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Synthesis, Structural Characterization and Antiangiogenic Activity of Polyfluorinated Benzamides

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The introduction of fluorine into bioactive molecules is a matter of importance in medicinal chemistry. In this study, representatives of various chemical entities of fluoroaromatic compounds have been synthesized. Depending on the reaction conditions, either tetrafluorophthalimides or ammonium tetrafluorophthalamates are accessible from tetrafluorophthalic anhydride and primary amines. Tetrafluorophthalamic acids undergo thermal decarboxylation to yield tetrafluorobenzamides. These could be successfully converted upon treatment with primary amines, in the course of an aromatic nucleophilic substitution, to 2,3,5-trifluorobenzamides with respective amino substituents at 4-position. The five structure types have been characterized by means of spectroscopic and crystallographic methods. The synthesized compounds were evaluated as inhibitors of angiogenesis by measuring the microvessel outgrowth in the rat aortic ring assay. The biological activity was maintained throughout these different polyfluorinated chemotypes.

The importance of tetrafluorination of simple phthalimides to achieve antiangiogenic activity has been shown.^[3] Similarly, compound **I** (Figure 1), a polyfluorinated phthalimide derivative, leads to a strongly enhanced inhibition of the microvessel outgrowth in an angiogenesis model,^[4] and of the TNF- α production by monocytes.^[5] It possesses antimyeloma capacity, activates the stress kinase p38 pathway, affects blood vessels depending on their state of maturity,^[6] and was active in a mouse xenograft model at low doses in combination with flavopiridol.^[7] Whereas phthalimidobarbituric acids of type **II** failed to act as antiangiogenic agents, the fluorinated analogs **III** showed a strong decrease in microvessel outgrowth in rat aortic ring explants.^[8,9] The intact fused five-membered ring was not essential since the activity was retained in case of the tetrafluorobenzamide derivatives **IV**.^[8,9]

Introduction

Angiogenesis, the formation of new blood vessels from existing ones, is driven by a finely tuned balance between factors which constitute a functional network. The process of angiogenesis is important for several physiological and pathological conditions, including cancer, where functional vessels are required to supply nutrients and oxygen, and to sustain tumor growth and metastatic dissemination. Angiogenesis can be inhibited by blocking pro-angiogenic or raising antiangiogenic factors or by killing tumor-activated endothelial cells. Antiangiogenic drugs have been shown to slow tumor progression and metastasis.^[1] Accordingly, therapies based on such drugs represent a promising approach for the treatment of cancer diseases and there remains a need for novel agents against angiogenesis.^[2]

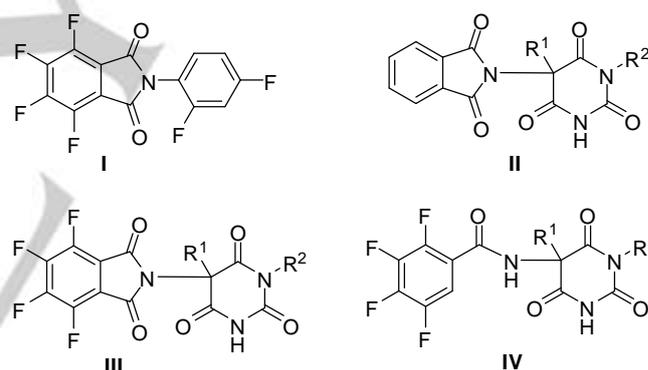


Figure 1. Structures of (fluorinated) phthalimide and benzamide derivatives as antiangiogenic agents.

