 118.32 (C-4), 115.43 (C-3′,5′), 112.75 (C-7), 66.29 (C-1″), 51.49 (C-2″), 47.50 (C-3″,3‴), 11.74 (C-4″,4‴). IR (ν, cm^{-1}) 3144, 2966, 2824, 1611, 1488, 1421, 1252, 1178, 804. EI^+ mode: m/z = 343.9, $[M^+]$ (calcd for $C_{19}H_{22}ClN_3O$ = 343.8).

4-(2-(4-(6-Chloro-1H-benzo[d]imidazol-2-yl)phenoxy)ethyl)morpholine ***16***. Compound **16** was synthesized according to the general procedure 3.2.3. from 4-(2-morpholinoethoxy)benzaldehyde **2** (216 mg, 0.92 mmol), $Na_2S_2O_5$ (210 mg, 1.10 mmol) and 4-chlorobenzene-1,2-diamine (131 mg, 0.92 mmol). After 3 hours, the reaction was completed crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **16** was isolated as a brown powder (211 mg, 84.1%, m.p. = 190–191 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.92 (s, 1H, NH), 8.09 (d, J = 8.8 Hz, 2H, H-2′,6′), 7.63 (m, 1H, H-7), 7.49 (d, J = 8.7 Hz, 1H, H-4), 7.18 (m, 1H, H-5), 7.12 (d, J = 8.9 Hz, 2H, H-3′,5′), 4.17 (t, J = 5.7 Hz, 2H, H-1″), 3.58 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.72 (t, J = 5.7 Hz, 2H, H-2″), H-3″,3‴ overlapped by the DMSO signal. ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 160.57 (C-4′), 153.42 (C-2), 145.35 (C-4a), 136.22 (C-7a), 128.66 (C-2′,6′), 126.79 (C-6), 122.65 (C-1′), 122.57 (C-5), 120.16 (C-4), 115.43 (C-3′,5′), 112.76 (C-7), 66.65 (C-4″,4‴), 66.02 (C-1″), 57.41 (C-2″), 54.09 (C-3″,3‴). IR (ν, cm^{-1}) 3195, 2944, 2799, 1609, 1492, 1419, 1250, 11 006, 865, 836. EI^+ mode: m/z = 357.8, $[M^+]$ (calcd for $C_{19}H_{20}ClN_3O_2$ = 357.8).

2-(4-(6-Chloro-1H-benzo[d]imidazol-2-yl)phenoxy)-1-morpholinoethan-1-one ***17***. Compound **17** was synthesized according to the general procedure 3.2.3. from 4-(2-morpholino-2-oxoethoxy)benzaldehyde **3** (162 mg, 0.84 mmol), $Na_2S_2O_5$ (100 mg, 0.70 mmol) and 4-chlorobenzene-1,2-diamine (100 mg, 0.70 mmol). After 3 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **17** was isolated as a white powder (81 mg, 31.1%, m.p. = 228–230 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 8.09 (d, J = 8.9 Hz, 2H, H-2′,6′), 7.58 (d, J = 2.0 Hz, 1H, H-7), 7.54 (d, J = 8.5 Hz, 1H, H-4), 7.16 (dd, J = 8.5, 2.0 Hz, 1H, H-5), 7.10 (m, 2H, H-3′,5′), 4.94 (s, 2H, H-1″), 3.60 (m, 4H, H-4″,4‴), 3.47 (s, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 166.25 (C-2″), 160.05 (C-4′), 153.65 (C-2), 128.49 (C-2′,6′), 126.28 (C-6), 123.40 (C-1′), 122.18 (C-5), 115.54 (C-3′,5′), 66.54 (C-4″), 66.49 (C-4‴), 66.21 (C-1″), 45.16 (C-3″), 42.08 (C-3‴). IR (ν, cm^{-1}) 2970, 2862, 1660, 1644, 1612, 1434, 1226, 1113, 1029, 838, 805. EI^+ mode: m/z = 371.8, $[M^+]$ (calcd for $C_{19}H_{18}ClN_3O_3$ = 371.8).

N,N-Diethyl-2-(4-(6-fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)ethan-1-amine ***18***. Compound **18** was synthesized according to the general procedure 3.2.3. from 4-(2-(diethylamino)ethoxy)benzaldehyde **1** (174 mg, 0.79 mmol), $Na_2S_2O_5$ (180 mg, 0.95 mmol) and and 4-fluorobenzene-1,2-diamine (100 mg, 0.79 mmol). After 4 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **18** was isolated as a white powder (162 mg, 62.2%,

m.p. = 169–170 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.90 (s, 1H, NH), 8.10 (d, J = 8.0 Hz, 2H, H-2′,6′), 7.54 (m, 1H, H-4), 7.34 (m, 1H, H-7), 7.12 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.02 (m, 1H, H-5), 4.17 (s, 2H, H-1″), 2.97 (m, 2H, H-2″), 2.70 (m, 4H, H-3″,3‴), 1.04 (t, J = 6.9 Hz, 6H, H-4″,4‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 159.10 (d, $J_{C-F}{}^1$ = 235.8 Hz, C-6/C-6·), 158.86 (d, $J_{C-F}{}^1$ = 233.3 Hz, C-6/C-6·) 153.57 (C-2/C-2·), 152.77 (C-2/C-2·), 144.78 (d, $J_{C-F}{}^3$ = 11.7 Hz, C-7a/C-7a·), 141.01 (C-4a/C-4a·), 135.55 (d, $J_{C-F}{}^3$ = 12.3 Hz, C-7a/C-7a·), 132.13 (C-4a/C-4a·), 128.58 (C-2′6′/-2′·6′·), 128.45 (C-2′6′/-2′·6′·), 123.03 (C-1′/C-1′·), 122.98 (C-1′/C-1′·), 119.67 (d, $J_{C-F}{}^3$ = 11.4 Hz, C-4/C-4·), 115.40 (C-3′,5′), 112.07 (C-4/C-4·), 110.41 (d, $J_{C-F}{}^2$ = 25.7 Hz, C-5/C-5·), 109.87 (d, $J_{C-F}{}^2$ = 24.3 Hz, C-5/C-5·), 104.45 (d, $J_{C-F}{}^2$ = 23.9 Hz, C-7/C-7·), 97.85 (d, $J_{C-F}{}^2$ = 28.0 Hz, C-7/C-7·), 66.15 (C-1″), 51.46 (C-2″), 47.53 (C-3″,3‴), 11.61 (C-4″,4‴). IR (ν, cm^{-1}) 2969, 2926, 2804, 1615, 1446, 1439, 1253, 1179, 1143, 838, 798. EI$^+$ mode: m/z = 327.9, [M$^+$] (calcd for $C_{19}H_{22}FNO_3$ = 327.4).

