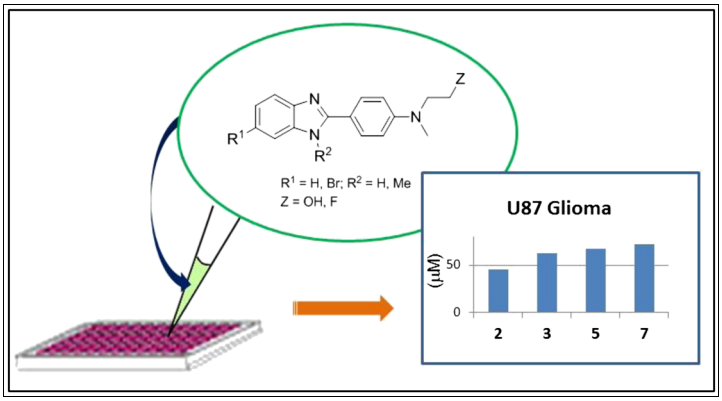
Synthesis and Biological Evaluation of Novel 2-Aryl Benzimidazoles as Chemotherapeutic Agents

Goreti Ribeiro Morais [1],[a]* Elisa Palma,[a,b] Fernanda Marques,[a] Lurdes Gano,[a] Maria Cristina Oliveira,[a] Antero Abrunhosa,[c] Hugo Vicente Miranda,[d,e] Tiago F. Outeiro,[d,e,f,g] Isabel Santos,[a] and Antonio Paulo[a]*

[a]Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Bobadela, Portugal
[b]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal
[c]ICNAS, Instituto de Ciências Nucleares Aplicadas à Saúde, Universidade Coimbra, Coimbra, Portugal
[d]Instituto de Medicina Molecular, Lisboa, Portugal
[e]CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal
[f]Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[g]Department of Neurodegeneration and Restorative Research, University Medical Center Göttingen, Göttingen, Germany
*E-mail: apaulo@ctn.ist.utl.pt; gribeiro@bradford.ac.uk

Additional Supporting Information may be found in the online version of this article.
Received June 28, 2015
DOI 10.1002/jhet.2575
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).



Here, we describe the synthesis and preliminary biological evaluation of novel *N*-unsubstituted and *N*-methylated 2-aryl benzimidazole derivatives that contain fluorinated or hydroxylated alkyl substituents in the 4-*N*-aryl position and different substitution patterns (H vs Br vs I) in the benzimidazole ring. For the selected compounds and for comparison purposes, the congener benzothiazoles were also tested. The cytotoxic effect of 11 benzazole derivatives was evaluated in a panel of human cancer cell lines, such as breast (MCF7), melanoma (A375), cervix (HeLa), and glioblastoma (U87). In general, the compounds exerted a moderate cytotoxic activity against all cells tested. In particular, for the A375 and HeLa cells, the *N*-unsubstituted benzimidazoles **2** and **3** displayed a better cytotoxic profile than the respective *N*-methylated benzimidazole congeners (**5** and **7**). The biodistribution of compound **2**, which has shown the highest cytotoxic activity active in the U87 glioblastoma cells ($IC_{50} = 45.2 \pm 13.0$), was evaluated in CD1 mice using its ^{18}F-labeled counterpart ([^{18}**F**]-**2**). These studies showed that compound **2** can cross the blood brain barrier with a reasonable brain uptake (1.24 and 2.81%I.A./g at 5 and 60 min p.i., respectively), which is a crucial issue for systemic chemotherapy of glioblastoma. Altogether, the *in vitro* antitumoral activity of benzimidazole **2** against the U87 cells and the ability of its ^{18}F-congener to cross the blood brain barrier provide a strong rationale to consider the reported fluoroalkylated 2-aryl benzimidazoles as lead candidates for the generation of chemotherapeutic agents, in particular, against highly aggressive brain tumors such as glioblastoma.

INTRODUCTION

Benzimidazole derivatives started to be envisaged as compounds with biological and medical relevance since the discovery of 5,6-dimethyl-1-(α-D-ribofuranosyl) benzimidazole as part of the structure of vitamin B_{12} in 1950 [2]. Later on, this heterocycle-fused ring system was recognized as a relevant pharmacophore in drug design, and extensive research has been reported in this field profiting from the versatile modification of its scaffold. Since then, benzimidazole derivatives were shown to present a broad spectrum of biological activity, such as antimicrobial [3,4], anticancer [5–8], antiviral [9], antihypertensive [10], antioxidant [11], antidiabetic [12], and anti-inflammatory [13] properties, to cite a few examples. There is a wide structural diversity for benzimidazole derivatives displaying antitumoral activity. For example, Hoechst 33258 is a fluorescent dye used to stain DNA and a known topoisomerase inhibitor, which corresponds to a bis-benzimidazole with a hydroxy aryl substituent at the 2-position of one of the

heterorings [14]. More recently, the two 2-arylbenzimidazole derivatives NU1085 (2-(4-hydroxyphenyl)benzimidazole-4-carboxamide) and a 2-(4-oxadiazolyphenyl) analog were described as chemosensitizers and radiosensitizers, respectively [15,16]. Both compounds were shown to be potent poly (ADP-ribose) polymerase (PARP-1) inhibitors with remarkable chemotherapeutic effect.

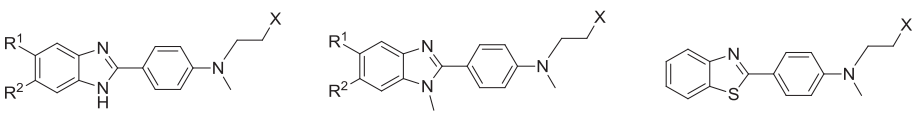
Following our work on antitumoral compounds [17–20], we focused our attention on novel fluoroalkylated benzimidazoles derivatives (Fig. 1). By including alkyl substituents in the structure of these compounds, we have considered the possibility of preparing the corresponding radiofluorinated congeners to evaluate their *in vivo* biological behavior. In fact, the generation of the radioactive counterparts of putative drugs is crucial in all stages of the drug development process, including the preclinical phase where promising leads are selected or excluded [21]. Besides classical biodistribution studies, the radioactive probes may also allow the *in vivo* imaging of drug biodistribution if the proper radionuclides are used. For *in vivo* imaging, there are two different nuclear imaging modalities: single photon emitted computed tomography (SPECT) and positron emission tomography (PET), which are highly sensitive techniques that allow the quantification and monitoring of drug distribution and its pharmacokinetics. SPECT measurements are mainly based on the use of gamma emitters such as radiometals (e.g., ^{99m}Tc), while PET explores positron emitter radionuclides such as ^{11}C and ^{18}F. Nowadays, ^{18}F is still the most relevant PET radionuclide because of its favorable nuclear decay properties ($T_{1/2} = 109.8$ min, $E\beta^+_{max} = 0.69$ MeV). Moreover, contrary to the use of radiometals, no deep structural change occurs when ^{18}F is incorporated into the compounds to be tested, and thus, no alterations of the pharmacokinetics are expected.

Herein, we describe the synthesis of a series of new arylbenzimidazoles that contain hydroxyalkyl or fluoroalkyl substituents in the 4-*N*-aryl position and different halogen (Br or I) substituents in the benzimidazole ring (Fig. 1), as well as the screening of their cytotoxic activity against different human tumor cell lines. *N*-methylated and benzothiazole congeners of some compounds were also synthesized and biologically evaluated using the same panel of cell lines, aiming to study the effect of the imidazolic proton and nature of the heterocycle in the antitumor properties of the compounds. The preclinical studies reported herein have also comprised biodistribution in mice for the ^{18}F-labeled analog ([^{18}F]-**2**) of compound 2-[*N*-methyl-*N*-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[*d*]imidazole (**2**), which has emerged as the most promising candidate for further evaluation as an anticancer drug.

RESULTS AND DISCUSSION

Chemistry and radiochemistry. We have considered a number of structurally related 1-H and 1-methyl 2-arylbenzimidazole derivatives (Fig. 1) whose aniline nitrogen atom is bi-substituted with a methyl group and with 2-hydroxyethyl or 2-fluoroethyl substituents. This new family of compounds includes unsubstituted (**1**, **2**, **4**, **5**) and halogenated 2-aryl benzimidazoles (**3**, **6–9**), as we sought to verify the effect of different substitution patterns on their antitumoral activity. To assess the role of the heterocycle core in the biological performance of the compounds, the benzothiazole congeners (**10** and **11**) of **1** and **2** were also prepared and evaluated.

The new benzazole derivatives were obtained based on a multistep synthesis that started with the formation of the heterocycle rings by the oxidative cyclocondensation of 1,2-diamine benzenes with adequate 4-substituted benzaldehydes (Scheme 1). For BOC-protected benzaldehyde derivatives, the cyclocondensation reactions were carried out in the presence of sodium metabisulfite in refluxing DMF, leading to compounds **17–19** that were obtained in high yield [11]. When an *O*-tosylated benzaldehyde was employed, no oxidant was used and the cyclocondensation was performed at lower temperatures (50°C) to limit hydrolysis processes. In this way, the respective benzimidazoles (**15** and **16**) were obtained in fair to good yields (Scheme 1). The *N*-methylated congeners of **17–19**, compounds **20–24**, were prepared by their reaction with methyl iodide under basic conditions (Scheme 1). In the case of the 5-substituted compounds, the *N*-methylation led to the formation of two pairs of regioisomers (**21/22** and **23/24**) that were obtained in equal ratio. The structures of regioisomers **21** and **22** were determined in solution by



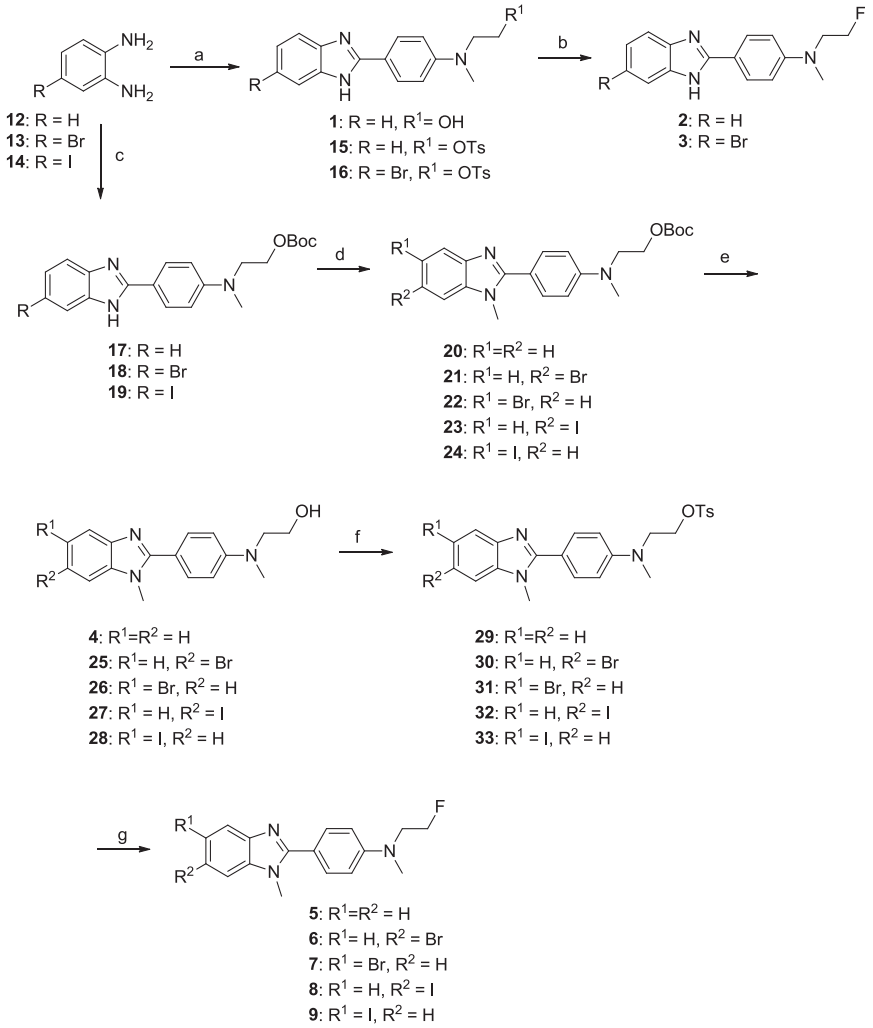
1 $R^1 = R^2 = H$, X = OH
2 $R^1 = R^2 = H$, X = F
3 R^1 = Br, $R^2 = H$, X = F

4 $R^1 = R^2 = H$, X = OH
5 $R^1 = R^2 = H$, X = F
6 $R^1 = H$, R^2 = Br, X = F
7 R^1 = Br, $R^2 = H$, X = F
8 $R^1 = H$, R^2 = I, X = F
9 R^1 = I, $R^2 = H$, X = F

10 Z = OH
11 Z = F

Figure 1. General chemical structures of the novel benzazole derivatives.

