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Novel Fluorinated Benzimidazole-Based Scaffolds and their Anticancer Activity *in vitro*

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In memory of Russ Bowman

ABSTRACT

A small library of twelve, structurally diverse, fluoroaryl benzimidazoles was prepared using a simple synthetic strategy employing S_NAr reactions. This allowed rapid assembly of heterocyclic structures containing linked and tethered fluoroaryl benzimidazoles. X-ray crystal structures of seven compounds were obtained including those of two macrocyclic compounds containing 21- and 24-membered rings. Three tethered fluoroaryl benzimidazole derivatives demonstrated micromolar inhibition against K-562 and MCF-7 cell lines. These compounds, in addition to 1-tetrafluoropyrid-4-yl-2-tetrafluoropyrid-4-ylsulfanyl-1*H*-benzimidazole, also demonstrated micromolar inhibition against G361 and HOS cell lines. Two of the compounds were found to activate caspases leading to apoptosis.

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Keywords: anticancer activity, C-F activation, fluorinated drug scaffolds, S_NAr substitution, benzimidazole

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1. Introduction

As part of an ongoing medicinal chemistry programme to develop synthetically accessible and modifiable scaffolds [1, 2, 3], the facile S_NAr reaction of perfluoroarenes [4] is being employed to generate fluorinated heterocyclic libraries [5] for screening. S_NAr reactions of perfluorinated aromatics occur predictably with a wide range of nucleophiles, proceed readily under mild

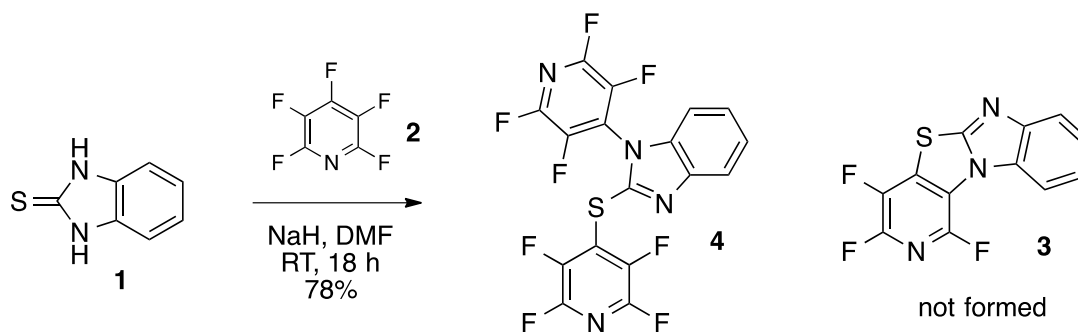
conditions, and without the need for transition metal catalysis [6]. Substitution patterns available by stepwise replacement of fluorines are different from those afforded by electrophilic aromatic substitution and allow preparation of diverse structural types [7, 8]. Potential for ring formation with bidentate nucleophiles [9, 10], control of regiochemistry through hard/soft nucleophilic interactions [11, 12,], and alternative methods for promoting reaction by ultrasound [13] continue to attract attention. Retaining fluorine in a molecule is also desirable from a medicinal chemistry perspective, since fluorine can improve resistance to metabolism due to the strong C–F bond (485 kJmol⁻¹) while the small size (van der Waals radius, 1.47 Å) and high electronegativity (3.98 Pauling scale) endow specific electronic properties that can dramatically alter the biological response of the compound [14, 15]. Benzimidazoles, amongst other aza-heterocycles, represent privileged substructures in medicinal chemistry and serve as desirable building blocks in the design and construction of bioactive compounds [16, 17, 18]. Here we report the synthesis of a number of *N*-fluoroaryl benzimidazole derivatives using S_NAr substitution of perfluorinated arenes and the screening of these for activity against breast carcinoma MCF-7, leukemia K562, melanoma G361 and osteosarcoma HOS cell lines. The structures of the fluoroaryl benzimidazole derivatives prepared were confirmed by NMR spectroscopy and X-ray crystallographic analysis.

2. Results and Discussion

2.1 Heterocyclic Synthesis and Characterisation

Benzimidazole [19, 20, 21] and related azoles [22, 23] are known to add to pentafluoropyridine and the aim of this work was to exploit the ready S_NAr reaction of benzimidazole derivatives with pentafluoropyridine as a building block to make composite linked and fused heterocyclic scaffolds. Furthermore, these scaffolds would have potential for further modification to allow synthesis of diverse libraries of molecules that can be screened for biological activity. Continuing our interest in preparing condensed polycyclic heteroarenes from perfluoroarenes [24] we first explored the reaction of pentafluoropyridine **2** with 1,3-dihydro-2*H*-benzimidazole-2-thione **1** (Scheme 1), expecting to obtain the tetracyclic-fused thiazole **3** by a tandem S_NAr addition. The thiolate group of the anion of **1** was expected to be the most nucleophilic centre and likely to attack C-4 of pentafluoropyridine first, leading to the ring fusion shown in **3**, if the ring nitrogen then effected a second substitution. Similar condensations with aminopyridines to form imidazopyridines have been reported [25]. We found however that the thione **1**, on treatment with sodium hydride in DMF, reacted instead with two molecules of pentafluoropyridine to give the di-arylated benzimidazole **4** in which both the sulfur atom and a ring nitrogen of **1** had added to the 4-position of separate pentafluoropyridine molecules. Compound **4** was formed even when one equivalent of pentafluoropyridine was used,

and the best yield of **4** was obtained with three equivalents. Sodium hydride is a convenient base for these reactions and can be pre-mixed with the fluoroarene prior to addition of the nucleophile. It allows rapid, quantitative deprotonation of the NH group of heterocyclic nucleophiles. The structure of compound **4** was supported by ^{19}F -NMR spectroscopy, which exhibited four signals of equal intensity (AA'BB'CC'DD' system) consistent with four pairs of fluorine atoms, rather than three signals expected for tetracycle **3**. Confirmation of the structure was obtained by single crystal X-ray diffraction analysis, and the molecular structure is shown in Figure 1 [26; crystal data in Table 3]. Addition of pentafluoropyridine to both ring nitrogen and exocyclic amine groups has also been observed with 2-aminopyridine derivatives [27].



Scheme 1. Reaction of 1,3-dihydro-2H-benzimidazole-2-thione with pentafluoropyridine.

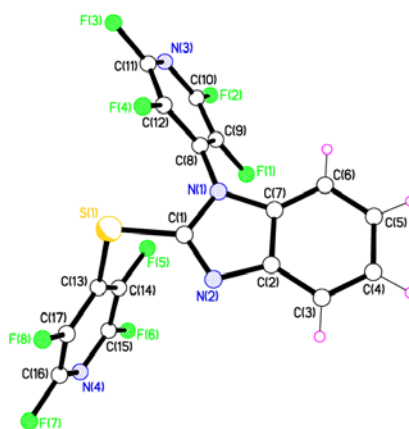


Figure 1. X-ray crystal structure of **4**. One of two similar molecules in the asymmetric unit.

Subsequently, we investigated the preparation of linked benzimidazole derivative **5** [28] (Scheme 2) as a nucleophilic partner to add to pentafluoropyridine to form tethered heterocyclic scaffolds, as linked compounds [29] were of interest due to their potential for acting as *bis*-intercalating [30, 31] and cross linking agents [32, 33, 34] for DNA binding. The bis-benzimidazole **5** (Scheme 2) (X-ray structure shown in Figure 2), with a four-methylene spacer chain, reacted readily with excess pentafluoropyridine **2**, in the presence of sodium hydride, to form **6**.