4-(2-(4-(6-Fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)ethyl)morpholine ***19***. Compound **19** was synthesized according to the general procedure 3.2.3. from 4-(2-morpholinoethoxy)benzaldehyde **2** (186 mg, 0.79 mmol), $Na_2S_2O_5$ (180 mg, 0.95 mmol) and 4-fluorobenzene-1,2-diamine (100 mg, 0.79 mmol). After 5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **19** was isolated as a white powder (61 mg, 22.6%, m.p. = 175–176 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.85 (d, J = 3.8 Hz, 1H, NH), 8.10 (m, 2H, H-2′,6′), 7.54 (ddd, J = 54.6, 8.7, 4.9 Hz, 1H, H-4), 7.34 (ddd, J = 53.2, 9.4, 2.2 Hz, 1H, H-7), 7.11 (d, J = 8.5 Hz, 2H, H-3′,5′), 7.02 (td, J = 10.7, 2.3 Hz, 1H, H-5), 4.17 (t, J = 5.7 Hz, 2H, H-1″), 3.58 (t, J = 4.6 Hz, 4H, H-3″,3‴), 2.71 (t, J = 5.7 Hz, 2H, H-2″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 160.48 (C-4′/C-4′·), 160.36 (C-4′/C-4′·), 159.10 (d, $J_{C-F}{}^1$ = 235.8 Hz, C-6/C-6·), 158.86 (d, $J_{C-F}{}^1$ = 233.3 Hz, C-6/C-6·), 153.60 (C-2/C-2·), 152.79 (C-2/C-2·), 144.79 (d, $J_{C-F}{}^3$ = 12.9 Hz, C-7a/C-7a·), 141.01 (C-4a/C-4a·), 135.53 (d, $J_{C-F}{}^3$ = 14.1 Hz, C-7a/C-7a·), 132.12 (C-4a/C-4a·), 128.55 (C-2′6′/-2′·6′·), 128.42 (C-2′6′/-2′·6′·), 122.92 (C-1′/C-1·), 122.83 (C-1′/C-1·), 119.73 (d, $J_{C-F}{}^3$ = 10.1 Hz, C-4/C-4·), 115.40 (C-3′,5′), 112.04 (d, $J_{C-F}{}^3$ = 10.4 Hz, C-4/C-4·), 110.40 (d, $J_{C-F}{}^2$ = 25.7 Hz, C-5/C-5·), 109.90 (d, $J_{C-F}{}^2$ = 24.7 Hz, C-5/C-5·), 104.48 (d, $J_{C-F}{}^2$ = 23.9 Hz, C-7/C-7·), 97.95 (d, $J_{C-F}{}^2$ = 26.8 Hz, C-7/C-7·), 66.65 (C-4″,4‴), 66.01 (C-1″), 57.42 (C-2″), 54.09 (C-3″,3‴). IR (ν, cm^{-1}) 2959, 2894, 2801, 1627, 1506, 1449, 1274, 1113, 1035, 871. EI$^+$ mode: m/z = 341.9, [M$^+$] (calcd for $C_{19}H_{20}FN_3O_2$ = 341.4).

2-(4-(6-Fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)-1-morpholinoethan-1-one ***20***. Compound **20** was synthesized according to the general procedure 3.2.3. from 4-(2-morpholino-2-oxoethoxy)benzaldehyde **3** (196 mg, 0.79 mmol), $Na_2S_2O_5$ (180 mg, 0.95 mmol) and 4-fluorobenzene-1,2-diamine (100 mg, 0.79 mmol). After 2 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **20** was isolated as a white powder (188 mg, 66.7%, m.p. = 218–220 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.85 (d, J = 4.2 Hz, 1H, NH), 8.07 (m, 2H, H-2′,6′), 7.55 (ddd, J = 54.1, 8.8, 4.9 Hz, 1H, H-4), 7.34 (ddd, J = 53.2, 9.5, 2.4 Hz, 1H, H-7), 7.10 (d, J = 8.9 Hz, 2H, H-3′,5′), 7.03 (m, 1H, H-5), 4.94 (d, J = 1.4 Hz, 2H, H-1″), 3.61 (m, 4H, H-4″,4‴), 3.47 (s, 4H, H-3″,3‴). ^{13}C-NMR (151 MHz, DMSO-d_6) (δ/ppm) 165.74 (C-2″), 159.57 (C-4′/C-4′·), 159.45 (C-4′/C-4′·), 158.61 (d, $J_{C-F}{}^1$ = 236.3 Hz, C-6/C-6·), 158.39 (d, $J_{C-F}{}^1$ = 233.9 Hz, C-6/C-6·), 153.04 (C-2/C-2·), 152.23 (C-2/C-2·), 144.27 (d, $J_{C-F}{}^3$ = 13.2 Hz, C-7a/C-7a·), 140.49 (C-4a/C-4a·), 135.02 (d, $J_{C-F}{}^3$ = 13.3 Hz, C-7a/C-7a·), 131.60 (C-4a/C-4a·), 127.90 (C-2′6′/2′·6′·), 127.76 (C-2′6′/2′·6′·), 122.74 (C-1′·/C-1·), 122.65 (C-1′·/C-1·) 119.27 (d, $J_{C-F}{}^3$ = 9.8 Hz, C-4/C-4·), 115.06 (C-3′,5′), 111.57 (d, $J_{C-F}{}^3$ = 10.5 Hz, C-4/C-4·), 109.94 (d, $J_{C-F}{}^2$ = 25.9 Hz, C-5/C-5·), 109.42 (d, $J_{C-F}{}^2$ = 24.8 Hz, C-5/C-5·), 104.00 (d, $J_{C-F}{}^2$ = 23.7 Hz, C-7/C-7·), 97.47 (d, $J_{C-F}{}^2$ = 26.7 Hz, C-7/C-7·), 66.03 (C-1″), 65.97 (C-4″), 65.70 (C-4‴), 44.66 (C-3″), 41.58 (C-3‴). IR (ν, cm^{-1}) 3599, 2972, 2885, 1660, 1639, 1440, 1427, 1227, 1110, 1030, 837, 802. EI$^+$ mode: m/z = 355.9, [M$^+$] (calcd for $C_{19}H_{18}FN_3O_3$ = 355.3).