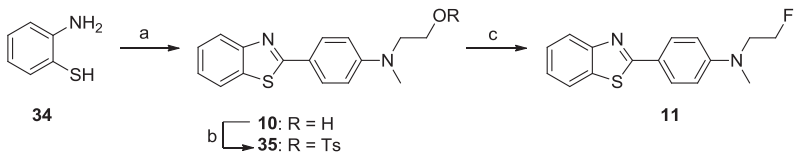
Scheme 1. Reaction reagents and conditions: (a) for **1**: *N*-methyl-*N*-(2′-hydroxyethyl)-4-aminobenzaldehyde, $Na_2S_2O_5$, DMF, 80°C; for **15** and **16**: *N*-methyl-*N*-(2′-*O*-tosyloxyethyl)-4-aminobenzaldehyde, DMF, 50°C; (b) TBAF, THF, 65°C; (c) *N*-methyl-*N*-(2′-*O*-*tert*-butylcarbonate)ethyl)-4-aminobenzaldehyde, $Na_2S_2O_5$, DMF, 120°C; (d) MeI, acetone, NaOH, water; (e) TFA, CH_2Cl_2; (f) *p*-TsCl, Et_3N, CH_2Cl_2; and (g) TBAF, THF, reflux.



NMR NOESY experiments and in solid state by X-ray diffraction studies, as described elsewhere [22]. Assignment of the structures of the related iodinated regioisomers **21** and **22** was achieved by comparing their ^{1}H NMR data with those of the brominated counterparts **24** and **25**. The final fluorinated benzimidazoles (**2**, **3,** and **5–9**) were prepared by nucleophilic substitution of the correspondent *O*-tosylated precursors in good to excellent yields (η = 59–85%). If compared with the *N*-methylated counterparts, the fluorination of the *N*-unsubstituted benzimidazoles, **15** and **16**, was achieved using a lower amount of fluoride ions (1.5 vs 5 eq) and lower reaction temperature in order to minimize competitive nucleophilic alkylation reactions involving the imidazole nitrogen. Under these conditions, fluorinated benzimidazoles **2** and **3** were obtained in fair to good yield (η = 59–71%) (Scheme 1).

Similarly, the benzothiazole scaffold of compound **10** was generated by cyclocondensation of *o*-aminothiophenol with

Scheme 2. Reaction reagents and conditions: (a) *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde, pyridine, reflux; (b) *p*-TsCl, Et_3N, CH_2Cl_2, RT; (c) TBAF, THF, reflux.



the adequate benzaldehyde under basic conditions (Scheme 2). Thereafter, its sequential tosylation and fluorination afforded the desired fluoroalkylated 2-arylbenzothiazole derivative (compound **11**).

As previously mentioned, the presence of fluorine atoms in the structures of this new family of benzazole derivatives offered the possibility of synthesizing the corresponding [^{18}F]-labeled congeners. Such congeners may be applied in biodistribution and metabolism studies to corroborate the application of the synthesized compounds in the design of new anticancer drugs. Thus, we selected compound **2** because it showed the most promising biological profile among the tested compounds, as discussed later. As shown in Scheme 3, the radiofluorinated counterpart of **2** (compound [**^{18}F**]**2**) was prepared by direct aliphatic nucleophilic substitution of the tosylated group in **15** using activated radiofluoride ion ($^{18}F^-$) in the form of [^{18}F]KF/$K_{2.2.2}$ complex.

[**^{18}F**]**2** was synthesized and purified in 70 min total synthesis time with a 30% radiochemical yield (decay corrected) from [^{18}F]fluoride, and with radiochemical purity >99% at the end of synthesis. The chemical identification of [**^{18}F**]**2** was performed by HPLC comparison with the non-radioactive congener **2** as shown in Figure 2. To our knowledge, [**^{18}F**]**2** is a relatively rare example of radiofluorinated benzimidazole derivatives that has been obtained using a direct labeling strategy.

Biological studies: cytotoxicity assays and biodistribution. *In vitro* preliminary screening of the cytotoxic activity of the 2-arylbenzimidazole (**1–9**) and 2-arylbenzothiazole (**10** and **11**) derivatives was assessed in a panel of representative human tumor cells such as MCF7, A375, HeLa and U87 from breast, melanoma, cervix carcinoma, and glioblastoma, respectively. Cells were treated with 100 and 200 μM of the tested compounds for 48 h, and the cellular viability evaluated by MTT, a metabolic assay in which the cellular reduction of a yellow tetrazolium salt yields a purple formazan in proportion to the number of viable cells [23]. At 100 μM concentration and for the large majority of compounds, the A375 and HeLa cells were the less sensitive and the U87 glioblastoma cells the less resistant ones (Fig. 3). In general, the benzimidazoles **2**, **3**, **5**, **6,** and **7** exhibited a more favorable cytotoxicity profile against all cancer cells, particularly against glioblastoma in a dose-dependent manner.

Compounds **2** and **3** were more active than the *N*-methylated counterparts **5** and **7** in almost all cancer cell lines, suggesting that there is a negative effect of the *N*-methylation on the antiproliferative activity. The presence of different substituents at the benzimidazole ring also influences the activity of the compounds. This can be verified by comparing the antiproliferative activity of the iodinated benzimidazole **9** with the brominated congener **7** (Fig. 3), which shows that the replacement of a bromo by an iodo substituent tends to decrease the cytotoxicity of the compounds. The nature of the different *N*-methylated regioisomers (**6/7** and **8/9**) also affects the biological activity of the compounds, particularly in the case of the iodinated benzimidazoles. In fact, the 6-iodinated compound **8** has a higher antiproliferative activity than the corresponding 5-iodinated regioisomer **9** in all tested cell lines. Finally, we confirmed that there is an influence of the fluoroalkylated group on the antiproliferative activity of the compounds, as the fluorinated benzimidazoles **2** and **3** are more active than their hydroxyl-alkylated counterpart, **4** and **5,** respectively. By comparing the antiproliferative activity of the fluorinated derivatives **2** with **11**, it was possible to confirm that the presence of a benzimidazole ring, instead of a benzothiazole, enhances the cytotoxicity of the compounds. This finding suggests that the nature of the heterocycle ring has an important influence on the biological activity of the reported benzazoles.

Based on the preliminary screening of the *in vitro* antiproliferative activity of the different compounds, we have selected **2**, **3**, **5,** and **7** to determine the concentration that

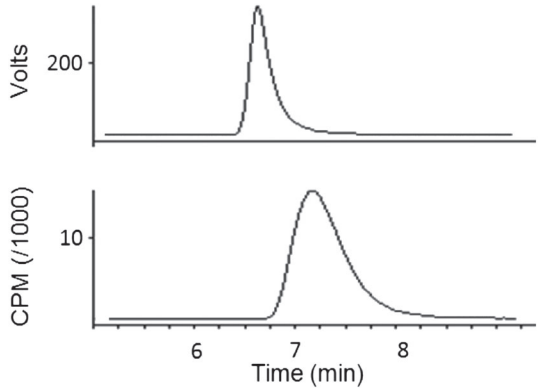
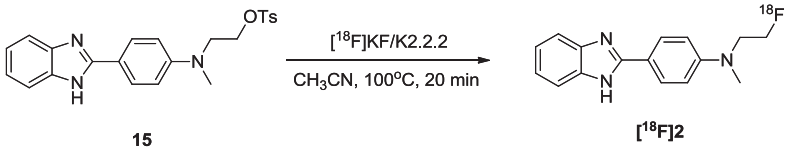


Figure 2. High-performance liquid chromatography profile of compound **2** (top) and [**^{18}F**]**2** (bottom). HPLC conditions: CH_3CN/0.1% triethylamine (pH = 10.2) 40/60, 1 mL/min, 254 nm, t_R = (UV) 6.88 min, (γ) 7.17 min. The slight difference in retention time between the radioactive peak and the UV peak is due to difference in the void volume of the detector system.

Scheme 3. Radiosynthesis of [**^{18}F**]**2**.



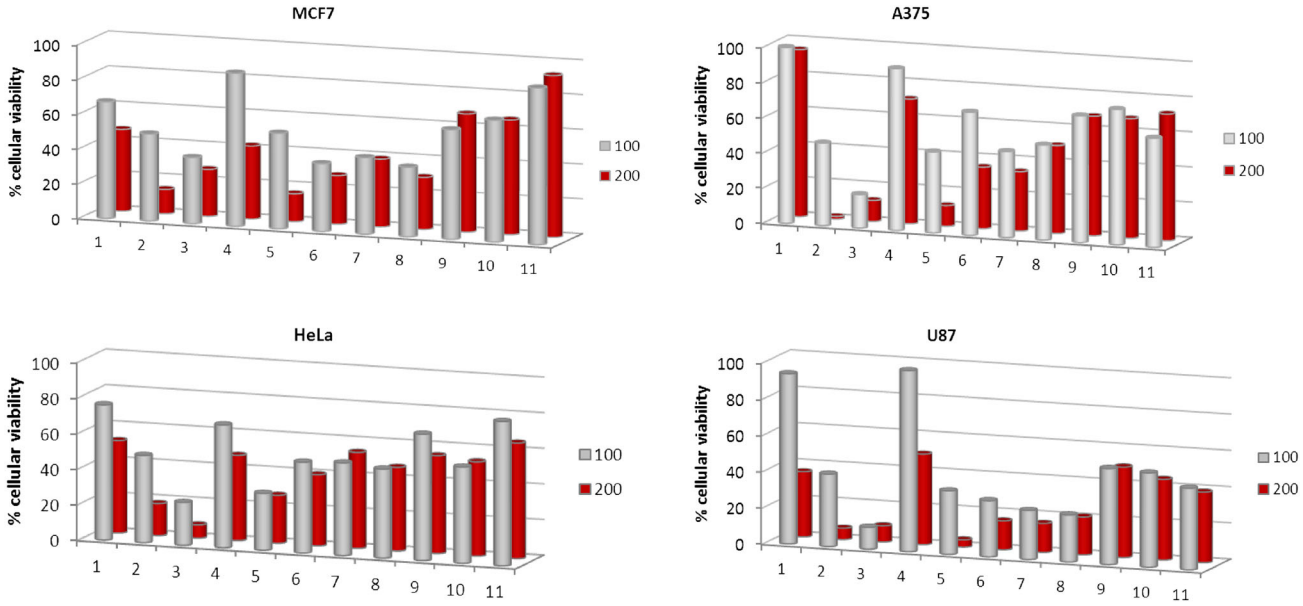


Figure 3. *In vitro* anticancer screening of 2-arylbenzimidazoles (**1–9**) and 2-arylbenzothiazoles (**10** and **11**) against a panel of human tumor cell lines (MCF7, A375, HeLa, and U87). Data are presented as the percentage of cellular viability compared with controls (cells with no treatment) obtained after 48 h treatment with the compounds at 100 and 200 μM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

causes a 50% reduction of the cellular viability (IC_{50} values) in the same cancer cell lines (MCF7, A375, HeLa, and U87). The IC_{50} values found for the selected compounds are shown in Table 1.