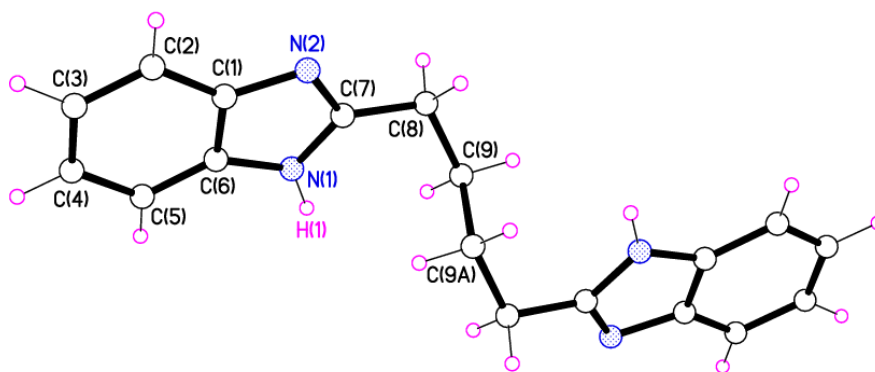
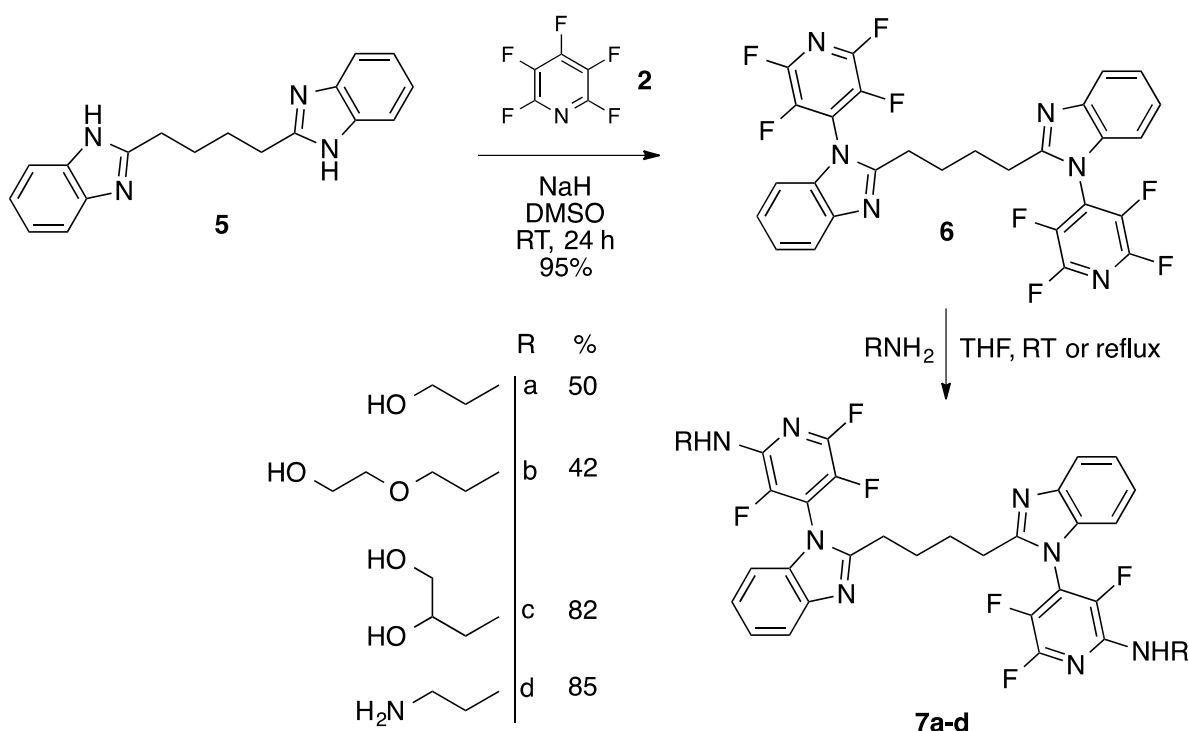


Figure 2. X-ray structure of tetramethylene linked bis-benzimidazole precursor **5**.

During initial screening (see section 2.2) compound **6** exhibited good activity against the four cancer cell lines studied, and so was pursued as a candidate for further functionalisation. Reactions with three amino alcohols and a diamine were investigated with the aim of improving water solubility. The amines all reacted at the next most electrophilic position in the tetrafluoropyridine rings, adding to C-2, forming the aminopyridine derivatives **7a-d**.



Scheme 2. Synthesis of linked benzimidazole-containing scaffolds.

The product derived from addition of ethylenediamine **7d** retained good activity against two of the cell lines, but was slightly less active against G361 and HOS. Treatment of **6** with the longer diamines, 2-(2-aminoethoxy)ethylamine or 2,2'-(ethylenedioxy)bis(ethylamine) surprisingly led to macrocycle formation affording compounds **8a** and **8b** which contain 21- and 24-membered rings

respectively (Figure 3). Both 14- and 21-membered macrocycles have been formed from tetrafluoropyrimidine and pentafluoropyridine previously [35]. The structures of compounds **8a** and **8b** were confirmed by X-ray crystallography as shown in Figures 4 and 5. The macrocycle **8a** formed a mono-ethanol solvate, **8a·EtOH** on recrystallisation, while **8b** could be crystallised as the hydrate **8b·2½H₂O**, or the chloroform solvate **8b·CHCl₃**. Diffraction data for **8b·2½H₂O** required the use of high intensity synchrotron radiation due to small crystal size. Reaction of the linked benzimidazole **5** with methyl pentafluorobenzoate afforded **9** with the imidazole nitrogen of **5** adding *para* to the ester groups which represent sites for further derivatisation. The X-ray crystal structure of the ester compound **9** is shown in Figure 5.

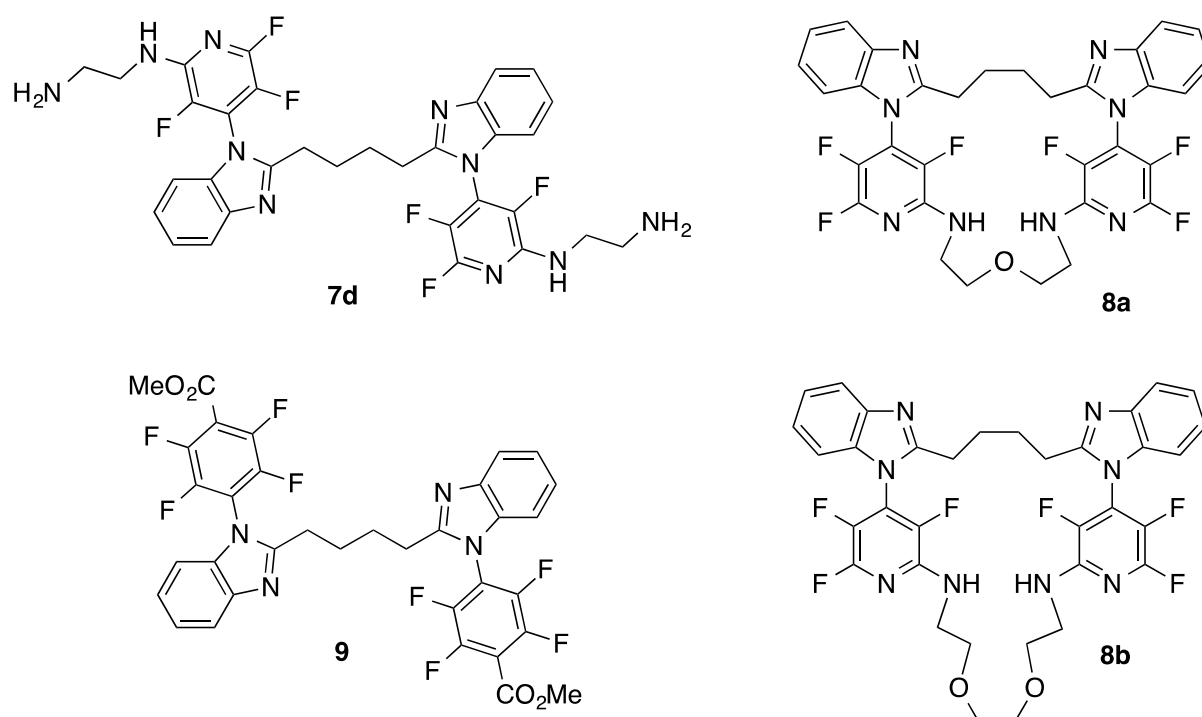


Figure 3. Linked and macrocyclic fluoroaryl benzimidazoles.

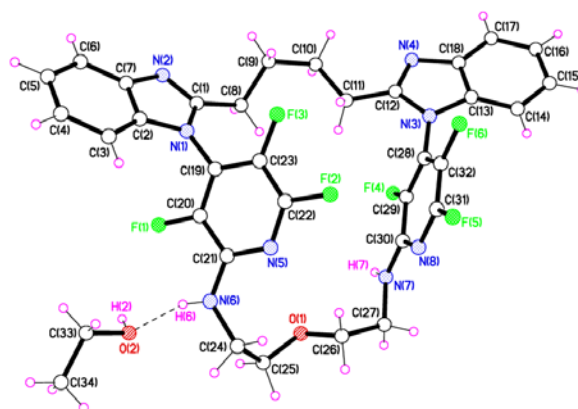


Figure 4. X-ray crystal structure of **8a** showing the 21-membered macrocycle with a hydrogen-bonded ethanol solvate molecule.