4-(2-(4-(1H-Benzo[d]imidazol-2-yl)-2-fluorophenoxy)ethyl)morpholine ***21***. Compound **21** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-(2-morpholinoethoxy)benzaldehyde **5** (116 mg, 0.46 mmol), $Na_2S_2O_5$ (105 mg, 0.55 mmol) and 1,2-diaminobenzene (50 mg, 0.46 mmol). After 4 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **21** was isolated as a white powder (28 mg, 17.5%, m.p. = 192–194 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.81 (s, 1H, NH), 7.96 (m, 2H, H-4,7), 7.63 (d, J = 7.1 Hz, 1H, H-6′), 7.51 (dd, J = 6.8, 1.5 Hz, 1H, H-2′), 7.39 (t, J = 8.9 Hz, 1H, H-5′), 7.19 (pd, J = 7.1, 1.3 Hz, 2H, H-5,6), 4.26 (t, J = 5.7 Hz, 2H, H-1″), 3.58 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.75 (t, J = 5.7 Hz, 2H, H-2″), H-3″,3‴ overlapped by the DMSO signal. ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 152.08 (d, $J_{C-F}{}^1$ = 243.5 Hz, C-3′), 150.68 (C-2), 148.19 (d, $J_{C-F}{}^2$ = 10.9 Hz, C-4′), 144.21 (C-4a), 135.41 (C-7a), 123.69 (d, $J_{C-F}{}^3$ = 6.5 Hz, C-1′), 122.92 (C-5), 122.15 (C-6), 119.16 (C-4), 115.77 (C-6′), 114.37 (d, $J_{C-F}{}^2$ = 20.3 Hz, C-2′), 111.66 (C-6), 67.25 (C-1″), 66.67 (C-3″,3‴), 57.29 (C-2″), 54.07 (4″,4‴). IR (ν, cm^{-1}) 3063, 2959, 2799, 1626, 1506, 1449, 1273, 1113, 1036, 1012, 871. EI$^+$ mode: m/z = 341.9, [M$^+$] (calcd for $C_{19}H_{20}FN_3O_2$ = 341.4).

2-(4-(1H-Benzo[d]imidazol-2-yl)-2-fluorophenoxy)-1-morpholinoethan-1-one ***22***. Compound **22** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-(2-morpholino-2-oxoethoxy)benzaldehyde **6** (294 mg, 1.11 mmol), $Na_2S_2O_5$ (251 mg, 1.33 mmol) and 1,2-diaminobenzene (120 mg, 1.11 mmol). After 7 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **22** was isolated as a pale yellow powder (152 mg, 46.2%, m.p. > 250 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.82 (s, 1H, NH), 7.98 (dd, J = 12.5, 2.0 Hz, 1H, H-4), 7.92 (m, 1H, H-7), 7.64 (d, J = 7.3 Hz, 1H, H-6′), 7.51 (d,

J = 7.1 Hz, 1H, H-2′), 7.26 (t, J = 8.7 Hz, 1H, H-5′), 7.19 (dq, J = 7.1, 6.0 Hz, 2H, H-5,6), 5.06 (s, 2H, H-2″), 3.62 (m, 4H, H-4″,4‴), 3.47 (s, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 165.86 (C-2″), 151.96 (d, $J_{C-F}{}^1$ = 244.1 Hz, C-3′), 150.68 (C-2), 147.83 (d, $J_{C-F}{}^2$ = 10.5 Hz, C-4′), 144.22 (C-4a), 135.44 (C-7a), 123.94 (d, $J_{C-F}{}^3$ = 7.0 Hz, C-1′), 123.27 (d, $J_{C-F}{}^3$ = 2.2 Hz, C-5′), 122.94 (C-1′), 122.16 (C-6), 119.18 (C-4), 115.91 (C-6′), 114.44 (d, $J_{C-F}{}^2$ = 20.2 Hz, C-2′), 111.68 (C-7), 66.61 (C-1″), 66.52 (C-4″), 66.43 (C-4‴), 45.07 (C-3″), 42.09 (C-3‴). IR (ν, cm^{-1}) 3181, 3159, 2971, 1650, 1498, 1423, 1238, 1113, 748. EI$^+$ mode: m/z = 355.9, [M$^+$] (calcd for $C_{19}H_{18}FN_3O_3$ = 355.4).

*2-(4-(6-Chloro-1H-benzo[d]imidazol-2-yl)-2-fluorophenoxy)-N,N-diethylethan-1-amine **23**.* Compound **23** was synthesized according to the general procedure 3.2.3. from 4-(2-(diethylamino)ethoxy)-3-fluorobenzaldehyde **4** (168 mg, 0.70 mmol), $Na_2S_2O_5$ (160 mg, 0.84 mmol) and 4-chlorobenzene-1,2-diamine (100 mg, 0.70 mmol). After 3 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **23** was isolated as a white powder (211 mg, 34.3%, m.p. = 201–203 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 13.11 (d, J = 10.3 Hz, 1H, NH), 8.00 (m, 2H, H-2′,6′), 7.61 (m, 2H, H-4,7), 7.41 (t, J = 8.8 Hz, 1H, H-5′), 7.21 (m, 1H, H-5), 4.35 (s, 2H, H-1″), 3.19 (m, 2H, H-2″), 2.89 (s, 4H, H-3″,3‴), 1.12 (t, J = 6.2 Hz, 6H, H-4″,4‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 152.03 (d, $J_{C-F}{}^1$ = 244.2 Hz, C-3′/C-3′·), 152.03 (d, $J_{C-F}{}^1$ = 38.7 Hz, C-4′/C-4′·), 148.12 (C-2/C-2·), 148.06 (C-2/C-2·), 145.14 (C-7a/C-7a·), 143.01 (C-4a/C-4a·), 136.21 (C-7a/C-7a·), 134.28 (C-4a/C-4a·), 127.21 (C-6/C-6·), 126.54 (C-6/C-6·), 123.82 (C-5′), 123.48 (C-1′), 123.01 (C-5/C-5·), 122.55 (C-5/C-5·), 120.39 (C-4/C-4·), 118.53 (C-4/C-4·), 115.78 (C-6′), 114.58 (d, $J_{C-F}{}^2$ = 20.2 Hz, C-2′), 113.03 (C-7/C-7·), 111.45 (C-7/C-7·), 51.03 (C-2″), 47.75 (C-3″,3‴), 10.85 (C-4″,4‴).