The MTT assay showed that the fluorinated aryl-benzimidazoles **2**, **3**, **5,** and **7** inhibited the cell growth of MCF7, HeLa, and U87 cells after 48 h treatment. In general, the compounds exerted less cytotoxic effect on the A375 melanoma cells. In particular, compound 2 showed the highest antiproliferative activity (IC_{50} = 45.2 ± 13.0 μM) against the U87 glioblastoma cells, which are representative of an aggressive malignancy often characterized by resistance to cytotoxic agents [24,25]. Doxorubicin (DOX) a widely used antitumor compound was used as a positive control for the U87 cells. Although important toxicity, together with poor distribution and limited penetration into solid tumors, has limited its full therapeutic potential, several studies have indicated its significant activity against a variety of human cancers, including glioblastoma [26,27]. Using the MTT assay and the same experimental conditions used for the benzimidazole derivatives (i.e., 48 h treatment at 37°C) the IC_{50} value found for DOX in the U87 cells was 16.6 ± 2.5 μM (from two experiments performed with six replicates).

The promising cytotoxic profile of compound **2** against the U87 glioblastoma cells (45.2 ± 13.0 μM) prompted us to proceed with the radiosynthesis of its radioactive counterpart [^{18}**F**]**2** in order to assess its biodistribution, particularly the capability of crossing the BBB to reach the brain.

The biodistribution studies of [^{18}**F**]**2** were performed in CD1 Charles River mice, at 5 and 60 min post-injection (p.i.) times to assess its ability to cross the BBB. The obtained biodistribution data (Table 2) showed moderate initial uptake in most of the organs, including the brain. Most importantly, the ^{18}F-radioactivity uptake in the brain increases over time (1.26 ± 0.47%ID/g and 2.81 ± 0.15%ID/g at 5 and 60 min p.i., respectively), suggesting that the compound can reach the central nervous system.

Table 1

Cytotoxic activity of selected fluorinated aryl-benzimidazoles measured by the MTT assay. Data shown are the IC_{50} values obtained after 48 h treatment with the compounds at serial concentrations. Results are mean (±SD) of two experiments carried out with six replicates.

	IC_{50} (μM)			
Compound	MCF7	A375	HeLa	U87
2	71.1 ± 18.0	106 ± 44.0	62.7 ± 15.0	45.2 ± 13.0
3	90.2 ± 15.0	91.6 ± 31.0	17.8 ± 3.00	62.8 ± 18.0
5	45.5 ± 16.0	104 ± 64.0	75.1 ± 19.0	67.8 ± 25.0
7	68.4 ± 32.0	>200	122 ± 37.0	71.9 ± 30.0

Table 2
Biodistribution data of [^{18}F]**2** in CD1 Charles River mice ($n = 4$).

	% I.A./g ± SD	
Organs	5 min p.i.	60 min p.i.
Blood	2.54 ± 0.25	2.00 ± 0.54
Liver	2.39 ± 0.80	2.83 ± 0.32
Intestine	2.22 ± 1.30	2.37 ± 0.09
Spleen	2.52 ± 1.45	2.86 ± 0.12
Lung	2.15 ± 1.1	2.39 ± 0.29
Kidneys	3.64 ± 0.56	2.89 ± 0.13
Femur	1.50 ± 0.64	2.84 ± 0.16
Pancreas	1.92 ± 1.18	1.90 ± 0.15
Brain	1.26 ± 0.47	2.81 ± 0.15
Brain/blood	0.49	1.40

Moreover, [^{18}F]**2** displayed a rather slow clearance from the blood compartment (2.54 ± 0.25% and 2.00 ± 0.54% at 5 and 60 min p.i., respectively) and main organs. Owing to the slow clearance from most organs, except the kidneys, the excretion pathway cannot be clearly defined at these early time points. However, the activity in the liver and intestines suggests a relevant contribution of the hepatobiliar excretion with a small contribution of the urinary route. Such biodistribution profile can probably be attributed to the high lipophilic character of [^{18}F]**2** (LogP = 3.34). Nevertheless the slow blood clearance points out to a rather large *in vivo* half-life of the non-radioactive congener, compound **2**, favoring its delivery to organ and tissues.

CONCLUSIONS

In summary, we have introduced a set of new heterocycles, including nine structurally related benzimidazoles and two benzothiazole derivatives. Their cytotoxicity was screened against a panel of representative human cancer cell lines. Considering the cellular viability results, four compounds (**2**, **3**, **5**, and **7**) were selected for further determination of their IC_{50} values. The results showed that compound **2** displayed the most promising *in vitro* properties to be applied in the design of new anticancer drugs for treatment of glioblastoma. In fact, compound **2,** having a non-substituted benzimidazole core and a 2-fluoroethyl chain at the aniline nitrogen, presented a reasonable cytotoxic activity against the U87 glioblastoma cell line ($IC_{50} = 45.2 \pm 13.0\ \mu M$) if compared with DOX ($IC_{50} = 16.6 \pm 2.5\ \mu M$), which is a widely used antitumor drug. This compound was selected for further *in vivo* evaluation, using its radiofluorinated congener [^{18}F]**2** as a surrogate. [^{18}F]**2** was synthesized in fair radiochemical yield and high radiochemical purity. [^{18}F]**2** represents a rare example of an *N*-unprotected benzimidazole that was obtained based on a direct radiofluorination strategy. Biodistribution studies showed that [^{18}F]**2** has a significant brain uptake, which increases over time. Altogether, our results represent a strong starting point for the generation of a novel class of chemotherapeutic agents. In particular, the satisfactory *in vitro* antitumoral activity of **2** against the U87 glioma cell line, together with its ability to cross the BBB, suggests this compound might be used in the design of drugs for the glioblastoma treatment.

EXPERIMENTAL

Chemistry. Diamine benzenes **12** and **13** were commercially obtained from Sigma-Aldrich (Taufkirchen, Germany). 4-Iodo-diaminebenzene (**14**) was prepared by reducing 4-iodo-2-nitroaniline ($SnCl_2$, conc HCl, EtOH). *N*-Methyl-*N*-(2-*O*-tosyloxyethyl)-4-aminobenzaldehyde and *N*-methyl-*N*-(2′-*O*-*tert*-butylcarbonate)ethyl)-4-aminobenzaldehyde were prepared as described [28]. Synthesis and characterization of compounds **18**, **21,** and **22** have been described elsewhere [22]. Melting points were measured in a Stuart SMP3 apparatus. NMR spectra were recorded on a Varian Unity 300 NMR spectrometer at the frequencies of 300 MHz (^{1}H), 75 MHz (^{13}C), and 282 MHz (^{19}F). Chemical shifts are reported in parts per million. ^{1}H and ^{13}C chemical shifts (δ) are reported in ppm relative to residual solvent signals ($CDCl_3$: 7.26 ppm for ^{1}H NMR, 77.0 ppm for ^{13}C NMR). ^{19}F chemical shifts were referenced externally to α,α',α''-trifluorotoluene (0.05% in C_6D_6; $\delta = -63.3$). Electrospray ionization mass spectrometry (ESI-MS) was performed on a Bruker HCT quadrupole ion trap instrument in positive ionization mode. High-resolution mass spectrometry (HRMS) measurements were performed on an Extrel–Finnigan Fourier transform ion cyclotron resonance instrument by electron ionization. Elemental analyses of the tested compounds were recorded on an CE Instruments EA 1110. Analytical thin-layer chromatography was performed on precoated silica plates 60 F_{254} (Merck). Visualization of the plates was carried out using UV light (254 and 365 nm) and/or in an iodine chamber. Column chromatography was carried out on silica gel (Merck).

***2-[N*-methyl-*N*-(2-hydroxyethyl)-4′-aminophenyl]-1H-benzo[*d*]imidazole (1).** A mixture of **12** (200 mg, 1.85 m*M*), *N*-methyl-*N*-(2′-hydroxyethyl)-4-aminobenzaldehyde (330 mg, 1.85 m*M*), and $Na_2S_2O_5$ (351 mg, 1.85 m*M*) in DMF (8 mL) was refluxed for 2 h. Ice water (50 mL) was added, and the precipitate formed was collected by filtration, washed with water, and dried under vacuum to give **1** in quantitative yield. R_f (CH_2Cl_2:MeOH 95:5) = 0.14; ^{1}H NMR (CD_3OD, 300 MHz) δ 3.06 (s, 3H, $N\underline{CH_3}$), 3.57 (t, 2H, ^3J 5.5 Hz, $N\underline{CH_2}CH_2OH$), 3.77 (t, 2H, ^3J 5.5 Hz, $NCH_2\underline{CH_2}OH$), 6.87 (d, 2H, ^3J 7.5 Hz,

H3′and H5′), 7.18–7.21 (m, 2H, H5 and H6), 7.52–7.55 (m, 2H, H4 and H7), 7.93 (d, 2H, ^3J 7.5 Hz, H2′ and H6′); ^{13}C NMR (CD_3OD, 75 MHz) δ 39.31 (N$\underline{C}H_3$), 55.32 (CH_2), 60.21 (CH_2), 112.84 (2C, C_{arom}), 115.16 (2C,C_{arom}), 117.86 (2C,C_{arom}), 123.20 (2C, C_{arom}), 129.00 (2C, C_{quart}), 152.28 (C_{quart}), 154.50 (C_{quart}); ES$^+$ MS $C_{16}H_{17}N_3O$ (267.1) m/z 268.0 $[M+H]^+$; HRMS (EI +) found 267.13646, calcd for $C_{16}H_{17}N_3O$ 267.13661 $[M]^+$.

General procedure for the preparation of benzimidazole derivatives 15 and **16**. A mixture of 1,2-diaminebenzene (**12** or **13**) (1 m*M*) and *N*-methyl-*N*-(2-tosyloxyethyl)-4-aminobenzaldehyde (1 m*M*) in DMF (3 mL) was stirred at 50°C for 2 h. Then the solvent was concentrated under vacuum, and the residue was submitted to column chromatography on silica gel (CH_2Cl_2:CH_3OH 99:1) to afford compounds **15** or **16**.