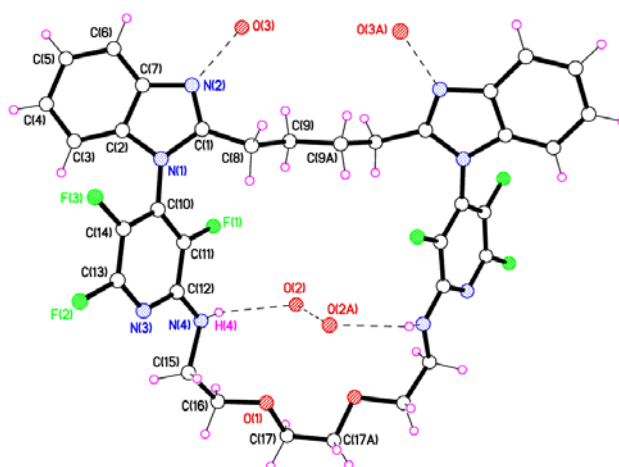


Figure 5. X-ray crystal structure of **8b**·2½H₂O showing the 24-membered macrocycle with 2½ hydrogen bonded water molecules. The molecule lies on a 2-fold axis. O(3) and O(3A) are each ¼ occupied.

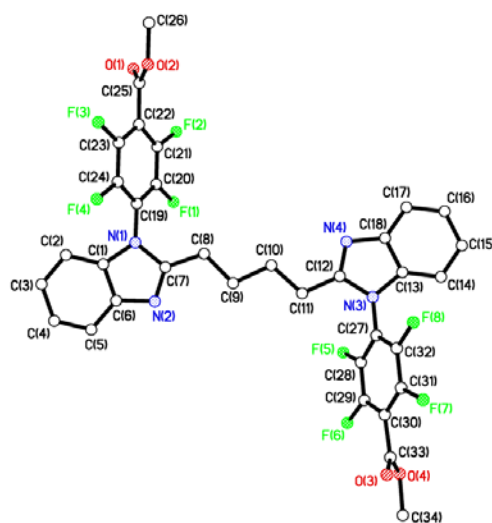
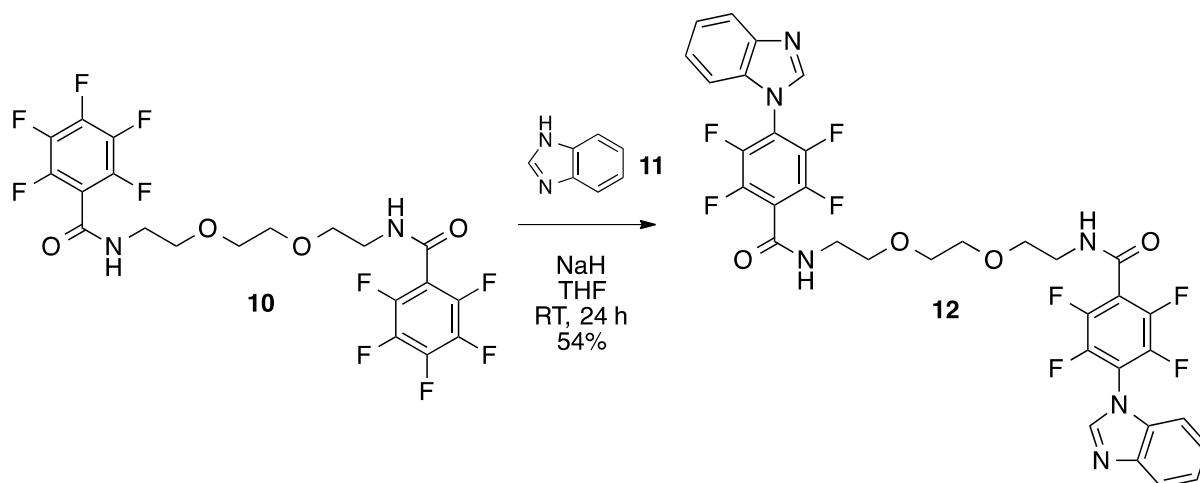


Figure 6. X-ray structure of linked tetrafluorobenzoate scaffold **9**.

A further scaffold (Scheme 3) containing tethered benzimidazole units was readily prepared by reaction of pentafluorobenzoyl chloride with 2,2'-(ethylenedioxy)bis(ethylamine) forming bis-amide **10**. Reaction with the anion of benzimidazole **11** generated with sodium hydride led to substitution of the *para* fluorine atom in each pentafluorobenzamide ring, affording scaffold **12**. The structure was confirmed by ¹H and ¹⁹F NMR spectroscopy. X-ray crystallography was also used to confirm the structure of the tethered pentafluorobenzamide **10** (Figure 7). Diffraction data for **10** required synchrotron radiation due to small crystal size.



Scheme 3. Reaction of tethered *bis*-pentafluorobenzamide **10** with benzimidazole.

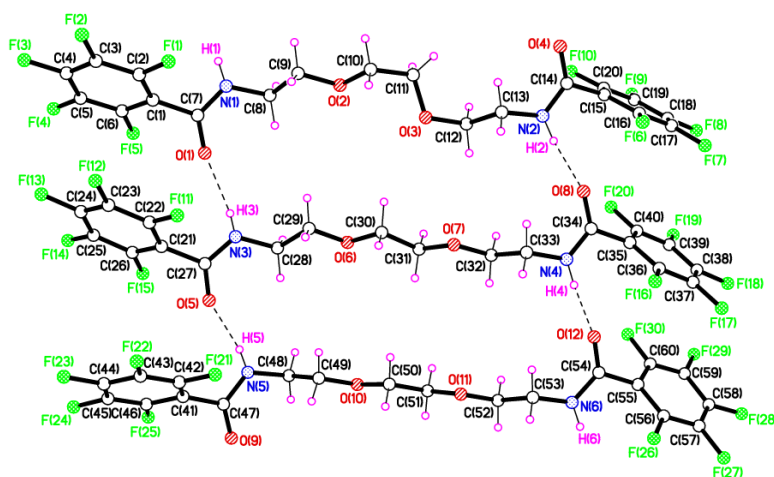


Figure 7. X-ray structure of amide linked scaffold **10** showing stacked H-bonded molecules in a ladder motif. These three molecules comprise the asymmetric unit.

The ^{19}F NMR spectra of the 4-substituted 2,3,5,6-tetrafluoro-aromatic compounds produced complex, highly second-order multiplets, that only yielded accurate coupling constants via analysis and iterative simulation. It was imperative that the correct sign of coupling constants were explored, used and optimised. An excellent starting point was the analysis of 60 MHz (for ^1H) NMR spectra by Dean and McFarlane [36]. Experimental ^{19}F NMR spectra were obtained on a JEOL-ECS-400 MHz (for ^1H) NMR spectrometer ensuring that sufficient data points were acquired in the FID with sufficient signal-to-noise to allow resolution-enhancement by application of a Gaussian function. SpinWorks-4 [37] was used to process the FID and simulation/optimisation was achieved using the NUMARIT algorithm [38] re-coded to NUMMRIT in C++ by Rudy Sebastian. The $^3J_{\text{FF}}$ couplings are generally negative (approx. -21 Hz) and the para- $^5J_{\text{FF}}$ are generally positive (approx. 10 Hz), with the $^4J_{\text{FF}}$ AA' and BB' couplings generally much smaller and of either sign (approx. ± 5 Hz).

Iterative simulation, and manual intervention of the sign of small couplings, allowed 19 assigned transitions producing a RMS of 0.217 after three iterations, yielding excellent simulated spectra (see supplementary information). For example, analysis of compound **6** produced: AA' = 76.58 ppm, BB' = 18.44 ppm, $^4J_{AA'} = -3.17 \pm 0.19$ Hz, $^3J_{AB} = -28.71 \pm 0.79$ Hz, $^5J_{AB'} = 19.82 \pm 0.80$ Hz, $^5J_{A'B} = 21.82 \pm 0.75$ Hz, $^3J_{A'B'} = -29.30 \pm 0.73$ Hz, $^4J_{BB'} = 14.17 \pm 0.14$ Hz. The ABCX spin system for the disubstituted trifluoropyridine derivatives (**7**, **8**) produced a simple set of doublet-of-doublets and doublets that yield chemical shifts and couplings directly from the peak-picking values on the spectrum. The only uncertainty was the small long-range $^4J_{FH}$ coupling from F-3 to the hydrogen of the NH group of the 2-substituent, for which little evidence was available from the 1H NMR and which is estimated as ~ 3 Hz. A good match between experimental and simulated NMR spectra was achieved for macrocycle **8b**: A = 73.96 ppm (± 1 Hz), B = 0.2 ppm (± 1 Hz), C = 12.9 ppm (± 1 Hz), $^3J_{FAFB} = -31.5 \pm 1.4$ Hz, $^5J_{FAFC} = 6.79 \pm 1.4$ Hz, $^4J_{BFC} = 22.9 \pm 1.4$ Hz, $^4J_{FCH} = 2.7 \pm 2$ Hz (estimated). Analysis of the ^{13}C - ^{19}F couplings in **4** is given in the supplementary information.