*4-(2-(4-(6-Chloro-1H-benzo[d]imidazol-2-yl)-2-fluorophenoxy)ethyl)morpholine **24**.* Compound **24** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-(2-morpholinoethoxy)benzaldehyde **5** (162 mg, 0.85 mmol), $Na_2S_2O_5$ (150.1 mg, 0.79 mmol) and 4-chlorobenzene-1,2-diamine (101 mg, 0.71 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **24** was isolated as a white powder (69 mg, 26.2%, m.p. = 159–161 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.95 (s, 1H, NH), 7.95 (m, 2H, H-2′,6′), 7.56 (m 1H, H-4), 7.36 (m, 2H, H-7,5′), 7.05 (m, 1H, H-5), 4.25 (t, J = 5.7 Hz, 2H, H-1″), 3.58 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.74 (t, J = 5.7 Hz, 2H, H-2″), H-3″,3‴ overlapped by the DMSO signal. ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 152.08 (d, $J_{C-F}{}^2$ = 78.4 Hz, C-4′), 152.06 (d, $J_{C-F}{}^1$ = 243.8 Hz, C-3′), 148.44 (C-2), 148.34 (C-2·), 144.62 (C-7a/C-7a·), 140.86 (C-4a/C-4a·), 135.53 (C-7a/C-7a·), 132.12 (C-4a/C-4a·), 123.66 (C-2′/C-2′·), 123.53 (C-2′/C-2′·), 123.36 (C-6/C-6·), 123.28 (C-6/C-6·), 120.01 (C-4/C-4·), 115.94 (C-6′), 114.47 (d, $J_{C-F}{}^3$ = 9.6 Hz, C-5′/C-5′·), 114.27 (d, $J_{C-F}{}^3$ = 9.5 Hz, C-5′/C-5′·), 112.28 (C-4/C-4·), 110.87 (C-5/C-5·), 110.23 (C-5/C-5·), 104.64 (C-7/C-7·), 98.11 (C-7/C-7·), 67.24 (C-1″), 66.66 (C-3″,3‴), 57.27 (C-2″), 54.06 (C-4″,4‴). IR (ν, cm^{-1}) 2977, 2901, 1626, 1502, 1435, 1280, 1109, 801, 725. EI$^+$ mode: m/z = 375.8, [M$^+$] (calcd for $C_{19}H_{19}ClFN_3O_2$ = 375.8).

*2-(4-(6-Chloro-1H-benzo[d]imidazol-2-yl)-2-fluorophenoxy)-1-morpholinoethan-1-one **25**.* Compound **25** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-(2-morpholino-2-oxoethoxy)benzaldehyde **6** (225 mg, 0.84 mmol), $Na_2S_2O_5$ (190 mg, 1.00 mmol) and 4-chlorobenzene-1,2-diamine (120 mg, 0.84 mmol). After 4.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **25** was isolated as a white powder (128 mg, 46.9%, m.p. = 239–241 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 13.02 (s, 1H, NH), 7.97 (dd, J = 12.4, 2.0 Hz, 1H, H-2′), 7.91 (m, 1H, H-6′), 7.61 (m, 2H, H-4,7), 7.27 (t, J = 8.7 Hz, 1H, H-5′), 7.22 (d, J = 6.9 Hz, 1H, H-5), 5.07 (s, 2H, H-1″), 3.61 (m, 4H, H-3″), 3.46 (s, 4H, H-4″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 165.82 (C-2″), 152.09 (d, $J_{C-F}{}^2$ = 36.3 Hz, C-4′), 151.92 (d, $J_{C-F}{}^1$ = 244.1 Hz, C-3′), 148.19 (C-2/C-2·), 148.09 (C-2/C-2·), 145.16 (C-4a/C-4a·), 143.04 (C-7a/C-7a·), 136.16 (C-4a/C-4a·), 134.26 (C-7a/C-7a·), 127.18 (C-6/C-6·), 126.54 (C-6/C-6·), 123.49 (C-5′), 123.34 (C-1′), 122.98 (C-5/C-5·), 122.53 (C-5/C-5·), 120.40 (C-4/C-4·), 118.56 (C-4/C-4·), 115.93 (C-6′), 114.59 (d, $J_{C-F}{}^2$ = 20.3 Hz, C-2′), 112.99 (C-7/C-7·), 111.41 (C-7/C-7·), 66.58 (C-1″), 66.52 (C-4″), 66.42 (C-4‴), 45.05 (C-3″), 42.09 (C-3‴). IR (ν, cm^{-1}) 3148, 2972, 2864, 1650, 1500, 1422, 1276, 1238, 1112, 1029. EI$^+$ mode: m/z = 371.8, [M$^+$] (calcd for $C_{19}H_{18}ClN_3O_3$ = 371.8).

*N,N-Diethyl-2-(2-fluoro-4-(6-fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)ethan-1-amine **26**.* Compound **26** was synthesized according to the general procedure 3.2.3. from 4-(2-(diethylamino)ethoxy)-3-fluorobenzaldehyde **4** (190 mg, 0.79 mmol), $Na_2S_2O_5$ (180 mg, 0.95 mmol) and 4-fluorobenzene-1,2-diamine (100 mg, 0.79 mmol). After 7.5 hours, the reaction was complete and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **26** was isolated as a white powder (207 mg, 64.8%, m.p. = 209–211 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 13.05 (s, 1H, NH), 8.00 (m, 2H, H-2′,6′), 7.58 (m, 1H, H-4), 7.37 (m, 2H, H-7,5′), 7.05 (m, 1H, H-5), 4.35 (t, J = 5.5 Hz, 2H, H-1″), 6.39 (m, 2H, H-2″), 2.91 (s, 4H, H-3″,3‴), 1.12 (t, J = 7.2 Hz, 6H, H-4″,4‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 159.12 (d, $J_{C-F}{}^1$ = 201.5 Hz, C-6/C-6·), 158.89 (d, $J_{C-F}{}^1$ = 220.9 Hz, C-6/C-6·), 152.04 (d, $J_{C-F}{}^1$ = 244.0 Hz, C-3′), 151.98 (d, $J_{C-F}{}^2$ = 77.2 Hz, C-4′), 147.92 (C-2/C-2·), 147.84 (C-2/C-2·), 144.60 (d, $J_{C-F}{}^3$ = 13.7 Hz, C-7a/C-7a·), 140.93 (C-4a/C-4a·), 135.56 (d, $J_{C-F}{}^3$ = 10.8 Hz, C-7a/C-7a·), 132.14 (C-4a/C-4a·), 123.79 (C-1′), 123.68 (C-2′/C-2′·), 123.57 (C-2′/C-2′·), 120.02 (C-4/C-4·), 115.78 (C-6′), 114.47 (d, $J_{C-F}{}^3$ = 15.3 Hz, C-5′/C-5′·), 112.31 (C-4/C-4·), 110.91 (d, $J_{C-F}{}^2$ = 23.6 Hz, C-5/C-5·), 110.27 (d, $J_{C-F}{}^2$ = 25.9 Hz, C-5/C-5·), 104.63 (d, $J_{C-F}{}^2$ = 22.9 Hz, C-7/C-7·), 98.14 (d, $J_{C-F}{}^2$ = 25.4 Hz, C-7/C-7·), 66.24 (C-1″), 51.00 (C-2″), 47.74 (C-3″,3‴), 10.79 (C-4″,4‴).