2-[N-methyl-N-(2′-O-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (15). Yield: 63%; R_f (*n*-hexane/EtOAc 1:2) = 0.47; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.32 (s, 3H, CH_3), 2.87 (s, 3H, N$\underline{C}H_3$), 3.60 (t, 2H, ^3J 5.7 Hz, N$\underline{C}H_2CH_2$OTs), 4.14 (t, 2H, ^3J 5.7 Hz, NCH$_2\underline{C}H_2$OTs), 6.53 (d, 2H, ^3J 8.8 Hz, H3′ and H5′), 7.16–7.21 (m, 4H, H5, H6 and H3″and H5″), 7.54–7.57 (m, 2H, H4 and H7), 7.64 (d, 2H, ^3J 8.1 Hz, H2″ and H6″), 7.85 (d, 2H, ^3J 8.8 Hz, H2′ and H6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.58 ($\underline{C}H_3$), 38.94 (N$\underline{C}H_3$), 50.86 (CH_2), 66.84 (CH_2), 111.75 (2C, C_{arom}), 114.68 (2C, C_{arom}), 117.63 (C_{quart}), 122.37 (2C, C_{arom}), 127.75 (2C, C_{arom}), 127.85 (2C, C_{arom}), 129.83 (2C, C_{arom}), 132.39 (2C, C_{quart}), 145.06 (C_{quart}), 149.46 (C_{quart}), 152.29 (C_{quart}); ES$^+$ MS $C_{23}H_{23}N_3O_3S$ (421.1) m/z 422.0 $[M+H]^+$.

6-Bromo-2-[N-methyl-N-(2′-O-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (16). Yield: 42%; R_f (*n*-hexane/EtOAc 1:2) = 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.31 (s, 3H, CH_3), 2.83 (s, 3H, N$\underline{C}H_3$), 3.56 (t, 2H, ^3J 5.7 Hz, N$\underline{C}H_2CH_2$OTs), 4.11 (t, 2H, ^3J 5.7 Hz, NCH$_2\underline{C}H_2$OTs), 6.46 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.17–8. 21 (m, 4H, H5, H6 and H3″ and H5″), 7.36 (d, 1H, ^3J 8.4 Hz, H4), 7.61 (s, 1H, H7), 7.63 (d, 2H, ^3J 8.4 Hz, H2″ and H6″), 7.82 (d, 2H, ^3J 8.7 Hz, H2′ and H6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.60 (CH_3), 38.85 (N$\underline{C}H_3$), 50.82 (CH_2), 66.88 (CH_2), 111.67 (2C, C_{arom}), 115.45 (C_{arom}), 115.69 (C_{quart}), 117.20 (C_{quart}), 125.88 (C_{arom}), 127.76 (2C, C_{arom}), 128.25 (2C, C_{arom}), 129.89 (2C, C_{arom}), 132.38 (C_{quart}), 145.15 (C_{quart}), 150.07 (C_{quart}), 152.69 (C_{quart}); ES$^+$ MS $C_{23}H_{22}BrN_3O_3S$ (499.0 calcd for ^{81}Br) *m/z* 500.2 $[M+H]^+$.

General procedure for the fluorination of *N*-unsubstituted benzimidazole derivatives (2 and **3**). A solution of tosylate precursor (**15** or **16**) (1 m*M*) and anhydrous TBAF (1.5 m*M*) in anhydrous THF (40 mL) was stirred at 65°C for 25 min. Thereafter, the solvent was concentrated, and the crude product was taken up in CH_2Cl_2 (50 mL). The organic phase was extracted with sat sol $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was subjected to column chromatography on silica gel (*n*-hexane/EtOAc 1:1) to afford compounds **2** or **3**.

2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (2). Yield = 71%; R_f (*n*-hexane/EtOAc 1:1) = 0.24; m.p. = 230–235°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.06 (s, 3H, N$\underline{C}H_3$), 3.69 (dt, 2H, $^3J_{H,H}$ 4.8 Hz, $^3J_{H,F}$ 24.6 Hz, N$\underline{C}H_2CH_2$F), 4.60 (dt, 2H, $^3J_{H,H}$ 4.8 Hz, $^2J_{H,F}$ 47.1 Hz, NCH$_2\underline{C}H_2$F), 6.72 (d, 2H, ^3J 8.1 Hz, H3′ and H5′), 7.19–7.21 (m, 2H, H5 and H6), 5.57-7.60 (m, 2H, H4 and H7), 7.95 (d, 2H, ^3J 8.1 Hz, H2′ and H6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.04 (N$\underline{C}H_3$), 52.26 (d, $J_{C,F}$ 20.85 Hz, N$\underline{C}H_2CH_2$F), 81.42 (d, $J_{C,F}$ 169.2 Hz, NCH$_2\underline{C}H_2$F), 110.90 (C_{quart}), 111.91 (2C, C_{arom}), 115.10 (C_{arom}), 115.67 (C_{arom}), 117.21 (C_{arom}), 128.10 (2C, C_{arom}), 150.60 (C_{quart}), 153.05 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz) -222.642 (m); ES$^+$ MS $C_{16}H_{16}FN_3$ (269.1) m/z 270.0 $[M+H]^+$; HRMS (EI+) found 269.13212, calcd for $C_{16}H_{16}FN_3$ 269.13228 [M]+; Anal. calcd. for $C_{16}H_{16}FN_3.0.4H_2O$: C 69.50, H 6.12, N 15.20; found C 69.09, H 7.84, N 15.53.

6-Bromo-2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (3). Yield = 59%; R_f (*n*-hexane/EtOAc 1:1) = 0.37; m.p. = 207–210°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02 (s, 3H, N$\underline{C}H_3$), 3.63 (dt, 2H, $^3J_{H,H}$ 4.8 Hz, $^3J_{H,F}$ 24.6 Hz, N$\underline{C}H_2CH_2$F), 4.56 (dt, 2H, $^3J_{H,H}$ 4.8 Hz, $^2J_{H,F}$ 47.1 Hz, NCH$_2\underline{C}H_2$F), 6.65 (d, 2H, ^3J 8.4 Hz, H3′ and H5′), 7.26 (d, 1H, ^3J 8.4 Hz, H5), 7.38 (d, ^3J 8.4 Hz, H4), 7.65 (s, 1H, H7), 7.90 (d, 2H, ^3J 8.4 Hz, H2′ and H6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.04 (N$\underline{C}H_3$), 52.26 (d, $J_{C,F}$ 20.85 Hz, N$\underline{C}H_2CH_2$F), 81.58 (d, $J_{C,F}$ 169.2 Hz, NCH$_2\underline{C}H_2$F), 111.78 (C_{arom}), 111.91 (2C, C_{arom}), 115.59 (C_{arom}), 115.77 (C_{quart}), 117.31 (C_{quart}), 125.80 (C_{arom}), 128.25 (2C, C_{arom}), 150.65 (C_{quart}), 152.94 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -222.81$ (m); ES$^+$ MS $C_{16}H_{15}BrFN_3$ (347.0 calcd for ^{79}Br) *m/z* 348.1 $[M+H]^+$; Anal. calcd. for $C_{16}H_{15}BrFN_3$: C 55.19, H 4.34, N 12.07; found C 55.20, H 4.91, N 11.68.

General procedure for the preparation of benzimidazole derivatives 17 and **19**. Compounds **17** and **19** were obtained as previously described [22]. Briefly a mixture of 1,2-diaminebenzene (**12** or **14**) (1 m*M*), *N*-methyl-*N*-((2′-*O*-*tert*-butylcarbonate)ethyl)-4-aminobenzaldehyde (1 m*M*), and $Na_2S_2O_5$ (1 m*M*) in DMF (3 mL) was refluxed. After 2 h iced water was added and the formed precipitate filtered. Then, the filtrate was redissolved in MeOH, the solvent was concentrated, and the residue was submitted to column chromatography on silica gel (*n*-hexane/EtOAc 1:1) to afford compounds **17** or **19**.

2-[N-methyl-N-(2′-O-tert-butylcarbonatethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (17). Yield: quantitative; R_f (*n*-hexane/EtOAc 1:2) = 0.66; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.43 (s, 9H, C($\underline{C}H_3$)$_3$), 3.10 (s, 3H, N$\underline{C}H_3$), 3.75 (t, 2H, ^3J 5.5 Hz, N$\underline{C}H_2CH_2$OBoc), 4.31 (t, 2H, ^3J 5.5 Hz, NCH$_2\underline{C}H_2$OBoc), 6.93 (d, 2H, ^3J 9.0 Hz, H3′and H5′),

7.21–7.24 (m, 2H, H5 and H6), 7.57 (b, 2H, H4 and H7), 7.97 (d, 2H, ^3J 9.0 Hz, H2′and H6′); ^{13}C NMR (CD_3OD, 75 MHz) δ 27.92 (C($\underline{CH_3}$)$_3$), 38.89 (N$\underline{CH_3}$), 51.75 (CH_2), 65.09 (CH_2), 82.93 ($\underline{C}(CH_3)_3$), 112.99 (2C, C_{arom}), 118.30 (2C, C_{arom}), 123.24 (2C, C_{arom}), 129.02 (2C, C_{arom}), 151.99 (C_{quart}), 154.40 (C_{quart}), 155.05 (C=O); ES$^+$ MS $C_{21}H_{25}N_3O_3$ (367.2) m/z 368.0 $[M+H]^+$; HRMS (EI+) found 367.18874, calcd for $C_{21}H_{25}N_3O_3$ 367.18904 $[M]^+$.

5-Iodo-2-[N-methyl-N-(2′-O-tert-butylcarbonatethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (19). Yield = 77%; R_f (*n*-hexane/EtOAc 1:1) = 0.52; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H, C($\underline{CH_3}$)$_3$), 3.00 (s, 3H, N$\underline{CH_3}$), 3.62 (t, 2H, ^3J 6.0 Hz, N$\underline{CH_2}CH_2$OBoc), 4.20 (t, 2H, ^3J 6.0 Hz, NCH$_2\underline{CH_2}$OBoc), 6.67 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.31 (d, 1H, ^3J 8.4 Hz, H7), 7.45 (dd, 1H, ^4J 1.5 Hz, ^3J 8.4 Hz, H6), 7.86 (d, 2H, ^3J 9.0 Hz, H2′ and H6′), 7.87 (d, 1H, ^4J 1.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.68 (3C, C($\underline{CH_3}$)$_3$), 38.73 (N$\underline{CH_3}$), 50.71 (CH_2), 63.45 (CH_2), 82.56 ($\underline{C}(CH_3)_3$), 85.89 (C_{quart}), 111.70 (2C, C_{arom}), 114.88 (C_{arom}), 123.08 (C_{arom}), 128.34 (2C, C_{arom}), 131.57 (C_{quart}), 150.70 (C_{quart}), 152.29 (C_{quart}), 153.30 (C=O); ES$^+$ MS $C_{21}H_{24}IN_3O_3$ (493.1) *m/z* 494.2 $[M+H]^+$.