Covalently bound fluorine has attracted much attention in drug development, although it is an unusual element in natural products. The substitution of hydrogen by fluorine in bioactive molecules produces only minor changes in the 3D structure due to the similar van der Waals radii of fluorine and hydrogen. Strong dipole moments of the carbon-fluorine bond result from the large electronegativity of fluorine and may contribute to inter- and intramolecular interactions.^[10]

The property of covalently bound fluorine in organic molecules as a hydrogen-bond acceptor has been a controversial issue. Meanwhile, a number of spectroscopic, crystallographic and computational studies provided several lines of evidence for weak C-F \cdots H-X hydrogen bonds. Hydrogen bonds to fluorine have the potential to effectively alter the binding events of fluorinated compounds to biological target structures.^[11] In addition to an enhanced target affinity, increased metabolic stability can be attained; in particular, labile sites of metabolic oxidation can be blocked by fluorination.^[10]

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The high electronegativity of fluorine and the resulting polarization of the C-F bond lead to a remarkable alteration of the physicochemical properties, such as acidity or basicity of adjacent functional groups. Moreover, the addition of fluorine or trifluoromethyl groups on aryl or heteroaryl groups can increase the lipophilicity and enhance the membrane permeability in comparison with the non-fluorinated parent compounds.^[10]

In the course of the determination of favorable (fluorophilic) and unfavorable (fluorophobic) environments within target proteins, inhibitors of serine and metallo proteases,^[12] cysteine proteases,^[13] and protein kinases^[14] have been established.

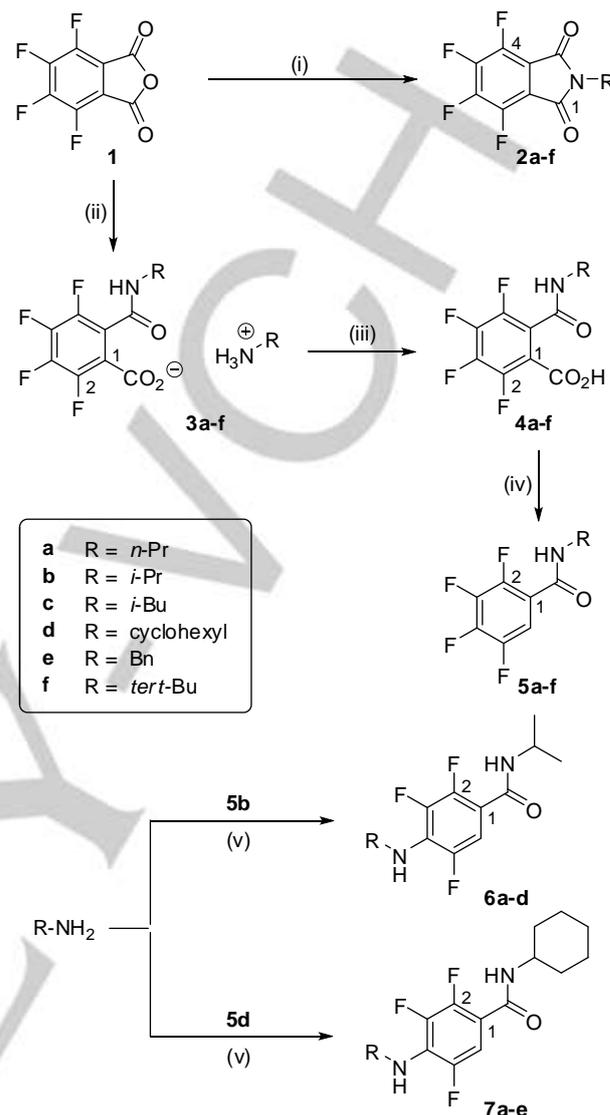
In this study, it was intended to incorporate several simplified amino components into tetrafluorophthalimides **III** and tetrafluorobenzamides **IV** and to extend the series of test compounds by two types of related polysubstituted fluorobenzene derivatives decorated with the same amino components. The resulting compounds were investigated on the basis of spectroscopic and crystallographic data. Finally, the entire library of fluoro compounds was subjected to the examination of antiangiogenic properties.