2-(2-Fluoro-4-(6-fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)-1-morpholinoethan-1-one ***27***. Compound **27** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-(2-morpholino-2-oxoethoxy)benzaldehyde **6** (211 mg, 0.79 mmol), $Na_2S_2O_5$ (180 mg, 0.95 mmol) and 4-fluorobenzene-1,2-diamine (100 mg, 0.79 mmol). After 5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4-OH = 50 : 1 : 0.1). Compound **27** was isolated as a white powder (221 mg, 74.7%, m.p. > 250 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.96 (s, 1H, NH), 7.97 (dd, J = 12.5, 2.1 Hz, 1H, H-2′), 7.91 (dt, J = 8.7, 1.5 Hz, 1H, H-6′), 7.57 (s, 1H, H-4), 7.39 (s, 1H, H-7), 7.27 (t, J = 8.7 Hz, 1H, H-5′), 7.10 (m, 1H, H-5), 5.07 (s, 2H, H-1″), 3.62 (m, 4H, H-4″,4‴), 3.47 (m, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 165.84 (C-2″), 159.30 (d, $J_{C-F}{}^1$ = 236.0 Hz, C-6/C-6·), 158.95 (d, $J_{C-F}{}^1$ = 232.7 Hz, C-6/C-6·), 152.06 (d, $J_{C-F}{}^2$ = 79.4 Hz, C-4′), 151.94 (d, $J_{C-F}{}^1$ = 244.0 Hz, C-3′), 148.09 (C-2/C-2·), 147.98 (C-2/C-2·), 144.62 (d, $J_{C-F}{}^3$ = 14.5 Hz, C-7a/C-7a·), 140.88 (C-4a/C-4a·), 135.53 (d, $J_{C-F}{}^3$ = 14.6 Hz, C-7a/C-7a·), 132.12 (C-4a/C-4a·), 123.61 (C-1), 123.36 (C-2′/C-2′·), 123.24 (C-2′/C-2′·), 120.03 (d, $J_{C-F}{}^3$ = 9.5 Hz, C-4/C-4·), 115.93 (C-6′), 114.48 (d, $J_{C-F}{}^2$ = 20.3 Hz, C-5′/C-5′·), 112.36 (d, $J_{C-F}{}^3$ = 10.1 Hz, C-4/C-4·), 110.90 (d, $J_{C-F}{}^2$ = 26.0 Hz, C-5/C-5·), 110.25 (d, $J_{C-F}{}^2$ = 24.0 Hz, C-5/C-5·), 104.67 (d, $J_{C-F}{}^2$ = 23.7 Hz, C-7/C-7·), 98.13 (d, $J_{C-F}{}^2$ = 26.5 Hz, C-7/C-7·), 66.59 (C-1″), 66.43 (C-4‴), 55.52 (C-4″), 45.06 (C-3″), 42.09 (C-3‴). IR (ν, cm^{-1}) 3164, 2969, 2865, 1646, 1499, 1421, 1241, 1115, 1034, 765. EI$^+$ mode: m/z = 373.8, [M$^+$] (calcd for $C_{19}H_{17}F_2N_3O_3$ = 373.3).

4-(2-(4-((4-(1H-Benzo[d]imidazol-2-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine ***28***. Compound **28** was synthesized according to the general procedure 3.2.3. from 4-((1-(2-morpholinoethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-benzaldehyde **10** (291 mg, 0.92 mmol), $Na_2S_2O_5$ (210 mg, 1.10 mmol) and 1,2-diaminobenzene (100 mg, 0.92 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 50 : 1 : 0.1). Compound **28** was isolated as a white powder (270 mg, 72.2%, m.p. = 212–213 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.74 (s, 1H, NH), 8.26 (s, 1H, H-9′), 8.12 (d, J = 8.9 Hz, 2H, H-2′,6′), 7.57 (m, 2H, H-4,7), 7.22 (d, J = 8.9 Hz, 2H, H-3′,5′), 7.18 (d, J = 4.6 Hz, 2H, H-5,6), 5.26 (s, 2H, H-7′), 4.51 (t, J = 6.3 Hz, 2H, H-1″), 3.54 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.75 (t, J = 6.3 Hz, 2H, H-2″), 2.41 (t, J = 4.6 Hz, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 159.83 (C-4′), 151.28 (C-2), 142.64 (C-8′), 128.45 (C-2′,6′), 125.48 (C-9′), 123.47 (C-1′), 122.59 (C-6), 121.94 (C-5), 119.03 (C-4), 115.63 (C-3′,5′), 111.52 (C-7), 66.57 (C-4″,4‴), 61.75 (C-7′), 57.83 (C-1″), 53.42 (C-3″,3‴), 47.00 (C-2″).

4-(2-(4-((4-(6-Chloro-1H-benzo[d]imidazol-2-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine ***29***. Compound **29** was synthesized according to the general procedure 3.2.3. from 4-((1-(2-morpholinoethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **10** (291 mg, 0.92 mmol), $Na_2S_2O_5$ (210 mg, 1.10 mmol) and 4-chlorobenzene-1,2-diamine (131 mg, 0.92 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **29** was isolated as a white powder (247 mg, 80.4%, m.p. = 189–190 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.88 (d, J = 4.5 Hz, 1H, NH), 8.26 (s, 1H, H-9′), 8.11 (m, 2H, H-2′,6′), 7.55 (m, 1H, H-7), 7.36 (m, 1H, H-4), 7.22 (m, 2H, H-3′,5′), 7.03 (m, 1H, H-5), 5.26 (s, 2H, H-7′), 4.51 (t, J = 6.3 Hz, 2H, H-1″), 3.52 (t, J = 4.4 Hz, 4H, H-4″,4‴), 2.75 (t, J = 6.3 Hz, 2H, H-2″), 2.41 (t, J = 4.4 Hz, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 160.11 (C-4′), 153.35 (C-2), 145.34 (C-4a), 142.60 (C-8′), 136.23 (C-7a), 128.64 (C-2′,6′), 126.83 (C-6), 125.49 (C-9′), 122.95 (C-1′), 122.62 (C-5), 120.20 (C-6), 115.70 (C-3′,5′), 112.79 (C-7), 66.56 (C-4″,4‴), 61.77 (C-7′), 57.83 (C-2″), 53.42 (4″,4‴), 47.00 (C-1″). IR (ν, cm^{-1}) 3217, 3159, 2865, 1622, 1494, 1433, 1251, 1191, 1114, 1044, 923, 816. EI$^+$ mode: m/z = 438.1, [M$^+$] (calcd for $C_{22}H_{23}ClN_6O_2$ = 438.1).