1-Methyl-2-[N-methyl-N-(2′-O-tert-butylcarbonatethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (20). To a solution of NaOH (230 mg, 5.6 m*M*) in water (0.5 mL) were added **17** (300 mg, 0.8 m*M*), DMF (1 mL), acetone (4 mL), and methyl iodide (60 μL, 0.97 m*M*), dropwise and using a cooling bath of ice water. The reaction mixture was stirred for 2 h at RT. Then, the solvents were evaporated, the reaction mixture was diluted with water (100 mL), and was extracted with CH_2Cl_2 (100 mL). The organic phase was dried over Na_2SO_4, filtered, and the filtrate was dried under vacuum to afford **20** (256 mg, 83%). R_f (CH_2Cl_2/MeOH 100:2) = 0.57; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 9H, C($\underline{CH_3}$)$_3$), 3.04 (s, 3H, N$\underline{CH_3}$), 3.66 (t, 2H, ^3J 6.3 Hz, N$\underline{CH_2}CH_2$OBoc), 3.84 (s, 3H, N$\underline{CH_3}$), 4.23 (t, 2H, ^3J 6.3 Hz, NCH$_2\underline{CH_2}$OBoc), 6.79 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.23–7.28 (m, 2H, H5 and H6), 7.32–7.35 (m, 1H, H7), 7.64 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.75–7.77 (m, 1H, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.65 (3C, C($\underline{CH_3}$)$_3$), 31.74 (N$\underline{CH_3}$), 38.62 (N$\underline{CH_3}$), 50.78 (CH_2), 63.45 (CH_2), 82.31 ($\underline{C}(CH_3)_3$), 109.25 (2C, C_{arom}), 111.53 (2C, C_{arom}), 117.60 (C_{quart}), 119.20 (C_{arom}), 122.05 (C_{arom}), 130.53 (2C, C_{arom}), 136.60 (C_{quart}), 142.91 (C_{quart}), 149.66 (C_{quart}), 153.33 (C_{quart}), 154.39 (C=O); ES$^+$ MS $C_{22}H_{27}N_3O_3$ (381.2) m/z 382.0 $[M+H]^+$.

Synthesis of the *N*-methylated regioisomers 23 and 24 [22]. To a solution of NaOH (280 mg, 7 m*M*) in water (0.5 mL) were added **19** (390 mg, 0.8 m*M*), acetone (4 mL), and methyl iodide (60 μL, 0.95 m*M*), dropwise and using a cooling ice water bath. The reaction mixture was stirred for 2 h 30 min at RT. Then, the solvents were evaporated, the reaction mixture was diluted with water (50 mL) and was extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was dried over Na_2SO_4, filtered, and the filtrate was concentrated under vacuum to give a mixture of regioisomers **23** and **24** (378 mg, 93%), in an approximate 1:1 M ratio. The regioisomers were separated by column chromatography on silica gel (*n*-hexane/EtOAc 2:1).

6-Iodo-1-methyl-2-[N-methyl-N-(2′-O-tert-butylcarbonatethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (23). R_f (*n*-hexane/EtOAc 1:1) = 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H, C($\underline{CH_3}$)$_3$), 3.05 (s, 3H, N$\underline{CH_3}$), 3.67 (t, 2H, ^3J 6.3 Hz, N$\underline{CH_2}CH_2$OBoc), 3.81 (s, 3H, NCH$_3$), 4.24 (t, 2H, ^3J 6.3 Hz, NCH$_2\underline{CH_2}$OBoc), 6.80 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.52 (s, 2H, H4 and H5), 7.63 (d, 2H, ^3J 9.0 Hz, H2′ and H6′), 7.66 (s, 1H, H7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.71 (3C, C($\underline{CH_3}$)$_3$), 31.96 (NCH$_3$), 38.70 (NCH^3), 50.82 (CH_2), 63.45 (CH_2), 82.42 ($\underline{C}(CH_3)_3$), 85.23 (C_{quart}), 104.73 (C_{arom}), 111.62 (2C, C_{arom}), 118.45 (C_{quart}), 120.90 (C_{arom}), 130.63 (2C, C_{arom}), 131.12 (C_{quart}), 142.27 (C_{quart}), 149.96 (C_{quart}), 153.37 (C_{quart}); ES$^+$ MS $C_{22}H_{26}IN_3O_3$ (507.1) *m/z* 508.2 $[M+H]^+$.

5-Iodo-1-methyl-2-[N-methyl-N-(2′-O-tert-butylcarbonatethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (24). R_f (*n*-hexane/EtOAc 1:1) = 0.72; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 9H, C($\underline{CH_3}$)$_3$), 3.04 (s, 3H, N$\underline{CH_3}$), 3.66 (t, 2H, ^3J 6.3 Hz, N$\underline{CH_2}CH_2$OBoc), 3.80 (s, 3H, NCH$_3$), 4.23 (t, 2H, ^3J 6.3 Hz, NCH$_2\underline{CH_2}$OBoc), 6.79 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.08 (d, 1H, ^3J 8.5 Hz, H7), 7.50 (dd, 1H, ^4J 1.8 Hz and ^3J 8.5 Hz, H6), 7.61 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 8.08 (d, 1H, ^4J 1.8 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.67 (3C, C($\underline{CH_3}$)$_3$), 31.92 (NCH$_3$), 38.68 (NCH$_3$), 50.75 (CH_2), 63.41 (CH_2), 82.40 ($\underline{C}(CH_3)_3$), 85.26 (C_{quart}), 111.08 (C_{arom}), 111.56 (2C, C_{arom}), 116.68 (C_{arom}), 128.01 (C_{quart}), 130.61 (2C, C_{arom}), 136.09 (C_{arom}), 144.54 (C_{quart}), 149.90 (C_{quart}), 153.33 (C_{quart}), 154.98 (C=O); ES$^+$ MS $C_{22}H_{26}IN_3O_3$ (507.1) *m/z* 508.2 $[M+H]^+$.

General procedure for the hydrolysis of *t*-Boc group: synthesis of 4 and **25–28**. A solution of **20–24** (1 m*M*) in CH_2Cl_2 (8 mL) and TFA (2 mL) was stirred at RT for 1 h. Then, the solvents were concentrated under vacuum. The residue was taken up in CH_2Cl_2 (100 mL), and NEt_3 was added until neutral pH. The organic phase was extracted with sat sol $NaHCO_3$ (100 mL), was dried over Na_2SO_4, was filtered, and the filtrate was concentrated. The crude product was subjected to column chromatography on silica gel (CH_2Cl_2/MeOH 94:6) to afford the title compounds.

1-Methyl-2-[N-methyl-N-(2′-hydroxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (4). Yield = 86%; R_f (CH_2Cl_2/MeOH 94:6) = 0.45; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.03 (s, 3H, N$\underline{CH_3}$), 3.54 (t, 2H, ^3J 6.0 Hz, N$\underline{CH_2}CH_2$OH), 3.82 (t, 2H, ^3J 6.0 Hz, NCH$_2\underline{CH_2}$OH), 3.83 (s, 3H, NCH$_3$), 6.77 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.26–7.31 (m, 2H, H5 and H6), 7.32–7.36 (m, 1H, H7), 7.59 (d, 2H,

^3J 8.7 Hz, H2′ and H6′), 7.7–7.8 (m, 1H, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.77 (NCH_3), 38.74 (NCH_3), 54.75 (CH_2), 59.41 (CH_2), 109.33 (C_{arom}), 111.67 (2C, C_{arom}), 116.82 (C_{arom}), 118.94 (C_{quart}), 122.14 (C_{arom}), 122.20 (C_{arom}), 130.44 (2C, C_{arom}), 136.47 (C_{quart}), 142.60 (C_{quart}), 150.49 (C_{quart}), 154.51 (C_{quart}); ES$^+$ MS $C_{17}H_{19}N_3O$ (281.2) m/z 282.0 $[M+H]^+$; Anal. calcd. for $C_{17}H_{19}N_3O$ C 72.57, H 6.81, N 14.94; found C 72.02, H 7.66, N 14.99.

6-Bromo-1-methyl-2-[N-methyl-N-(2′-hydroxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (25). Yield = 74%; R_f (*n*-hexane/EtOAc 1:2) = 0.13; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.04 (s, 3H, $N\underline{CH_3}$), 3.56 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OH$), 3.81 (s, 3H, NCH_3), 3.84 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OH$), 6.82 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.36 (dd, 1H, ^4J 1.8 Hz, ^3J 8.4 Hz, H5), 7.49 (d, 1H, ^4J 1.8 Hz, H7), 7.61 (d, 2H, ^3J 9.0 Hz, H2′ and H6′), 7.62 (d, 1H, ^3J 8.4 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 32.11 (NCH_3), 38.84 (NCH_3), 54.67 (CH_2), 60.08 (CH_2), 104.75 (C_{arom}), 111.99 (2C, C_{arom}), 119.99, 125.94 (C_{arom}), 130.62 (2C, C_{arom}), 150.98 (C_{quart}); ES$^+$ MS $C_{17}H_{18}BrN_3O$ (359.1 calcd for ^{79}Br) *m/z* 360.2 $[M+H]^+$.

5-Bromo-1-methyl-2-[N-methyl-N-(2′-hydroxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (26). Yield = 93%; R_f (*n*-hexane/EtOAc 1:2) = 0.18; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.04 (s, 3H, $N\underline{CH_3}$), 3.55 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OH$), 3.82 (s, 3H, NCH_3), 3.83 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OH$), 6.82 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.19 (d, 1H; ^3J 8.7 Hz, H7), 7.35 (dd, 1H, ^4J 1.8 Hz and ^3J 8.7 Hz, H6), 7.61 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.9 (d, 1H, ^4J 1.8 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 32.07 (NCH_3), 38.87 (NCH_3), 54.69 (CH_2), 60.03 (CH_2), 110.62 (C_{arom}), 111.95 (2C, C_{arom}), 115.48 (C_{arom}), 121.68 (C_{arom}), 125.36 (C_{quart}), 130.64 (2C, C_{arom}), 135.35 (C_{quart}), 150.92 (C_{quart}), 155.18 (C_{quart}); ES$^+$ MS $C_{17}H_{18}BrN_3O$ (359.1 calcd for ^{79}Br) *m/z* 360.2 $[M+H]^+$; HRMS (EI+) found 359.06264, calcd for $C_{17}H_{18}BrN_3O$ 359.06277 [M]+.

6-Iodo-1-methyl-2-[N-methyl-N-(2′-hydroxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (27). Yield = quantitative; R_f (*n*-hexane/EtOAc 1:2) = 0.14; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.96 (bs, 1H), 3.05 (s, 3H, $N\underline{CH_3}$), 3.56 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OH$), 3.81 (s, 3H, NCH_3), 3.84 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OH$), 6.83 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.54 (s, 2H, H4 and H5), 7.63 (d, 2H, ^3J 9.0Hz, H2′ and H6′), 7.68 (s, 1H, H7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 32.08 (NCH_3), 38.87 (NCH_3), 54.67 (CH_2), 60.07 (CH_2), 111.97 (2C, C_{arom}), 118.58 (C_{arom}), 120.51 (C_{quart}), 130.67 (2C, C_{arom}), 131.49 (C_{arom}), 135.54 (C_{quart}), 147.17 (C_{quart}), 150.95 (C_{quart}); ES$^+$ MS $C_{17}H_{18}IN_3O$ (407.0) *m/z* 407.9 $[M+H]^+$.