The ease of such S_NAr reactions with a range of benzimidazole nucleophiles demonstrates that this synthetic approach can be utilised to rapidly generate libraries of compounds with latent functionality for biological screening. The composite heterocycles prepared were then examined for anti-cancer activity to identify scaffolds that could be further refined into potential drug candidates.

2.2 Anticancer activity in vitro

The anticancer activities of all new compounds were determined against several breast carcinoma MCF-7 and leukemia K562 cell lines after 72 h of incubation using the Calcein assay, and the CDK inhibitor, roscovitine, as a control compound. The results obtained (Table 1), show that compounds **4**, **6**, **7d** and **8a** display cytotoxicity in which IC_{50} values reached low micromolar ranges.

Table 1. Cytotoxicity of fluoroarylbenzimidazole derivatives.

Compound	Cell line IC_{50} (μM)	
	K-562	MCF-7
4	13.2 ± 1.3	>25
6	2.8 ± 0.4	7.8 ± 2.0
7a	>12.5	>12.5
7b	>12.5	>12.5
7d	3.4 ± 0.1	8.0 ± 2.6

7c	>12.5	>12.5
8a	7.2 ± 1.5	4.8 ± 0.4
9	>12.5	>12.5
12	>12.5	>12.5
roscovitine	42 ± 3	11 ± 1

These four compounds were further screened against melanoma G361 and osteosarcoma HOS cell lines to expand information about selectivity towards various types of cancers (Table 2). Concentration-dependent activity was observed in all cases with these four compounds. Three of the active compounds contained the tetramethylene bis-benzimidazole linker group **5** with tri- or tetrafluorinated pyridine rings, whilst the smaller molecule **4** also contains two fluorinated pyridine rings, separated by the benzimidazole ring. This suggests that two fluorinated pyridine rings separated between 4-6 Å is desirable for activity. Initial studies have shown possible interaction with double-stranded DNA with a small increase in melting temperature and decrease in fluorescence in ethidium bromide competition experiments, but the mechanism of the activity is not yet clear.

Table 2. Cytotoxicity of lead derivatives.

Compound	IC ₅₀ (μM)	
	G361	HOS
4	4.4 ± 0.6	2.5 ± 0.9
6	2.0 ± 0.1	1.8 ± 0.1
7d	19.9 ± 1.2	16.3 ± 3.0
8a	6.0 ± 0.1	8.3 ± 2.0
roscovitine	22.4 ± 0.2	24.3 ± 0.2

Further experiments were conducted to gain information about the mechanism underlying the observed cytotoxicity. First, we analyzed the effect of compounds **6**, **7d** and **8a** on the cell cycle of K562 and MCF-7 cells. Asynchronously growing cells were treated for 24 h with compounds in two doses, corresponding to 1× and 3× IC₅₀ values followed by analysis via flow cytometry. As shown in Figure 8, the compounds markedly influenced cell cycle profiles in both cell lines although each compound demonstrated this effect in a different way. Treatment of MCF-7 cells with a low dose of **6** led to a decrease in the S phase, whilst a higher dose significantly decreased G1-phase population and increased sub-G1 population in both K562 and MCF-7 cell lines. Compound **7d** had no effect at the lower dose used, but

the higher dose significantly increased G2/M population in MCF-7 cells. Interestingly, treated K562 cells completely altered the profile, with apparent block of S phase. Macrocyclic compound **8a** markedly reduced S and G2/M phases in MCF7 cells, while the cell cycle profile in K562 was not changed at all.

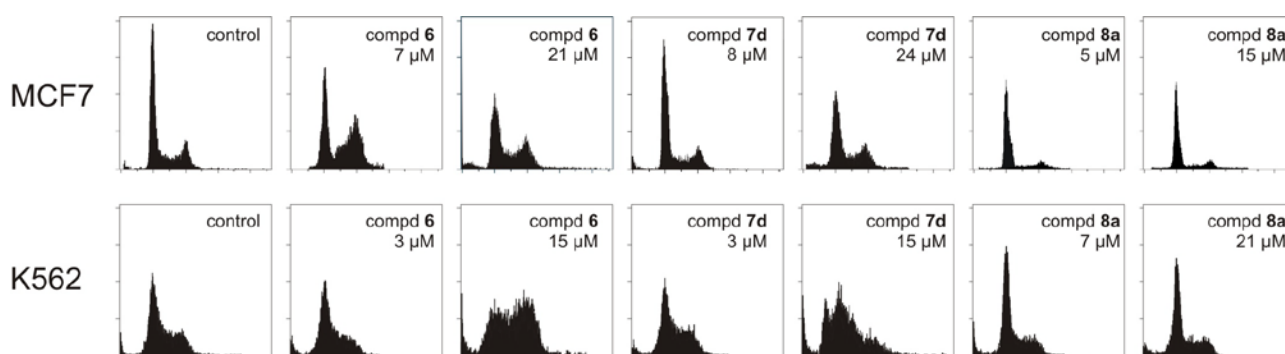


Figure 8. Effect of studied compounds on the cell cycle of MCF7 and K562 cells treated for 24 h.

Flow cytometric analysis revealed that some compounds increase sub-G1 population, which is a well accepted indicator of ongoing apoptotic cell death. We therefore analyzed treated K562 cells for activity of caspases 3 and 7, proteases that are activated during apoptotic cell death. Figure 9 shows the results of biochemical assay of caspase 3/7 activity in cells treated for 24 h with the studied compounds at doses corresponding to 1× and 3× IC₅₀ values. While **7d** was inactive in the assay, compounds **6** and **8a** significantly stimulated activation of the caspases, with the increase in activity more than twenty-fold, and five-fold, over untreated control cells respectively. Based on flow cytometric results and biochemical assay of caspases we conclude that **6** and **8a** induce apoptosis in treated cells. However, the flow cytometric experiments suggest that the active compounds exhibit their toxicity through different cellular targets. Further work is ongoing to identify the mode of action of these fluorinated compounds.

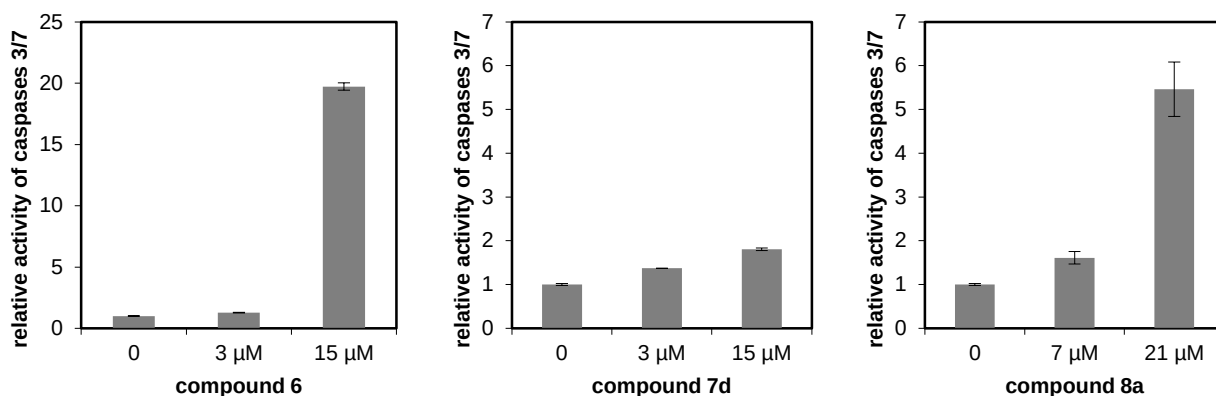


Figure 9. Fluorimetric caspase activity assay. K562 cells treated with studied compounds for 24 h were lysed and the activities of caspases were measured using the fluorogenic substrate Ac-DEVD-AMC and normalised to untreated control.