4-(2-(4-((4-(6-Fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine ***30***. Compound **30** was synthesized according to the general procedure 3.2.3. from 4-((1-(2-morpholinoethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **10** (291 mg, 0.92 mmol), $Na_2S_2O_5$ (210 mg, 1.10 mmol) and 4-fluorbenzene-1,2-diamine (116 mg, 0.92 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **30** was isolated as a white powder (150 mg, 44.8%, m.p. = 189–190 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.90 (m, 1H, NH), 8.26 (s, 1H, H-9′), 8.11 (m, 2H, H-2′,6′), 7.46 (m, 2H, H-4,7), 7.23 (m, 2H, H-3′,5′), 7.03 (m, 1H, H-5), 5.26 (s, 2H, H-7′) 4.51 (t, J = 6.3 Hz, 2H, H-1″), 3.53 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.75 (t, J = 6.3 Hz, 2H, H-2″), 2.41 (t, J = 4.6 Hz, 4H, H-3″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 142.62 (C-8′), 128.64 (C-2′6′/C-2·′6′·), 128.52 (C-2′6′/C-2·′6′·), 125.49 (C-9′), 123.07 (C-1′/C-1′·), 119.97 (d, $J_{C-F}{}^2$ = 23.7 Hz, C-4/C-4′·), 115.67 (C-2′), 110.45 (d, $J_{C-F}{}^2$ = 25.5 Hz, C-5/C-5·), 109.82 (d, $J_{C-F}{}^2$ = 24.8 Hz, C-5/C-5·), 104.63 (d, $J_{C-F}{}^2$ = 24.2 Hz, C-7/C-7·), 98.0 (d, $J_{C-F}{}^2$ = 29.3 Hz, C-7/C-7·), 66.56 (C-4″,4‴), 61.76 (C-7′), 57.83 (C-1″), 53.41 (C-3″,3‴), 47.00 (C-2″). IR (ν, cm^{-1}) 3194, 3144, 2982, 1611, 1490, 1448, 1248, 1111, 1003, 845, 809. EI$^+$ mode: m/z = 422.1, [M$^+$] (calcd for $C_{22}H_{23}FN_6O_2$ = 422.1).

4-(2-(4-((4-(1H-Benzo[d]imidazol-2-yl)-2-fluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine ***31***. Compound **31** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-((1-(2-morpholinoethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **11** (308 mg, 0.92 mmol), $Na_2S_2O_5$ (210 mg, 1.10 mmol) and 1,2-diaminobenzene (100 mg, 0.92 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 20 : 1 : 0.1). Compound **31** was isolated as a white powder (241 mg, 61.7%, m.p. = 189–190 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.84 (s, 1H, NH), 8.98 (m, 2H, H-4,7), 8.28 (s, 1H, H-9′), 7.58 (m, 3H, H-2′,5′,6′), 7.19 (d, J = 3.9 Hz, 2H, H-5,6), 5.34 (s, 2H, H-7′), 4.51 (t, J = 6.3 Hz, 2H, H-1″),

3.51 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.73 (t, J = 6.3 Hz, 2H, H-2″), 2.40 (t, J = 4.6 Hz, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 152.18 (d, $J_{C-F}{}^{1}$ = 243.9 Hz, C-3′), 150.63 (C-2), 147.63 (d, $J_{C-F}{}^{2}$ = 10.6 Hz, C-4′), 142.17 (C-8′), 125.78 (C-9′), 124.05 (d, $J_{C-F}{}^{3}$ = 7.1 Hz, C-1′), 123.44 (d, $J_{C-F}{}^{4}$ = 3.0 Hz, C-6′), 122.97 (C-5), 122.19 (C-6), 119.22 (C-4), 116.18 (C-6′), 114.47 (d, $J_{C-F}{}^{2}$ = 20.3 Hz, C-2′), 111.69 (C-7), 66.56 (C-4″,4‴), 62.63 (C-7′), 57.80 (C-1″), 53.40 (C-3″,3‴), 47.02 (C-2″). IR (ν, cm^{-1}) 3160, 2961, 2800, 1623, 1501, 1279, 1133, 1113, 850, 802. EI$^+$ mode: m/z = 423.0, [M$^+$] (calcd for $C_{22}H_{23}FN_6O_2$ = 422.4).

4-(2-(4-((4-(6-Chloro-1H-benzo[d]imidazol-2-yl)-2-fluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine ***32***. Compound **32** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-((1-(2-morpholinoethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **11** (237 mg, 0.71 mmol), $Na_2S_2O_5$ (162 mg, 0.85 mmol) and 4-chlorobenzene-1,2-diamine (102 mg, 0.71 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 25 : 1 : 0.1). Compound **32** was isolated as a white powder (126 mg, 39.4%, m.p. = 194–196 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 13.04 (d, J = 12.0 Hz, 1H, NH), 8.29 (s, 1H, H-9′), 7.98 (m, 2H, H-4,7), 7.61 (m, 3H, H-2′,5′,6′), 7.23 (m, 2H, H-5,6), 5.35 (s, 2H, H-7′), 4.52 (t, J = 6.2 Hz, 2H, H-1″), 3.52 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.75 (t, J = 6.2 Hz, 2H, H-2″), 2.41 (s, 4H, H-3″,3‴). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 152.23 (C-2), 152.14 (d, $J_{C-F}{}^{2}$ = 20.2 Hz, C-3′), 147.93 (C-4′), 145.16 (C-4a), 142.12 (C-8′), 136.20 (C-7a), 127.23 (C-6), 125.80 (C-9′), 123.68 (C-5′), 123.50 (C-1′), 123.02 (C-5), 120.42 (C-6), 118.54 (C-4), 116.19 (C-5′), 114.61 (d, $J_{C-F}{}^{2}$ = 20.2 Hz, C-2′), 113.00 (C-7), 66.56 (C-4″,4‴), 62.64 (C-7′), 57.80 (C-1″), 53.40 (C-3″,3‴), 47.02 (C-2″). IR (ν, cm^{-1}) 3159, 2940, 2811, 1629, 1503, 1454, 1284, 1150, 1111, 1044, 925, 805. EI$^+$ mode: m/z = 456.9, [M$^+$] (calcd for $C_{22}H_{22}ClFN_6O_2$ = 456.9).