5-Iodo-1-methyl-2-[N-methyl-N-(2′-hydroxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (28). Yield = quantitative; R_f (*n*-hexane/EtOAc 1:2) = 0.16; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.90 (bs, 1H), 3.05 (s, 3H, $N\underline{CH_3}$), 3.56 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OH$), 3.84–3.86 (m, 5H, NCH_3 and $NCH_2\underline{CH_2}OH$), 6.83 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.11 (d, 1H ^3J 8.4 Hz, H7), 7.54 (d, 1H, ^3J 8.4 Hz, H6), 7.65 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 8.13 (s, 1H, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 32.17 (NCH_3), 38.89 (NCH_3), 54.64 (CH_2), 60.07 (CH_2), 111.29 (C_{arom}), 111.79 (C_{quart}), 112.00 (2C, C_{arom}), 127.38 (C_{arom}), 130.78 (2C, C_{arom}), 131.27 (C_{arom}); ES$^+$ MS $C_{17}H_{18}IN_3O$ (407.0) *m/z* 408.0 $[M+H]^+$.

General procedure for the *O*-tosylation of *N*-methylbenzimidazole derivatives: synthesis of 29–33. A solution of **4/25-28** (1 m*M*), NEt_3 (0.5 mL), and *p*-TsCl (3 mmol) in CH_2Cl_2 (12 mL) was stirred at RT for 4 h. Thereafter, the reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic phase was extracted with a saturated solution of $NaHCO_3$ (100 mL). The organic extract was dried over Na_2SO_4, filtered, and the filtrate was concentrated. The residue was subjected to column chromatography on silica gel (CH_2Cl_2:MeOH 98:2) to afford the title compounds.

1-Methyl-2-[N-methyl-N-(2′-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (29). Yield = 80%; R_f CH_2Cl_2:MeOH 98:2) = 0.45; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.40 (s, 3H, CH_3), 2.98 (s, 3H, $N\underline{CH_3}$), 3.70 (t, 2H, ^3J 6.0 Hz, $N\underline{CH_2}CH_2OTs$), 3.85 (s, 3H, NCH_3), 4.21 (t, 2H, ^3J 6.0 Hz, $NCH_2\underline{CH_2}OTs$), 6.69 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.26–7.30 (m, 4H, H5, H6, H3″ and H5″), 7.35–7.38 (m, 1H, H7), 7.63 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.73 (d, 2H, ^3J 8.4 Hz, H2″ and H6″), 7.78–7.81 (m, 1H, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.65 (CH_3), 31.84 (NCH_3), 39.00 (NCH_3), 51.03 (CH_2), 66.73 (CH_2), 109.37 (2C, C_{arom}), 111.59 (2C, C_{arom}), 119.20 (C_{quart}), 122.31 (2C, C_{arom}), 127.82 (2C, C_{arom}), 129.87 (2C, C_{arom}), 130.61 (2C, C_{arom}), 132.59 (C_{quart}), 136.50 (C_{quart}), 145.04 (C_{quart}), 149.14 (C_{quart}), 154.12 (C_{quart}); ES$^+$ MS $C_{24}H_{25}N_3O_3S$ (435.2) m/z 436.0 $[M+H]^+$.

6-Bromo-1-methyl-2-[N-methyl-N-(2′-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (30). Yield = 54%; R_f (*n*-hexane/EtOAc 1:1) = 0.26; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.32 (s, 3H, CH_3), 2.89 (s, 3H, $N\underline{CH_3}$), 3.61 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OTs$), 3.74 (s, 3H, NCH_3), 4.12 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OTs$), 6.60 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.21 (d, 2H, ^3J 8.7 Hz, H3″ and H5″), 7.29 (dd, 1H, ^4J 1.8 Hz, ^3J 8.7 Hz, H5), 7.43 (d, 1H, ^4J 1.8 Hz, H7), 7.53 (d, 2H, ^3J 9.0 Hz, H2′ and H6′), 7.54 (d, 1H, ^3J 8.7 Hz, H4), 7.64 (d, 2H, ^3J 8.7 Hz, H2″ and H6″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.60 (CH_3), 31.93 (NCH_3), 38.92 (NCH_3), 50.97 (CH_2), 66.69 (CH_2), 111.58 (2C, C_{arom}), 112.50 (C_{arom}), 115.22 (C_{quart}), 117.25 (C_{arom}), 120.38 (C_{quart}), 125.43 (C_{arom}), 127.77 (2C, C_{arom}), 129.84 (2C, C_{arom}), 130.50 (2C, C_{arom}), 132.59 (C_{quart}), 137.70 (C_{quart}), 141.79 (C_{quart}), 145.01 (C_{quart}), 149.28 (C_{quart}), 154.94 (C_{quart}); ES$^+$ MS $C_{24}H_{24}BrN_3O_3S$ (513.1 calcd for ^{79}Br) *m/z* 514.2 $[M+H]^+$.

5-Bromo-1-methyl-2-[N-methyl-N-(2′-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (31). Yield = 75%; R_f (*n*-hexane/EtOAc 1:1) = 0.36; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H, CH_3), 2.97 (s, 3H, $N\underline{CH_3}$), 3.69 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OTs$), 3.84 (s, 3H, NCH_3), 4.19 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OTs$), 6.68 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.21 (d, 1H, ^3J 8.4 Hz, H7), 7.27 (d, 2H, ^3J 8.4 Hz, H3″ and H5″), 7.37 (dd, 1H, ^4J 1.5 Hz and ^3J 8.4 Hz, H6), 7.62 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.70 (d, 2H, ^3J 8.4 Hz, H2″ and H6″), 7.92 (d, 1H, ^4J 1.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.65 (CH_3), 32.08 (NCH_3), 38.99 (NCH_3), 51.02 (CH_2), 66.68 (CH_2), 110.65 (C_{quart}), 111.67 (2C, C_{arom}), 115.56 (C_{arom}), 121.76 (C_{quart}), 125.47 (C_{arom}), 127.82 (2C, C_{arom}), 129.90 (2C, C_{arom}), 130.71 (2C, C_{arom}), 132.61 (C_{quart}), 145.08 (C_{quart}); ES^+ MS $C_{24}H_{24}BrN_3O_3S$ (513.1 calcd for ^{81}Br) *m/z* 514.2 $[M+H]^+$.

6-Iodo-1-methyl-2-[N-methyl-N-(2′-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (32). Yield = 60%; R_f (*n*-hexane/EtOAc 1:1) = 0.33; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H, CH_3), 2.96 (s, 3H, $N\underline{CH_3}$), 3.69 (t, 2H, ^3J 5.8 Hz, $N\underline{CH_2}CH_2OTs$), 3.81 (s, 3H, NCH_3), 4.18 (t, 2H, ^3J 5.8 Hz, $NCH_2\underline{CH_2}OTs$), 6.67 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.27 (d, 2H, ^3J 8.1 Hz, H3″ and H5″), 7.54 (s, 2H, H4 and H5), 7.61 (d, 2H, ^3J 9.0 Hz, H2′ and H6′), 7.69 (s, 1H, H7), 7.70 (d, 2H, ^3J 8.1 Hz, H2″ and H6″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.90 (CH_3), 32.29 (NCH_3), 39.26 (NCH_3), 51.27 (CH_2), 66.95 (CH_2), 85.80 (C_{quart}), 111.91 (2C, C_{arom}), 118.83 (C_{arom}), 120.97 (C_{quart}), 128.08 (2C, C_{arom}), 130.15 (2C, C_{arom}), 130.94 (2C, C_{arom}), 131.67 (C_{arom}), 132.87 (C_{arom}), 149.74 (C_{quart}); ES^+ MS $C_{24}H_{24}IN_3O_3$ (561.1) *m/z* 562.2 $[M+H]^+$.

5-Iodo-1-methyl-2-[N-methyl-N-(2′-tosyloxyethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (33). Yield =41%; R_f (*n*-hexane/EtOAc 1:1) = 0.37; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H, CH_3), 2.96 (s, 3H, $N\underline{CH_3}$), 3.68 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OTs$), 3.81 (s, 3H, NCH_3), 4.18 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OTs$), 6.67 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.10 (d, 1H, ^3J 8.4 Hz, H7), 7.26 (d, 2H, ^3J 8.4 Hz, H3″ and H5″), 7.52 (dd, 1H, ^4J 1.5 Hz and ^3J 8.4 Hz, H6), 7.59 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.70 (d, 2H, ^3J 8.4 Hz, H2″ and H6″), 8.09 (d, 1H, ^4J 1.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.64 (CH_3), 31.96 (NCH_3), 38.98 (NCH_3), 51.00 (CH_2), 66.69 (CH_2), 85.41 (C_{quart}), 111.15 (C_{arom}), 111.62 (2C, C_{arom}), 127.80 (2C, C_{arom}), 128.04 (C_{arom}), 129.87 (2C, C_{arom}), 130.64 (2C, C_{arom}), 130.78 (C_{arom}), 132.58 (C_{quart}), 136.06 (C_{quart}), 145.05 (C_{quart}), 149.37 (C_{quart}), 154.75 (C_{quart}); ES^+ MS $C_{24}H_{24}IN_3O_3S$(561.1) *m/z* 562.2 $[M+H]^+$.

General procedure for the fluorination of *N*-methylbenzimidazole derivatives: synthesis of 5–9. A solution of **29–33** (1 m*M*) and anhydrous TBAF (5 m*M*) in anhydrous THF (40 mL) was refluxed for 10 min. Thereafter, the solvent was concentrated, and the crude was taken up in CH_2Cl_2 (50 mL). The organic phase was extracted with sat sol $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and the filtrate was concentrated. The resulting residue was subjected to column chromatography on silica gel (*n*-hexane/EtOAc 1:1).

1-Methyl-2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (5). Yield = 72%; R_f (*n*-hexane/EtOAc 1:1) = 0.25; m.p. = 118–120°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02 (s, 3H, $N\underline{CH_3}$), 3.65 (dt, 2H, $^3J_{H,H}$ 5.1 Hz, $^3J_{H,F}$ 24.3 Hz, $N\underline{CH_2}CH_2F$), 4.56 (dt, 2H, $^3J_{H,H}$ 5.1 Hz, $^2J_{H,F}$ 47.1 Hz, $NCH_2\underline{CH_2}F$), 6.73 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.18–7.22 (m, 2H, H5 and H6), 7.24–7.29 (m, 1H, H7), 7.59 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.70–7.73 (m, 1H, H4) Falta um $N\underline{CH_3}$; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.76 (NCH_3), 39.03 (NCH_3), 52.37 (d, $J_{C,F}$ 20.8 Hz, $N\underline{CH_2}CH_2F$), 81.65 (d, $J_{C,F}$ 169.2 Hz, $NCH_2\underline{CH_2}F$), 109.28 (C_{arom}), 111.70 (2C, C_{arom}), 117.84 (C_{arom}), 119.26 (C_{quart}), 122.08 (2C, C_{arom}), 130.57 (2C, C_{arom}), 136.63 (C_{quart}), 142.98 (C_{quart}), 149.66 (C_{quart}), 154.37 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz) $\delta = -222.681$ (m); ES^+ MS $C_{17}H_{18}FN_3$ (283.1) m/z 284.0 $[M+H]^+$; HRMS (EI+) found 283.14824, calcd for $C_{17}H_{18}N_3F$ 283.14793 [M]+; Anal. calcd. for $C_{17}H_{18}FN_30.3H_2O$: C 70.71, H 6.49, N 14.55; found C 71.08, H 6.87, N 14.28.