3. Conclusion

A library of twelve, structurally diverse, fluoroaryl benzimidazoles was prepared using a simple synthetic strategy employing S_NAr reactions. This allowed rapid assembly of heterocyclic structures containing linked and tethered rings. X-ray crystal structures of seven compounds were obtained including those of two macrocyclic compounds containing 21- and 24-membered rings. Three compounds **6**, **7d** and **8a** demonstrated micromolar inhibition against K-562 and MCF-7 cell lines. These compounds, in addition to **4**, also demonstrated micromolar inhibition against G361 and HOS cell lines. Compounds **6** and **8a** were found to activate caspases leading to apoptosis.

4. Experimental

4.1 General Methods

All reactions were conducted under anhydrous conditions using oven/flame-dried glassware under a nitrogen atmosphere. Commercially available solvents were used without further purification, except THF, which was distilled from sodium and benzophenone. DMF and DMSO were purchased dry from a commercial supplier. Light petroleum refers to the fraction boiling between 40-60 °C. NMR spectra were recorded (1H , ^{19}F and ^{13}C) using Bruker 500 and 400 MHz, or Jeol ECS-400 instruments with tetramethylsilane as internal standard or hexafluorobenzene for ^{19}F spectra. Chemical shifts are reported in ppm and coupling constants are recorded in hertz. 1H spectra were recorded at 400 MHz, ^{19}F spectra at 376 MHz and ^{13}C spectra were recorded at 125 or 100 MHz. DEPT was used to assign environment (CH, CH_2 , CH_3) in ^{13}C NMR spectra. The solvent is specified in brackets.

A Thermofisher Exactive (orbitrap) mass spectrometer was used to obtain high-resolution mass spectra, with ESI as the ionization mode. The solvent used for all samples was methanol. Other high-resolution mass spectra were recorded at the EPSRC UK National Mass Spectrometry Facility. GC-MS was performed on a Fisons 8060 with a DB5MS column of 30 m length and split-less injection.

A Perkin-Elmer spectrum 65FT-IR spectrophotometer was used to obtain infrared spectra. Liquid samples were acquired as thin films on NaCl. Solid samples were acquired as KBr discs.

Melting points were recorded using an Electro thermal-IA 9100 melting point instrument.

TLC analysis was carried out on Merck TLC silica gel 60 F₂₅₄ aluminum backed plates and were visualised under UV light at 254 nm or by vanillin stain. Column chromatography was carried out on Merck Kiesel 60 silica gel.

Elemental analysis was determined using a Perkin-Elmer 2400 analyser.

4.2. 1-Tetrafluoropyrid-4-yl-2-tetrafluoropyrid-4-ylsulfanyl-1H-benzimidazole **4**

A solution of pentafluoropyridine (1.01 g, 6 mmol) in DMF (1.5 mL) was added to NaH (60% dispersion in mineral oil) (0.16 g, 4 mmol) and the mixture treated dropwise with a solution of 2-mercaptobenzimidazole (0.30 g, 2 mmol) in DMF (1.5 mL). The mixture was shaken until gas evolution ceased and was then maintained at room temperature for 18 h. The reaction mixture was partitioned between ether (25 mL) and water (10 mL). The ether layer was separated, washed with water (2 × 2 mL) and saturated NaCl (aq) (2 mL), dried over MgSO₄, filtered and evaporated to give an off-white solid which was triturated with hexane to give **4** as a cream powder (0.64 g, 78 %), m.p. 112-115 °C; $\nu_{\max}/\text{cm}^{-1}$ (film) 1630, 1474, 1246, 975, 952; δ_{H} (400 MHz, CDCl₃) 7.85-7.80 (1H, m), 7.49-7.45 (2H, m), 7.23-7.19 (1H, m); δ_{C} (125 MHz, CDCl₃) 144.1 (dtd, $^1J_{\text{CF}}$ 248, $^2J_{\text{CF}}$ 15, $^4J_{\text{CF}}$ 4), 143.5 (dddd, $^1J_{\text{CF}}$ 247, $^2J_{\text{CF}}$ 18, $^3J_{\text{CF}}$ 13, $^4J_{\text{CF}}$ 3), 143.4, 142.0, 141.2 (dm, $^1J_{\text{CF}}$ 260), 138.1 (dm, $^1J_{\text{CF}}$ 266), 135.0, 126.1, 126.0 (tt, $^2J_{\text{CF}}$ 13, $^3J_{\text{CF}}$ 5), 125.0, 124.9 (tt, $^2J_{\text{CF}}$ 18, $^3J_{\text{CF}}$ 3), 120.7, 110.1; δ_{F} (376 MHz, CDCl₃) 77.4-77.1 (2F, AA'), 74.4-74.0 (2F, BB'), 27.0-26.9 (2F, CC'), 19.3-19.1 (2F, DD'); HRMS (ESI) found m/z 446.9962 (M-H)⁻, C₁₇H₃F₈N₄S requires 446.9956; Anal. (%) calcd for C₁₇H₄F₈N₄S (448): C, 45.55; H, 0.90; N, 12.49; found C, 45.41; H, 0.92; N, 12.00.

4.3. 1,4-bis-1-Tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane **6**

A solution of pentafluoropyridine **2** (2.5 g, 15 mmol) in DMSO (2 mL) was added to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (0.6 g, 15 mmol) in DMSO (2 mL). 1,4-bis-1H-Benzimidazol-2-ylbutane **5** [21] (1.45 g, 5 mmol) in DMSO (4 mL) was then

added dropwise using a syringe pump and the mixture left at room temperature under N₂ for 24 h. After quenching with a few drops of methanol, distilled water (10 mL) was added, and the precipitated solid formed collected by suction filtration to give compound **6** (2.79 g, 95%) as a shiny white solid, m.p. 195-198 °C; $\nu_{\max}/\text{cm}^{-1}$ (film), 2924, 1642, 1471, 1255, 1136, 972, 743; δ_{H} (400 MHz, CDCl₃) 7.81 (2H, d, $^3J_{\text{HH}}$ 7.6), 7.36 (4H, m), 7.07 (2H, d, $^3J_{\text{HH}}$ 7.6), 2.87 (4H, m), 2.06 (4H, m); δ_{C} (100 MHz, CDCl₃) 153.0, 144.2 (dd, $^1J_{\text{CF}}$ 230, $^2J_{\text{CF}}$ 26), 142.8, 136.8 (dd, $^1J_{\text{CF}}$ 267, $^2J_{\text{CF}}$ 36), 134.2, 127.2-128.1 (m), 124.2, 124.0, 120.1, 109.4, 29.8, 26.4; δ_{F} (376 MHz, CDCl₃) 76.4-76.1 (4F, AA'), 18.2-17.9 (4F, BB'); HRMS (ESI) found m/z 589.1376 (MH⁺), C₂₈H₁₇F₈N₆ requires 589.1381; Anal. (%) calcd for C₂₈H₁₆F₈N₆·½H₂O (597): C, 56.28; H, 2.85; N, 14.07; found: C, 56.38; H, 3.01; N, 13.86.

4.4. 1,4-bis-1-[2-(2-Hydroxyethylamino)-trifluoropyrid-4-yl]-1H-benzimidazol-2-ylbutane **7a**

A solution of ethanolamine (0.024 g, 0.4 mmol) in THF (4 mL) was added dropwise to a stirred solution of **6** (0.108 g, 0.2 mmol) in THF (4 mL) under N₂, and the mixture heated under reflux for 24 h. Distilled water (10 mL) was added and the mixture was extracted with CH₂Cl₂ (3 × 20 mL). The organic extracts were washed with saturated NaCl (aq) and dried over MgSO₄, filtered and evaporated to give a white solid (0.14 g). Recrystallisation from ethanol gave **7a** as white crystals (0.065 g, 50 %), m.p. 175-178 °C; $\nu_{\max}/\text{cm}^{-1}$ (film), 3425 (N-H), 2947, 1651, 1512, 1427, 1149, 1057, 987, 748; δ_{H} (400 MHz, (CD₃)₂CO) 7.70 (2H, dd, $^3J_{\text{HH}}$ 8.3, $^4J_{\text{HH}}$ 1.6), 7.32-7.25 (6H, m), 6.60 (2H, s), 4.12 (2H, broad s), 3.78 (4H, t, $^3J_{\text{HH}}$ 11.2), 3.60-3.58 (4H, m), 2.88 (4H, m), 1.99 (4H, m); δ_{F} (376 MHz, CDCl₃) 72.5 (2F, t, J_{FF} 26.5), 13.9 (2F, dd, $^3J_{\text{FF}}$ 29.6, $^4J_{\text{FF}}$ 9.9), 5.1 (2F, dd, $^3J_{\text{FF}}$ 25.5, $^4J_{\text{FF}}$ 9.9); HRMS (ESI) found m/z 669.2175 (M-H⁺), C₃₂H₂₇F₆N₈O₂ requires m/z 669.2161.