4-(2-(4-((2-Fluoro-4-(6-fluoro-1H-benzo[d]imidazol-2-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine ***33***. Compound **33** was synthesized according to the general procedure 3.2.3. from 3-fluoro-4-((1-(2-morpholinoethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **11** (264 mg, 0.79 mmol), $Na_2S_2O_5$ (180 mg, 0.95 mmol) and 4-fluorobenzene-1,2-diamine (100 mg, 0.79 mmol). After 3.5 hours, the reaction was completed and crude product was purified by column chromatography (CH_2Cl_2 : MeOH : NH_4OH = 25 : 1 : 0.1). Compound **33** was isolated as a white powder (8 mg, 22.3%, m.p. = 223–226 °C). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ/ppm) 12.98 (s, 1H, NH), 8.29 (s, 1H, H-9′), 7.98 (m, 2H, H-2′,6′), 7.58 (m, 2H, H-5′,4), 7.38 (ddd, J = 48.6, 9.5, 2.5 Hz, 1H, H-7), 7.05 (m, 1H, H-5), 5.35 (s, 2H, H-7′), 4.52 (t, J = 6.2 Hz, 2H, H-1″), 3.52 (t, J = 4.6 Hz, 4H, H-4″,4‴), 2.75 (t, J = 6.2 Hz, 2H, H-2″), 2.40 (t, J = 4.6 Hz, 4H, H-3″,3″). ^{13}C-NMR (101 MHz, DMSO-d_6) (δ/ppm) 159.31 (d, $J_{C-F}{}^{1}$ = 236.2 Hz, C-6/C-6·), 158.95 (d, $J_{C-F}{}^{1}$ = 233.9 Hz, C-6/C-6·), 152.15 (d, $J_{C-F}{}^{1}$ = 244.1 Hz, C-3′/C-3′·), 152.00 (d, $J_{C-F}{}^{2}$ = 78.5 Hz, C-4′/C-4′·), 147.77 (C-2/C-2·), 147.65 (C-2/C-2·), 144.62 (d, $J_{C-F}{}^{3}$ = 12.7 Hz, C-7a/C-7a·), 142.14 (C-8′), 140.87 (C-4a/C-4a·), 135.54 (d, $J_{C-F}{}^{3}$ = 13.7 Hz, C-7a/C-7a·), 132.12 (C-4a/C-4a·), 125.79 (C-9′), 123.55 (C-2′/C-2′·), 123.42 (C-2′/C-2′·), 120.11 (C-4/C-4·), 116.19 (C-6/C-6·), 114.57 (d, $J_{C-F}{}^{3}$ = 10.0 Hz, C-5′/C-5′·), 114.37 (d, $J_{C-F}{}^{3}$ = 9.8 Hz, C-5′/C-5′·), 112.32 (d, $J_{C-F}{}^{3}$ = 10.1 Hz, C-4/C-4·), 110.92 (d, $J_{C-F}{}^{2}$ = 25.8 Hz, C-5/C-5·), 110.27 (d, $J_{C-F}{}^{2}$ = 24.6 Hz, C-5/C-5·), 104.69 (C-7/C-7·), 98.13 (C-7/C-7·), 66.56 (C-4″,4‴), 62.63 (C-7′), 57.80 (C-1″), 53.40 (C-3″,3‴), 47.02 (C-2″). EI$^+$ mode: m/z = 440.9, [M$^+$] (calcd for $C_{22}H_{22}F_2N_6O_2$ = 440.4).

3.3. Biological assay

3.3.1. Antiproliferative evaluation.[64] For proliferation assays, adherent cell lines LN-229, HCT-116, NCI-H460, and Capan-1 cells were seeded in 384-well tissue culture plates (Greiner, Kremsmünster, Austria) at a density between 500 and 1500 cells per well (500 cells per well for Capan-1, 1000 cells per well for LN-229 and HCT-116, and 1500 cells per well for NCI-H460). The cells were treated with seven different concentrations of the test compounds in a 5-fold dilution series ranging from 100 to 0.006 μM after overnight incubation. Suspension cell lines HL-60, K-562, Z-138, and DND-41 were seeded at densities ranging from 2500 to 5500 cells per well (2500 cells per well for HL-60, K-562, and Z-138, and 5500 cells per well for DND-41) in 384-well culture plates containing the test compounds at the same concentration points. All conditions were incubated for 72 h before measuring the cell viability by the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WA, USA) according to the manufacturer's instructions. After 3 h, absorbance of all conditions was measured at 490 nm using a SpectraMax Plus 384 (Molecular Devices, San Jose, CA, USA), and the OD values were used to calculate the 50% inhibitory concentration (IC_{50}). Compounds were tested in two independent experiments.

3.3.2. Fluorescent intercalator displacement (FID) assay. For the experiment, 50 nM acridine orange and 6 μg mL^{-1} DNA (salmon sperm DNA, Invitrogen) were mixed with the test compounds in HEN buffer (10 mM HEPES pH 7.5, 1 mM EDTA, and 100 mM NaCl) and incubated for 20 minutes in the dark. Mitoxantrone (MTX, MedChemExpress) served as the positive control. The assay was performed in 384-well plates (Corning #3575) in a total reaction volume of 30 μL per well. Fluorescence polarization was measured using a Tecan Spark plate reader.