6-Bromo-1-methyl-2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (6). Yield = 87%; R_f (*n*-hexane/EtOAc 1:1) = 0.44; m.p. = 146–150°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.07 (s, 3H, $N\underline{CH_3}$), 3.71 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^3J_{H,F}$ 24.6 Hz, $N\underline{CH_2}CH_2F$), 3.80 (s, 3H, NCH_3), 4.62 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^2J_{H,F}$ 47.4 Hz, $NCH_2\underline{CH_2}F$), 6.77 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.34 (dd, 1H, ^4J 1.2 Hz and ^3J 8.4 Hz, H5), 7.47 (d, 1H, ^4J 1.2 Hz, H7), 7.60 (d, 1H, ^3J 8.4 Hz, H4), 7.63 (d, 2H, ^3J 8.7 Hz, H2′ and H6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.96 (NCH_3), 39.05 (NCH_3), 52.34 (d, $^2J_{C,F}$ 20.85 Hz, $N\underline{CH_2}CH_2F$), 81.63 (d, $^gJ_{C,F}$ 169.2 Hz, $NCH_2\underline{CH_2}F$), 111.70 (2C, C_{arom}), 112.49 (C_{quart}), 115.21 (C_{arom}), 116.93 (C_{arom}), 120.36 (C_{quart}), 125.44 (C_{arom}), 130.57 (2C, C_{arom}), 137.69 (C_{quart}), 141.71 (C_{quart}), 149.89 (C_{quart}), 155.05 (C_{quart}); ^{19}F ($CDCl_3$, 282 MHz) $\delta = -223.18$ (m); ES^+ MS $C_{17}H_{17}BrFN_3$ (361.1 calcd for ^{79}Br) *m/z* 362.1 $[M+H]^+$; Anal. calcd. for $C_{17}H_{17}BrFN_3$: C 56.36, H 4.73, N 11.60; found C 56.45, H 5.33, N 11.26.

5-Bromo-1-methyl-2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (7). Yield = 75%; R_f (*n*-hexane/EtOAc 1:1) = 0.55; m.p. = 154–156°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.08 (s, 3H, $N\underline{CH_3}$), 3.72 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^3J_{H,F}$ 24.6 Hz, $N\underline{CH_2}CH_2F$), 3.84 (s, 3H, NCH_3), 4.62 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^2J_{H,F}$ 47.1 Hz, $NCH_2\underline{CH_2}F$), 6.79 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.20 (d, 1H, ^3J 8.7 Hz, H7), 7.36 (dd, 1H, ^4J 1.8 Hz and ^3J 8.7 Hz, H6), 7.65 (d, 2H, ^3J 8.7 H2′ and H6′), 7.90 (d, 1H, ^4J 1.8 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 32.00 (NCH_3), 39.06 (NCH_3), 52.36 (d, $^2J_{C,F}$ 20.85 Hz,

$N\underline{CH_2}CH_2F$), 81.63 Hz (d, $^1J_{C,F}$ 169.2Hz, $NCH_2\underline{CH_2}F$), 110.54 (C_{quart}), 111.74 (2C, C_{arom}), 115.22 (C_{arom}), 121.90 (C_{arom}), 125.15 (C_{quart}), 130.65 (2C, C_{arom}), 135.52 (C_{quart}), 149.98 (C_{quart}), 155.27 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -223.00$ (m); ES$^+$ MS $C_{17}H_{17}BrFN_3$ (361.1 calcd for ^{79}Br) *m/z* 362.2 $[M+H]^+$; Anal. calcd. for $C_{17}H_{17}BrFN_3$: C 56.37, H 4.73, N 11.60; found C 56.28, H 5.31, N 11.36.

6-Iodo-1-methyl-2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (8). Yield = 65%; R_f (*n*-hexane/EtOAc 1:1) = 0.47; m.p. = 137–140°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.08 (s, 3H, $N\underline{CH_3}$), 3.71 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^3J_{H,F}$ 24.3 Hz, $N\underline{CH_2}CH_2F$), 3.80 (s, 3H, NCH_3), 4.62 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^2J_{H,F}$ 47.1 Hz, $NCH_2\underline{CH_2}F$), 6.78 (d, 2H, ^3J 9.0 Hz, H3′ and H5′), 7.52 (s, 2H, H4 and H5), 7.64 (d, 2H, ^3J 9.0 Hz, H2′ and H6′), 7.67 (s, 1H, H7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.95 (NCH_3), 39.07 (NCH_3), 52.36 (d, $^2J_{C,F}$ 21.4 Hz, $N\underline{CH_2}CH_2F$), 81.63 (d, $^1J_{C,F}$ 169.6 Hz, $NCH_2\underline{CH_2}F$), 85.27 (C_{quart}), 111.73 (2C, C_{arom}), 118.46 (C_{arom}), 120.90 (C_{quart}), 130.63 (2C, C_{arom}), 131.13 (C_{arom}), 138.21 (C_{arom}), 142.32 (C_{quart}), 142.79 (C_{quart}), 149.93 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -222.64$; ES$^+$ MS $C_{17}H_{17}FIN_3$ (409.0) *m/z* 409.9 $[M+H]^+$; HRMS (EI+) found 409.04447, calcd for $C_{17}H_{17}FIN_3$ 409.04457 [M] + Anal. calcd. for $C_{17}H_{17}FIN_30{,}15H_2O$: C 49.35, H 4.26, N 10.16; found C 49.31, H 4.75, N 9.87.

5-Iodo-1-methyl-2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-1H-benzo[d]imidazole (9). Yield = 49%; R_f (*n*-hexane/EtOAc 1:1) = 0–57; m.p. = 147–149°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.08 (s, 3H, $N\underline{CH_3}$), 3.71 (dt, 2H, $^3J_{H,H}$ 5.2 Hz, $^3J_{H,F}$ 24.6 Hz, $N\underline{CH_2}CH_2F$), 3.81 (s, 3H, NCH_3), 4.62 (dt, 2H, $^3J_{H,H}$ 5.2 Hz, $^2J_{H,F}$ 47.1 Hz, $NCH_2\underline{CH_2}F$), 6.78 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.08 (d, 1H, ^3J 8.4 Hz, H7), 7.51 (dd, 1H, ^4J 1.5 Hz, ^3J 8.4 Hz, H6), 7.63 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 8.09 (d, 1H, ^4J 1.5 Hz, H4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.92 (NCH_3), 39.06 (NCH_3), 52.35 (d, $J_{C,F}$ 20.8 Hz, $N\underline{CH_2}CH_2F$), 81.62 (d, $^2J_{C,F}$ 169.2 Hz, $NCH_2\underline{CH_2}F$), 85.29 (C_{quart}), 111.09 (C_{arom}), 111.73 (2C, C_{arom}), 128.08 (C_{arom}), 130.67 (2C, C_{arom}), 136.13 (C_{quart}), 149.93 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -222.73$ (m); ES$^+$ MS $C_{17}H_{17}FIN_3$ (409.0) *m/z* 409.9 $[M+H]^+$; HRMS (EI+) found 409.04446, calcd for $C_{17}H_{17}FIN_3$ 409.04457 [M]+; Anal. calcd. for $C_{17}H_{17}FIN_3$: C 48.89, H 4.19, N 10.27; found C 49.17, H 4.61, N 9.87.

2-[N-methyl-N-(2′-hydroxyethyl)-4′-aminophenyl]-benzothiazole (10). A solution of *o*-aminothiophenol (**34**) (550 mg, 4.4 m*M*) and *N*-methyl-*N*-(2-tosyloxyethyl)-4-aminobenzaldehyde (650 mg, 3.6 m*M*) in pyridine (6 mL) was refluxed for 32 h. After cooling, the reaction mixture was acidified by addition of 2 M HCl. The resulting precipitate was filtered off and dried under vacuum to afford **10** (805 mg, 79%) - R_f (EtOAc/*n*-hexane 1:1) = 0.24; m.p. = 185–187°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.78 (bs, 1H, OH), 3.07 (s, 3H, $N\underline{CH_3}$), 3.58 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OH$), 3.86 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OH$), 6.79 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.29 (dd, 1H, ^3J 8.1 Hz, ^3J 8.1 Hz, H5), 7.42 (dd, 1H, ^3J 8.1 Hz, ^3J 8.1 Hz, H6), 7.82 (d, 1H, ^3J 8.1 Hz, H4), 7.94 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.98 (d, 1H, ^3J 8.1 Hz, H7); ^{13}C ($CDCl_3$, 75 MHz) δ 39.05 (NCH_3), 54.67 (CH_2), 60.17 (CH_2), 104.72 (C_{quart}), 111.98 (2C, C_{arom}), 121.37 (C_{arom}), 122.15 (C_{arom}), 124.38 (C_{arom}), 126.14 (C_{arom}), 129.07 (2C, C_{arom}); ES$^+$ MS $C_{16}H_{16}N_2OS$ (284.1) *m/z* 284.9 $[M]^+$; Anal. calcd. for $C_{16}H_{16}N_2OS$: C 67.58, H 5.67, N 9.85, S 11.28; found C 67.17, H 6.26, N 9.74, S 11.41.

2-[N-methyl-N-(2′-O-tosyloxyethyl)-4′-aminophenyl]-benzothiazole (35). As described for the synthesis and purification of **20** – Yield = 96%; R_f (*n*-hexane/EtOAc 1:1) =0.42; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.41 (s, 3H, CH_3), 3.02 (s, 3H, $N\underline{CH_3}$), 3.74 (t, 2H, ^3J 5.7 Hz, $N\underline{CH_2}CH_2OTs$), 4.25 (t, 2H, ^3J 5.7 Hz, $NCH_2\underline{CH_2}OTs$), 6.63 (d, 2H, ^3J 8.5 Hz, H3′ and H5′), 7.28 (d, 2H, ^3J 7.8 Hz, H3″ and H5″), 7.37 (dd, 1H, ^3J 7.5 Hz, ^3J 7.5 Hz, H5), 7.50 (dd, 1H, ^3J 7.5 Hz, ^3J 8.1 Hz, H6), 7.73 (d, 2H, ^3J 7.8 Hz, H2″), 7.84 (d, 2H, ^3J 8.5 Hz, H2′ and H6′), 7.90 (d, 1H, ^3J 7.5 Hz, H4), 8.04 (d, 1H, ^3J 8.1 Hz, H7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.62 (CH_3), 39.20 (NCH_3), 50.90 (CH_2), 66.60 (CH_2), 111.55 (2C, C_{arom}), 121.39 (C_{arom}), 122.22 (C_{arom}), 124.45 (C_{arom}), 126.17 (C_{arom}), 127.77 (2C, C_{arom}), 128.98 (2C, C_{arom}), 129.82 (2C, C_{arom}); ES$^+$ MS $C_{23}H_{22}N_2O_3S_2$ (438.1) *m/z* 439.0 $[M+H]^+$.