4.5. 1,4-bis-1-{2-[2-(2-Hydroxyethoxy)ethylamino]-trifluoropyrid-4-yl]-1H-benzimidazol-2-ylbutane **7b**

A solution of 2-(2-aminoethoxy)ethanol (0.02 g, 0.2 mmol) in THF (4 mL) was added dropwise to a stirred solution of **6** (0.058 g, 0.1 mmol) in THF (4 mL) and the mixture stirred at room temperature for 24 h under N₂. Distilled water (10 mL) was added and the mixture extracted with ethyl acetate (3 × 25 mL). The organic extract was washed with saturated NaCl (aq), dried over MgSO₄, filtered and evaporated to give a colourless oil (0.07 g). Crystallisation from CH₂Cl₂ and light petroleum gave **7b** as white crystals (0.033 g, 42%), m.p. 168-170 °C; $\nu_{\max}/\text{cm}^{-1}$ (film), 3333 (N-H), 2870, 1651, 1512, 1427, 1388, 1273, 1118, 1072, 987, 740; δ_{H} (400 MHz, (CD₃)₂CO) 7.67 (2H, d, $^3J_{\text{HH}}$ 8.0), 7.46 (2H, s), 7.33-7.24 (6H, m), 4.68 (2H, t, $^3J_{\text{HH}}$ 4.8), 3.61 (8H, t, $^3J_{\text{HH}}$ 5.6), 3.54-3.47 (8H, m

(overlap with water peak)), 2.78 (4H, m), 1.84 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.2 (2F, t, J_{FF} 11.7), 13.6-13.3 (2F, m), -1.93-(-1.46) (2F, m); HRMS (ESI) found m/z 759.2829 (MH^+), $C_{36}H_{37}F_6N_8O_4$ requires 759.2836.

4.6. 1,4-bis-1-[2-(2,3-Dihydroxypropylamino)-trifluoropyrid-4-yl]-1H-benzimidazol-2-ylbutane **7c**

A solution of ($\pm$)-3-amino-1,2-propanediol (0.018 g, 0.2 mmol) in DMF (3 mL) was added dropwise to a stirred solution of **6** (0.058 g, 0.1 mmol) in THF (3 mL) and the mixture heated at 65 °C for 24 h under N_2 . Distilled water (10 mL) was added and the precipitated solid formed collected by suction filtration to give **7c** as a white solid (0.08 g, 82%), m.p. 123-126 °C; ν_{max}/cm^{-1} (film), 3417 (N-H), 2931, 1651, 1427, 1257, 1057, 979, 748; δ_H (400 MHz, $CDCl_3$) 7.64 (2H, d, $^3J_{HH}$ 7.6), 7.28-7.18 (6H, m), 4.25 (2H, s), 3.90 (4H, m), 3.84-3.56 (6H, m), 3.47-3.42 (2H, m), 2.90-2.75 (4H, m), 1.92-1.86 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.53 (2F, t, J_{FF} , 26.6), 14.02 (2F, dd, $^3J_{FF}$ 29.1, $^4J_{FF}$ 8.9), 5.09 (2F, dd, $^3J_{FF}$ 25.4, $^4J_{FF}$ 8.9); HRMS (ESI) found m/z 731.2516 (MH^+), $C_{34}H_{33}F_6N_8O_4$ requires 731.2523.

4.7. 1,4-bis-1-[2-(2-Aminoethylamino)-trifluoropyrid-4-yl]-1H-benzimidazol-2-ylbutane **7d**

A solution of ethylenediamine (0.025 g, 0.4 mmol) in THF (2 mL) was added dropwise to stirring solution of **6** (0.12 g, 0.2 mmol) in THF (3 mL) and the mixture stirred at room temperature for 24 h under N_2 . Distilled water (10 mL) was added and the mixture extracted with CH_2Cl_2 (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give **7d** as a white solid (0.11 g, 85%), m.p. 195-198 °C; ν_{max}/cm^{-1} (film), 3400 (br. NH), 2095, 1647, 1509, 1453, 1388, 1264; δ_H (400 MHz, $CDCl_3$) 7.80 (2H, d, $^3J_{HH}$ 7.6), 7.35-7.29 (4H, m), 7.11 (2H, d, $^3J_{HH}$ 7.6), 5.46 (2H, td, $^3J_{HH}$ 8.4, $^4J_{HF}$ 5.4), 3.57 (4H, dd, $^3J_{HH}$ 11.2, $^3J_{HH}$ 5.2), 3.05 (4H, t, $^3J_{HH}$ 5.6), 2.81 (4H, s), 1.99-1.95 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.3 (2F, m), 13.09 (2F, dd, J_{FF} 23.3, J_{FF} 8.6), 1.44 (2F, dd, J_{FF} 23.3, J_{FF} 8.6); HRMS (ESI) found m/z 669.2621 (MH^+), $C_{32}H_{31}F_6N_{10}$ requires 669.2632.

4.8. Reaction of 1,4-bis-1-tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane **6** with 2-(2-aminoethoxy)ethylamine: macrocycle **8a**

A solution of 2-(2-aminoethoxy)ethylamine (0.07 g, 0.4 mmol) in THF (3 mL) was mixed with a solution of triethylamine (0.08 g, 0.8 mmol) in THF (2 mL) and treated dropwise with a solution of **6** (0.12 g, 0.2 mmol) in THF (3 mL). The reaction mixture was heated under reflux for 24 h under N_2 . The solvent was evaporated and water (10 mL) was added to the residue, precipitating a yellow orange solid, which was filtered, (0.12 g). Column chromatography eluting with ethyl acetate and

light petroleum (9:1) gave macrocycle **8a** as white crystals of the monohydrate (0.04 g, 35 %), m.p. 203-205 °C; $\nu_{\max}/\text{cm}^{-1}$ (film) 3434 (N-H), 2099, 1648, 1550, 1453, 1260, 1126, 1011, 744; δ_{H} (400 MHz, CDCl_3) 7.75 (2H, d, $^3J_{\text{HH}}$ 8.0), 7.29-7.18 (4H, m), 6.99 (2H, d, $^3J_{\text{HH}}$ 8.4), 5.28 (2H, s), 4.05-4.00 (2H, m), 3.81-3.73 (4H, m), 3.45-3.39 (2H, m), 2.93-2.82 (2H, m), 2.68-2.63 (2H, m), 2.04-1.87 (4H, m); δ_{C} (100 MHz, CDCl_3) 153.8, 146.4 (dd, $^1J_{\text{CF}}$ 235, $^2J_{\text{CF}}$ 14), 142.9, 142.0 (t, $^2J_{\text{CF}}$ 13), 137.8 (dd, $^1J_{\text{CF}}$ 255, $^2J_{\text{CF}}$ 5), 134.9, 130.7 (dd, $^1J_{\text{CF}}$ 254, $^2J_{\text{CF}}$ 30), 123.6, 123.3, 123.5-123.0 (m), 119.9, 109.4, 68.6, 40.6, 27.0, 26.1; δ_{F} (376 MHz, CDCl_3) 72.6 (2F, dd, J_{FF} 29.5, J_{FF} 23.0), 11.7 (2F, d, J_{FF} 29.5), 0.55 (2F, dd, J_{FF} 22.5, J_{FF} 8.1); HRMS (ESI) found m/z 653.2196 (MH^+), $\text{C}_{32}\text{H}_{27}\text{F}_6\text{N}_8\text{O}$ requires 653.2207; Anal. (%) calcd for $\text{C}_{32}\text{H}_{26}\text{F}_6\text{N}_8\text{O}\cdot\text{H}_2\text{O}$ (670): C, 57.31; H, 4.17; N, 16.72; found: C, 57.38; H, 4.18; N, 16.41.