3.3.3. Cytotoxicity test on normal peripheral blood mononuclear cells.[65] Buffy coat preparations from healthy donors were obtained from the Blood Transfusion Center in Leuven, Belgium. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation with lymphoprep (d = 1.077 g mL^{-1}) (Nycomed, Oslo, Norway) and cultured in cell culture medium (DMEM/F12, Gibco Life Technologies, USA) containing 8% FBS. PBMCs were seeded at 28 000 cells per well in 384-well, black-walled, clear-bottomed tissue culture plates and treated with compounds at seven different concentrations ranging from 100 to 0.006

μM. Propidium iodide was added at a concentration of 1 μg mL^{-1} with IncuCyte® Caspase 3/7 Green Reagent as recommended by the supplier. Plates were incubated and monitored at 37 °C for 72 h in IncuCyte®. Images were captured every 3 h in bright field and green and red fluorescence channels, with one field captured per well under 10× magnification. By quantifying the fluorescent signal after 72 h in both channels using IncuCyte® image analysis software, the percentages of live, dead and apoptotic cells were calculated. Additionally, for determining the viability of the normal cells after 72 hour treatment, the CellTiter 96® AQueous One Solution Cell Proliferation Assay was employed according to the manufacturer's instructions. Absorbance of the samples was measured at 490 nm using a SpectraMax Plus 384 (Molecular Devices), and OD values were used to calculate the 50% inhibitory concentration (IC_{50}). All compounds were tested in two independent experiments on PMBC cells from two different donors.

3.3.4. Antibacterial activity assay.[66] 2-Arylbenzimidazole derivatives **12–33** were tested against Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212), and Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027) and *Klebsiella pneumoniae* (ATCC 27736) and activities are expressed as minimum inhibitory concentration (MIC). To assess the relative potency of the tested derivatives, the obtained MIC values were compared with those of standard antibiotics, ceftazidime (CAZ) and ciprofloxacin (CIP), which served as reference controls. This comparative analysis provides insight into the potential of the synthesized compounds as novel antibacterial agents, particularly in the context of emerging antibiotic resistance and the ongoing need for new therapeutic alternatives. All mentioned bacterial cultures are in the collection of the Department of Industrial Ecology, University of Zagreb Faculty of Chemical Engineering and Technology.

3.3.4.1. Preparation of stock solutions of synthesized compounds. The stock solution was prepared by dissolving 5.12 mg of the tested compound in 1 mL of concentrated DMSO. The mentioned solution was homogenized until the sample was completely dissolved. The initial concentration of the stock solution was 5120 μg mL^{-1}. The initial concentrations of the working solutions in the system were 256 μg mL^{-1}, 128 μg mL^{-1}, 64 μg mL^{-1}, 32 μg mL^{-1}, 16 μg mL^{-1}, 8 μg mL^{-1}, 4 μg mL^{-1}, 2 μg mL^{-1}, 1 μg mL^{-1} and 0.05 μg mL^{-1}. Preparation of solutions was carried out under sterile conditions.

3.3.4.2. Preparation of inoculum. Pure bacterial cultures were inoculated onto nutrient agar and incubated at 37 °C the day before setting up the experiment. A suspension of bacteria (*Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 25923)) was prepared in to Mueller-Hinton broth (MHB). Pure and separate colonies of the grown bacterial culture were transferred with a sterile microbiological loop to MHB. The concentration of the bacterial suspension that was inoculated into the test tubes was 1×10^6 st mL^{-1}, or in the system 5×10^5 st mL^{-1}.

3.3.4.3. Determination of the minimum inhibitory concentration (MIC) using the macrodilution method. The MIC was determined by the macrodilution method according to 03-CLSI-M07-A9-2012 (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically). Sterile glass test tubes measuring 13 × 100 mm were used for the experiment. 1 mL of the prepared suspension (1×10^6 st mL^{-1}) a certain volume of the tested compound and sterile water were added to each test tube. Inoculum and inoculum in 1% DMSO were used as positive control, and MHB as negative control. The total volume of suspension, test compound and sterile water was 2 mL. The test tubes are then homogenized on a homogenizer and incubated at a temperature of 37 °C for 16–20 hours. The optical density was observed at 600 nm after 24 h optical density measurement was carried out using spectrophotometer Hach, Model DR/2400, USA.

4. Conclusion

2-Arylbenzimidazole derivatives **12–27** were synthesized by ultrasound-assisted cyclocondensation reaction of prepared *O*-alkylated benzaldehydes **1–6** with *o*-phenylenediamines in the presence of $Na_2S_2O_5$, while 1,2,3-triazole derivatives of 2-arylbenzimidazoles **28–33** were synthesized by ultrasound-assisted reaction of 1,2,3-triazole derivatives of benzaldehyde **10** and **11** with appropriate *o*-phenylenediamines. The structures of the synthesized 2-arylbenzimidazole derivatives **12–33** were confirmed by one-dimensional ^{1}H- and ^{13}C-NMR spectroscopy. Additionally, the structure of benzimidazole derivative **20** was further validated using HSQC spectroscopy for single-bond correlations and HMBC spectroscopy for multiple-bond interactions.

Of all newly synthesized compounds evaluated for their efficacy against tumor cell lines compound **23** which is substituted with chlorine at the C-6 position of benzimidazole and fluorine at the *meta*-position of the benzene ring and an *N*,*N*-diethyl substituent at the *para*-position of the benzene ring showed the most pronounced antiproliferative activity within the concentration range of 2 to 9.4 μM. In order to determine the mechanism of action of the benzimidazole derivative **23**, which showed the highest activity across tested tumor cell lines, a high-throughput fluorescence polarization test for DNA binding molecules was performed as well as a cytotoxicity test against peripheral blood mononuclear cells (PBMC) from two healthy donors in order to determine selective antiproliferative activity against tumor cells. The obtained results indicated that compound **23** caused apoptosis in normal cells only at the highest applied concentration of 100 μM.

Of all tested 2-arylbenzimidazole derivatives **12–33**, the most pronounced selective antibacterial activity against the Gram-positive bacterium *Enterococcus faecalis* was shown by

derivatives **15–17** substituted with a chlorine atom in the C-6 position of benzimidazole and with an unsubstituted benzene ring (MIC = 0.25–1 μg mL^{-1}), while 1,2,3-triazole derivatives of 2-arylbenzimidazole **28** and **30** showed strong selective activity against the Gram-positive bacterium *Enterococcus faecalis* (MIC = 0.25 μg mL^{-1}).

The presented results indicate that 2-arylbenzimidazoles can be efficiently synthesized using ultrasound-assisted methods, which can also be applied to the preparation of other similar derivatives. Notably, benzimidazole derivative **23** exhibited the most pronounced antiproliferative activity, making it a promising candidate for further design and optimization.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its ESI† materials.

Author contributions

IS: synthesis and structural characterization of compounds, methodology, writing – original draft. AR: synthesis of compounds, contributed to the *in vitro* antibacterial evaluation; DKG: *in vitro* antibacterial evaluation, methodology, writing – review and editing; LP, DD: *in vitro* antiproliferative evaluation, methodology, writing – review and editing; TGK: conceptualization, supervision, writing – review and editing. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest in the publication of this article.

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