2-[N-methyl-N-(2′-fluoroethyl)-4′-aminophenyl]-benzothiazole (11). As described for the synthesis and purification of **5** – Yield = 87%; R_f (*n*-hexane/EtOAc 4:1) = 0.37; m.p. = 138–140°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.09 (s, 3H, $N\underline{CH_3}$), 3.72 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^3J_{H,F}$ 24.3 Hz, $N\underline{CH_2}CH_2F$), 4.62 (dt, 2H, $^3J_{H,H}$ 5.1 Hz and $^2J_{H,F}$ 47.1 Hz, $NCH_2\underline{CH_2}F$), 6.74 (d, 2H, ^3J 8.7 Hz, H3′ and H5′), 7.29 (dd, 1H, ^3J 7.2 Hz and ^3J 7.2 Hz, H5), 7.42 (dd, 1H, ^3J 7.2 Hz and ^3J 7.8 Hz, H6), 7.82 (d, 1H, ^3J 7.2 Hz, H4), 7.95 (d, 2H, ^3J 8.7 Hz, H2′ and H6′), 7.97 (d, 1H, ^3J 7.8 Hz, H7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.17 (NCH_3), 52.33 (d, $^2J_{C,F}$ 20.8 Hz, $N\underline{CH_2}CH_2F$), 81.61 (d, $^1J_{C,F}$ 169.7 Hz, $NCH_2\underline{CH_2}F$), 111.70 (2C, C_{arom}), 121.36 (C_{quart}), 122.29 (2C, C_{arom}), 124.31 (C_{arom}), 126.04 (C_{arom}), 129.04 (2C, C_{arom}), 134.34 (C_{quart}), 150.81 (C_{quart}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -222.77$; ES$^+$ MS $C_{16}H_{15}FN_2S$ (286.1) *m/z* 287.0 $[M+H]^+$; Anal. calcd. for $C_{16}H_{15}FN_2S$: C 67.11, H 5.28, N 11.20, S 11.20; found C 66.87, H 5.90, N 9.69, S 11.31.

Radiochemistry. No-carrier-added aqueous [^{18}F] fluoride was produced in a CYCLONE 18/9 cyclotron (IBA) by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction. Resolubilization of the aqueous [^{18}F] fluoride (0.8–1.0 GBq) was accomplished with Kryptofix® 2.2.2 and K_2CO_3 in a conical vial and

azeotropically removing water with acetonitrile in a stream of nitrogen [29]. Finally, the dried [^{18}F]KF was resolubilized in 500 μL of anhydrous acetonitrile and added to **15** (4.1 mg) in a conical glass vial. The vial was sealed and heated for 20 min at 100°C in an oil bath. After cooling, the mixture was subjected to semi-preparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5μm) using an isocratic eluent of 40/60 acetonintrile/water(0.1% NEt_3, pH = 10.2) with a flow rate of 6 mL/min originated by a Knauer K-501 pump. The products were monitored by UV detector (Knauer K-200) at 254 nm and by gamma detection with a scintillation detector (Bioscan Reform).

The radiolabeled product [^{18}**F**]**2** eluting at 10–11 min was collected, diluted with 30 mL of water, and the whole solution applied to a C18 cartridge. The cartridge was washed with 5 mL of water and the radiolabeled product [^{18}**F**]**2** eluted with 0.4 mL of ethanol and reconstituted with 1.6 mL of E153 electrolyte infusion solution. This solution was used for the biodistribution studies.

HPLC analyses of the radiolabeled product [^{18}**F**]**2** were performed using an Agilent 1200 Series system (Agilent Technologies, USA) equipped with a multi-wavelength UV detector and a GABIstar NaI(Tl) radiometric detector (Raytest Isotopenmessgeraete GmbH, Straubenhardt, Germany) using an Agilent Zorbax Eclipse XDB C18 column, 5 μm, 4.6 × 150 mm and the indicated isocratic 40/60 acetonitrile/water (0.1% NEt_3, pH = 10.2) with a flow rate of 1.0 mL/min.

Cytotoxicity studies. *Cell culture growth conditions*: The human cancer cell lines MCF7 breast, A375 melanoma, HeLa cervical, and U87 glioblastoma (American Type Culture Collection, ATCC) were used in this study. The cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) with Glutamax I (Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics (Invitrogen). Cells were maintained in flasks at 37°C in a 5%CO_2 incubator (Heraus, Germany) in a humidified atmosphere. For the assays, cells in exponential growth were detached with trypsin–EDTA, suspended in medium and seeded in 96-well plates at a density of between 1–3 × 10^4cells/well.

Cytotoxicity assays: For evaluation of cellular viability, cells were treated with selected concentrations of the compounds previously dissolved in DMSO (final concentration < 1%) diluted in medium and incubated for 48 h at 37°C, 5% CO_2. Analysis of cell survival was carried out by the MTT [MTT = [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay at 37 °C 48 h incubation. After incubation, the medium was discarded, and a solution of MTT dissolved in PBS (0.5 mg/mL) was added to each well (200 μL) and the plates incubated at 37°C for another 3–4 h. Then, 200 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm with a plate spectrophotometer (Power Wave Xs, Bio-TeK). Each experiment was repeated at least two times, and each concentration was tested in at least six replicates. Results are expressed as the percentage of cellular viability with respect to control wells (non-treated cells). The IC_{50} values were calculated from plots for cell survival (%) versus compound concentration with the GraphPad Prism software (version 4.0).

Biodistribution. Animal studies were carried out in conformity with the national law and with the EU Guidelines for Animal Care and Ethics in Animal Experimentation. The animals were housed in a temperature and humidity-controlled room with a 12 h light/dark schedule.

The biodistribution of the [^{18}**F**]**2** was studied in groups of four female CD-1 mice (randomly bred, Charles River) weighting approximately 30–35 g each. Animals were intravenously injected with 100 μL (0.7–1.7 MBq) of each preparation via the tail vein and maintained on normal diet *ad libitum*. At 5 min and 1 h after administration, each mice group was sacrificed by cervical dislocation. The radioactive dosage administered in the animal was measured in a dose calibrator (*Capintec CRC25R*). Blood samples were taken by cardiac puncture at sacrifice. Tissue samples of the main organs were removed, weighted, and counted in a well counter (*Capintec CRC-55tW*). Accumulation of radioactivity in the tissues was calculated and expressed as percentage of the injected radioactivity per gram of organ (% I.A./g) (Table 2).

Acknowledgments. The authors thank the Fundação para a Ciência e a Tecnologia (FCT) for the "Ciência 2008" program (GRM) and for the grants SFRH/BPD/80758/2011 (EP) and SFRH/BPD/64702/2009 (HVM). The authors thank Sergio do Carmo (ICNAS) for his support in the operation of the cyclotron and production of fluorine-18. Elizabete Correia and Vitor Alves are acknowledged for technical support in the biodistribution studies and HPLC, respectively. J. Marçalo is acknowledged for the mass spectrometry analyses performed on QIT and FTICR instruments that are part of RNEM - Rede Nacional de Espectrometria de Massa, supported by the FCT. TFO is supported by the DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB).

REFERENCES AND NOTES

[1] Present address: Institute of Cancer Therapeutics, University of Bradford, Bradford, UK.

[2] Townsend, L. B.; Revankar, G. R. Chem Rev 1970, 70, 389.

[3] Tuncbilek, M.; Kiper, T.; Altanlar, N. Eur J Med Chem 2009, 44, 1024.

[4] Ozkay, Y.; Tunali, Y.; Karaca, H.; Isikdag, I. Eur J Med Chem 2010, 45, 3293.

[5] Abdel-Mohsen, H. T.; Ragab, F. A. F.; Ramla, M. M.; El Diwani, H. I. Eur J Med Chem 2010, 45, 2336.

[6] Noolvi, M.; Agrawal, S.; Patel, H.; Badiyer, A.; Gaba, A. Arabian J Chem 2014, 7, 219.

[7] Styskala, J.; Styskalova, L.; Slouka, J.; Hajduch, M. Eur J Med Chem 2008, 43, 449.

[8] Hranjec, M.; Pavlovic, G.; Marjanovic, M.; Kralj, M.; Karminski-Zamola, G. Eur J Med Chem 2010, 45, 2405.

[9] Miller, J. F.; Turner, E. M.; Gudmundsson, K. S.; Jenkinson, S.; Spaltenstein, A.; Thomson, M.; et al. Bioorg Med Chem Lett 2010, 20, 2125.

[10] Shah, D. I.; Sharma, M.; Bansal, Y.; Bansal, G.; Singh, M. Eur J Med Chem 2008, 43, 1808.

[11] Kus, C.; Ayhan-Kilcigil, G.; Ozbey, S.; Kaynak, F. B.; Kaya, M.; Coban, T.; et al. Bioorgan Med Chem 2008, 16, 4294.

[12] Shingalapur, R. V.; Hosamani, K. M.; Keri, R. S. Eur J Med Chem 2009, 44, 4244.

[13] Leonards, T. J.; Jeyaseeli, L.; Kumar, M.; Sivakumar, R. Asian J Xhem 2006, 18, 1104.

[14] Singh, M. P.; Joseph, T.; Kumar, S.; Bathini, Y.; Lown, J. W. Chem Res Toxicol 1992, 5, 597.

[15] Bryant, H. E.; Helleday, T. Biochem Soc Trans 2004, 32, 959.

[16] Tong, Y. S.; Bouska, J. J.; Ellis, P. A.; Johnson, E. F.; Leverson, J.; Liu, X. S.; et al. J Med Chem 2009, 52, 6803.

[17] Gama, S.; Mendes, F.; Esteves, T.; Marques, F.; Matos, A.; Rino, J.; et al. Chembiochem 2012, 13, 2352.

[18] Gama, S.; Mendes, F.; Marques, F.; Santos, I. C.; Carvalho, M. F.; Correia, I.; et al. J Inorg Biochem 2011, 105, 637.

[19] Silva, F.; Marques, F.; Santos, I. C.; Paulo, A.; Rodrigues, A. S.; Rueff, J.; et al. J Inorg Biochem 2010, 104, 523.

[20] Goncalves, A. C.; Morais, T. S.; Robalo, M. P.; Marques, F.; Avecilla, F.; Matos, C. P.; et al. J Inorg Biochem 2013, 129, 1.

[21] Murphy, P. S.; McCarthy, T. J.; Dzik-Jurasz, A. S. K. Brit J Radiol 2008, 81, 685.

[22] Morais, G. R.; Santos, I. C.; Santos, I.; Paulo, A. J Chem Crystallogr 2012, 42, 1052.

[23] Fotakis, G.; Timbrell, J. A. Toxicol Lett 2006, 160, 171.

[24] Homet, B.; Ribas, A. J Pathol 2014, 232, 134.

[25] Wilson, T. A.; Karajannis, M. A.; Harter, D. H. Surg Neurol Int 2014, 5, 64.

[26] Thorn, C. F.; Oshiro, C.; Marsh, S.; Hernandez-Boussard, T.; McLeod, H.; Klein, T. E.; Altman, R. B. Pharmacogenet Genomics 2011, 21, 440.

[27] Yang, F. Y.; Teng, M. C.; Lu, M.; Liang, H. F.; Lee, Y. R.; Yen, C. C.; Liang, M. L.; Wong, T. T. Int J Nanomedicine 2012, 7, 965.

[28] Ribeiro Morais, G.; Miranda, H. V.; Santos, I. C.; Santos, I.; Outeiro, T. F.; Paulo, A. Bioorgan Med Chem 2011, 19, 7698.

[29] Coenen, H. H.; Klatte, B.; Knochel, A.; Schuller, M.; Stocklin, G. J Labelled Compd Rad 1986, 23, 455.