4.9. Reaction of 1,4-bis-1-tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane **6** with 2,2-(ethylenedioxy)bis(ethylamine): macrocycle **8b**

A solution of 2,2-(ethylenedioxy)bis(ethylamine) (0.06 g, 0.4 mmol) in THF (3 mL) was added dropwise to stirred solution of compound **6** (0.24 g, 0.4 mmol) in THF (5 mL), and the mixture heated under reflux for 24 h under N_2 . Distilled water (10 mL) was added and the mixture extracted with CH_2Cl_2 (3×20 mL). The organic extract was washed with saturated NaCl (aq), dried over MgSO_4 , filtered and evaporated to give a white solid, (0.23 g). Column chromatography eluting with ethyl acetate and light petroleum (1:1) gave colourless crystals of **8b** dihydrate (0.06 g, 22 %), m.p. 133-135 °C; $\nu_{\max}/\text{cm}^{-1}$ (film) 3311 (N-H), 2925, 2840, 1652, 1508, 1454, 1263, 1097, 990, 744; δ_{H} (400 MHz, CDCl_3) 7.79 (2H, d, $^3J_{\text{HH}}$ 7.6), 7.37-7.33 (2H, td, $^3J_{\text{HH}}$ 7.2, $^4J_{\text{HH}}$ 0.8), 7.30-7.26 (2H, td, $^3J_{\text{HH}}$ 8.0, $^4J_{\text{HH}}$ 1.2), 7.04 (2H, d, $^3J_{\text{HH}}$ 7.6), 5.37 (2H, t, $^3J_{\text{HH}}$ 4.8), 3.83-3.59 (12H, m), 2.82-2.63 (4H, m), 2.03-1.87 (4H, m); δ_{F} (376 MHz, CDCl_3) 72.9 (2F, dd, J_{FF} 29.5, J_{FF} 22.5), 11.95 (2F, d, J_{FF} 29.0), -0.74 (2F, dd, J_{FF} 22.5, J_{FF} 8.1); HRMS (ESI) found m/z 697.2462 (MH^+), $\text{C}_{34}\text{H}_{31}\text{F}_6\text{N}_8\text{O}_2$ requires 697.2469; Anal. (%) calcd for $\text{C}_{34}\text{H}_{30}\text{F}_6\text{N}_8\text{O}_2\cdot 2\text{H}_2\text{O}$ (732): C, 55.74; H, 4.64; N, 15.30; found: C, 55.88; H, 4.37; N, 14.91.

4.10. 1,4-bis-1-(4-Methoxycarbonyltetrafluorophenyl)-1H-benzimidazol-2-ylbutane **9**

A solution of methyl pentafluorobenzoate (0.452 g, 2 mmol) in DMSO (3 mL) was added to a stirred suspension of NaH (60 % dispersion in mineral oil) (0.08 g, 2 mmol) in DMSO (3 mL). A solution of **5** (0.29 g, 1 mmol) in DMSO (4 mL) was added dropwise by syringe pump and the mixture stirred at room temperature for 24 h under N_2 . The reaction mixture was quenched by dropwise addition of methanol, and distilled water (10 mL) added. A light yellow solid formed and was collected by suction filtration, (0.8 g) and recrystallised from ethanol to give **9** as colourless

crystals (0.41 g, 58 %), m.p. 195-198 °C; $\nu_{\max}/\text{cm}^{-1}$ (film), 2924, 1642, 1471, 1255, 1136, 972, 743; δ_{H} (400 MHz, CDCl_3) 7.81 (2H, d, $^3J_{\text{HH}}$ 7.6), 7.30-7.38 (m, 4H), 7.04 (2H, d, $^3J_{\text{HH}}$ 8.0), 4.09 (6H, 2), 2.87 (4H, m), 2.06 (4H, m); δ_{C} (100 MHz, CDCl_3) 159.3, 154.0, 145.4 (dd, $^1J_{\text{CF}}$ 263, $^2J_{\text{CF}}$ 15), 142.9, 143.3 (dd, $^1J_{\text{CF}}$ 250, $^2J_{\text{CF}}$ 15), 135.0, 123.8, 123.5, 119.9, 118.0 (t, $^2J_{\text{CF}}$ 15), 114.1 (t, $^2J_{\text{CF}}$ 17), 109.3, 53.8, 27.1, 26.5; δ_{F} (376 MHz, CDCl_3) 25.3-25.2 (4F, AA'), 19.3-19.2 (4F, BB'); HRMS (ESI) found m/z 703.1569 (MH^+), $\text{C}_{34}\text{H}_{23}\text{F}_8\text{N}_6\text{O}_4$ requires 703.1586.

4.11. 2,2-(Ethylenedioxy)-bis-ethyl pentafluorobenzamide **10**

A stirred solution of 2,2-(ethylenedioxy)-bis-(ethylamine) (0.148 g, 1 mmol) and Et_3N (0.202 g, 2 mmol) in THF (4 mL) was treated dropwise with pentafluorobenzoyl chloride (0.46 g, 2 mmol) in THF (2 mL) and the reaction mixture maintained at room temperature under the N_2 for 24 h. The solvent was evaporated and water (10 mL) added to the residue. A yellow orange solid precipitated, and was filtered, (0.5 g). Recrystallisation from CH_2Cl_2 and light petroleum gave **10** as shiny white crystals (0.38 g, 71 %); m.p. 128 - 129 °C; $\nu_{\max}/\text{cm}^{-1}$ (film), 3279 (N-H), 2872, 1658 (C=O), 1501, 1135, 1119, 992, 731; δ_{H} (400 MHz, CDCl_3) 6.55 (2H, s, NH), 3.70-3.66 (8H, m), 2.87 (4H, m); δ_{C} (100 MHz, CDCl_3) 157.6, 144.2 (dd, $^1J_{\text{CF}}$ 255, $^2J_{\text{CF}}$ 12), 142.3 (dd, $^1J_{\text{CF}}$ 260, $^2J_{\text{CF}}$ 25), 137.5 (dd, $^1J_{\text{CF}}$ 230, $^2J_{\text{CF}}$ 32), 111.5 (t, $^2J_{\text{CF}}$ 30), 70.8, 69.8, 40.1; δ_{F} (376 MHz, CDCl_3) 21.2-21.1 (4F, AA'), 11.26 (2F, t, $^3J_{\text{FF}}$ 20.3), 1.74-1.90 (4F, BB'); HRMS (ESI) found m/z 537.0867 (MH^+), $\text{C}_{20}\text{H}_{15}\text{F}_{10}\text{N}_2\text{O}_4$ requires 537.0867; Anal. (%) calcd for $\text{C}_{20}\text{H}_{14}\text{F}_{10}\text{N}_2\text{O}_4$ (536): C, 44.79; H, 2.63; N, 5.22; found: C, 44.78; H, 2.44; N, 5.25.

4.12. 2,2'-(Ethylenedioxy)-bis-ethyl 4-benzimidazol-1-yltetrafluorobenzamide **12**

A solution of benzimidazole (0.236 g, 2 mmol) in THF (3 mL) was added dropwise to a stirred suspension of NaH (60 % dispersion in mineral oil) (0.08 g, 2 mmol) in THF (3 mL). After 30 min a solution of **10** (0.536 g 1 mmol) in THF (3 mL) was added dropwise. The reaction mixture was left to stir at room temperature under N_2 for 24 h. Distilled water (10 mL) was added and the mixture extracted with CH_2Cl_2 (3 $\times$ 25 mL). The organic extract was washed with saturated NaCl (aq), dried over MgSO_4 , filtered and evaporated to give a white solid, (0.65 g). Column chromatography eluting with ethyl acetate and methanol (9:1) gave **12** as white crystals (0.4 g, 54 %); m.p. 115-117 °C; $\nu_{\max}/\text{cm}^{-1}$ (film) 3194 (N-H), 2877, 166 (C=O), 1550, 1418, 1280, 1134, 987, 710, 748; δ_{H} (400 MHz, CDCl_3) 8.03 (2H, s), 7.91-7.89 (2H, m), 7.43-7.38 (4H, m), 7.22 (2H, d, $^3J_{\text{HH}}$ 6), 7.00 (2H, s), 3.75-3.73 (12H, m); δ_{F} (376 MHz, CDCl_3) 22.6-22.5 (4F, AA'), 17.8-17.7 (4F, BB'); HRMS (ESI) found m/z 731.1657 (M-H^-), $\text{C}_{34}\text{H}_{23}\text{F}_8\text{N}_6\text{O}_4$ requires 731.1659.

4.13. Cell Cultures

Human cancer cell lines MCF-7, K562, G361 and HOS (purchased from the American Type Culture Collection) were maintained in DMEM supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). All cell lines were cultivated at 37 °C in 5% CO₂ as described previously [39, 40].

4.14. Cytotoxicity Assays

The cytotoxicity of the studied compounds was determined as described previously [39, 40]. Briefly, cells were assayed with compounds using three-fold dilutions in triplicate. Treatment lasted for 72 h, followed by addition of Calcein AM solution and measurement of the fluorescence of live cells at 485 nm/538 nm (excitation/emission) with a Fluoroskan Ascent microplate reader (Labsystems). The IC₅₀ value, the drug concentration lethal to 50% of the tumor cells, was calculated from the obtained dose response curves using GraphPad Prism version 5.0.

4.15. Flow cytometry

K562 cells (5×10^5 cells/mL) were incubated with test compounds for 24 h. After incubation, cells were centrifuged, washed in PBS, fixed with 70% ethanol, washed in PBS again, stained with propidium iodide and analysed by flow cytometry using a 488 nm laser (Cell Lab Quanta SC, Beckman Coulter).

4.16. Caspase activity assay

The cells were homogenised in an extraction buffer (10 mM KCl, 5 mM HEPES, 1 mM EDTA, 1 mM EGTA, 0.2% CHAPS, inhibitors of proteases, pH 7.4) on ice for 20 min. The homogenates were clarified by centrifugation at $10000 \times g$ for 30 min at 4 °C, and then the proteins were quantified and diluted to equal concentrations. Lysates were then incubated for 3 h with 100 µM Ac-DEVD-AMC as a substrate of caspases 3 and 7 in the assay buffer (25 mM PIPES, 2 mM EGTA, 2 mM MgCl₂, 5 mM DTT, pH 7.3). The fluorescence of the product was measured using a Fluoroskan Ascent microplate reader (Labsystems) at 355/460 nm (excitation/emission) as described previously [39, 40].

Table 3. Crystallographic data for compounds **4**, **5**, **8a·EtOH**, **8b·2½H₂O**, **8b·CHCl₃**, **9**, and **10**.

Compound	4	5	8a·EtOH	8b·2½H₂O	8b·CHCl₃
Formula	C ₁₇ H ₄ F ₈ N ₄ S	C ₁₈ H ₁₈ N ₄	C ₃₂ H ₂₆ F ₆ N ₈ O·C ₂ H ₆ O	C ₃₄ H ₃₀ F ₆ N ₈ O ₂ ·2½H ₂ O	C ₃₄ H ₃₀ F ₆ N ₈ O ₂ ·CHCl ₃
Formula weight	448.30	290.36	698.67	741.70	816.03
Crystal system	triclinic	orthorhombic	monoclinic	orthorhombic	monoclinic
Space group	<i>P</i> $\bar{1}$	<i>Pbca</i>	<i>C2/c</i>	<i>P2₁2₁2</i>	<i>P2₁/n</i>
Unit cell dimensions					
<i>a</i> (Å)	9.4800(9)	8.788(3)	14.1373(7)	22.3299(9)	13.8932(16)
<i>b</i> (Å)	11.4612(11)	9.340(3)	17.2114(8)	6.0739(3)	14.1669(16)
<i>c</i> (Å)	15.7757(15)	17.743(5)	27.3518(13)	12.2892(5)	18.231(2)
α (°)	75.3725(14)	90	90	90	90
β (°)	89.9135(15)	90	103.7199(8)	90	99.7139
γ (°)	80.8998(15)	90	90	90	90
<i>V</i> (Å ³)	1636.3(3)	1456.3(8)	6465.4(5)	1666.78(13)	3536.8(7)
<i>Z</i>	4	4	8	2	4
Temperature (K)	150(2)	150(2)	150(2)	100(2)	150(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.7749	0.71073
Calculated density (g·cm ⁻³)	1.820	1.324	1.436	1.478	1.532
Absorption coefficient (mm ⁻¹)	0.30	0.08	0.12	0.15	0.34
Transmission factors (max./min.)	0.948 and 0.855	0.988 and 0.914	0.992 and 0.971	0.997 and 0.985	0.888 and 0.839
Crystal size (mm ³)	0.54 × 0.43 × 0.18	1.13 × 0.23 × 0.15	0.25 × 0.18 × 0.07	0.10 × 0.03 × 0.02	0.54 × 0.54 × 0.36
θ (max) (°)	30.6	30.6	28.3	30.8	28.4
Reflections measured	26360	15966	33520	16401	35615
Unique reflections	9927	2226	8064	4015	8798
<i>R</i> _{int}	0.022	0.047	0.047	0.068	0.051
Reflections with <i>F</i> ² > 2σ(<i>F</i> ²)	8270	1773	5498	3344	5995
Number of parameters	573	136	477	278	278
<i>R</i> ₁ [<i>F</i> ² > 2σ(<i>F</i> ²)]	0.037	0.042	0.042	0.053	0.064
<i>wR</i> ₂ (all data)	0.098	0.115	0.101	0.140	0.209
GOOF, S	1.04	1.05	1.03	1.05	1.02
Largest difference peak and hole (e Å ⁻³)	0.46 and -0.45	0.34 and -0.21	0.24 and -0.21	0.29 and -0.38	0.68 and -0.86

Table 3 cont'd.

Compound	9	10
Formula	C ₃₄ H ₂₂ F ₈ N ₄ O ₄	C ₂₀ H ₁₄ F ₁₀ N ₂
Formula weight	702.55	536.33
Crystal system	monoclinic	monoclinic
Space group	<i>I</i> 2/ <i>a</i>	<i>P</i> 2 ₁ / <i>c</i>
Unit cell dimensions		
<i>a</i> (Å)	19.230(2)	17.4161(6)
<i>b</i> (Å)	8.3173(10)	14.3221(5)
<i>c</i> (Å)	38.209(5)	25.7502(9)
<i>α</i> (°)	90	90
<i>β</i> (°)	99.650(2)	101.207(2)
<i>γ</i> (°)	90	90
<i>V</i> (Å ³)	6024.7(12)	6300.5(4)
<i>Z</i>	8	12
Temperature (K)	150(2)	150(2)
Wavelength (Å)	0.71073	0.7749
Calculated density (g.cm ⁻³)	1.549	1.696
Absorption coefficient (mm ⁻¹)	0.14	0.17
Transmission factors (max./min.)	0.980 and 0.893	0.998 and 0.992
Crystal size (mm ³)	0.85 × 0.40 × 0.15	0.05 × 0.03 × 0.01
<i>θ</i> (max) (°)	30.6	25.6
Reflections measured	35137	44141
Unique reflections	9202	9083
<i>R</i> _{int}	0.038	0.056
Reflections with <i>F</i> ² > 2σ(<i>F</i> ²)	6386	6863
Number of parameters	539	991
<i>R</i> ₁ [<i>F</i> ² > 2σ(<i>F</i> ²)]	0.045	0.055
<i>wR</i> ₂ (all data)	0.124	0.142
GOOF, <i>S</i>	1.05	1.09
Largest difference peak and hole (e Å ⁻³)	0.39 and -0.24	0.83 and -0.32

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[26] Details of X-ray crystallography.

Diffraction data for **4**, **5**, **8a·EtOH**, **8b·CHCl₃** and **9**, were collected on a Bruker APEX 2 CCD diffractometer equipped with graphite-monochromated Mo-K α X-radiation. Diffraction data for **8b·H₂O** were collected on a Bruker APEX 2 CCD diffractometer equipped with a silicon 111 monochromator using narrow frame ω and ϕ scans on Station 11.3.1 at the ALS using synchrotron radiation [41]. Data for **10** were also collected at the ALS but with a Bruker D8 diffractometer and CMOS detector using shutterless ω -scans. Data were corrected for absorption and Lp effects [41]. Structures were solved by direct methods or charge flipping algorithms [42, 43] and refined by full-matrix least squares on F^2 [44, 45]. Further details are given in Table 3. In **4** there are two molecules in the asymmetric unit. In **8a·EtOH** atoms C(33) & C(34) were modelled as disordered over two sets of positions with major component 62.2(7)%. In **8b·2½H₂O** atoms C(15), C(16), C(17) & O(1) were modelled as disordered over two sets of positions with major component 75.6(11)%. Absolute structure parameter $x = 0.4(5)$, so was not reliably determined. H atoms on the water molecules could not be definitively located. Atom O(3) is only a quarter occupied due to the disorder in the chain. CCDC 1453894-1453900 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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