Results and Discussion

Six representatives of the chemotype of tetrafluorophthalimides (**2a-f**) were synthesized by condensation of tetrafluorophthalic anhydride (**1**) with different primary amines. The ring-opening/recyclization reactions were performed in AcOH at 118 °C (Scheme 1). Under different conditions, when treating **1** with primary amines in refluxing toluene, the corresponding ammonium tetrafluorophthalamates (**3a-f**) were obtained. In the absence of AcOH, the recyclization step is prevented because the products accumulate as salts without carbonyl reactivity and precipitate. Tetrafluorophthalamic acids **4a-f** were easily accessible by protonation of **3** in an aqueous medium. Chemotypes **3** and **4** constitute intermediate structures of a previously reported tandem ring opening/decarboxylation sequence.^[9] In the present study, we have isolated and characterized these compounds and provided those of type **4** to a biological evaluation.

Thermal decarboxylation of tetrafluorophthalamic acids **4** was achieved in DMSO at 153 °C to successfully produce **5a-f**. These tetrafluorobenzamides represent structurally simplified analogs of the aforementioned antiangiogenic barbituric acids **IV** (Figure 1).

To introduce a further point of diversity, we considered to subject tetrafluoro derivatives to a nucleophilic substitution with amines. The reaction of certain phthalimides of type **2** with primary amines led to mixtures of 4- and 5- substituted regioisomers, which influenced cellular vesicular systems, in particular the generation of lipids droplets.^[15] Mixtures of even four isomeric products might be formed from tetrafluorophthalamic acids **4** and amines. However, the transformation of tetrafluorobenzamides **5** is expected to proceed regioselectively. A corresponding conversion has been reported, even though in rare cases.^[16]



Scheme 1. Synthesis of polyfluorinated compounds **2-7**. Conditions (i) RNH₂, AcOH, reflux, 3 h; (ii) RNH₂, dry toluene, reflux, 3 h; (iii) H₂O, pH 1-2, 4 °C; (iv) 1. dry DMSO, 153 °C, 4 h, 2. H₂O, rt, 3 d; (v) Δ, 4 h.

We selected two tetrafluorobenzamides (**5b** and **5d**) for the reaction with primary amines, the isopropyl derivative **5b** because of its promising antiangiogenic activity (Table 1) and **5d** because the bulky cyclohexyl moiety was expected to be advantageous. The nucleophilic substitution occurred unsurprisingly at the *para* position. With the so substituted products **6a-d** and **7a-e**, we received nine highly functionalized benzene derivatives. This transformation could also be accomplished under microwave assisted conditions with similar yields, as exemplified for **7c** and **7d**.

The ¹³C NMR spectra of tetrafluorophthalimides **2** exhibited three distinct shifts of the carbon resonances of the fused benzene ring, C-3a/7a, C-4/7, and C5/6. Our assignments are in agreement with previous work^[9] and a detailed NMR investigation including *N*-phenyl-tetrafluorophthalimide.^[17] The

magnitude of the C-F coupling of carbons C-3a/7a varied from 7 to 10 Hz, although larger constants could be assumed for two-bond fluorine couplings, e.g. as described for C-3a/7a in *N*-phenyl-tetrafluorophthalimide ($^2J(\text{C},\text{F}) = 13.5$ Hz).^[17] However, smaller values ($^2J(\text{C},\text{F}) \sim 7$ Hz) have been reported for four derivatives of *N*-phenyl-tetrafluorophthalimide.^[18] The $^2J(\text{C},\text{F})$ coupling constants of 4-fluoroisindoline (**8**, Figure 2) of ~ 19 Hz for both C-3a and C-5 indicate that carbons which are engaged in ring-fusion do not necessarily produce small $^2J(\text{C},\text{F})$ constants. Thus, the fixed orientation of the carbonyl groups relative to the C-F bonds at positions 4 and 7 attributes to the small coupling constants in case of **2a-f**.

The C-1 and C-6 signals of ammonium tetrafluorophthalamates **3** derived from $^2J(\text{C},\text{F})$ coupling constants of either around 22 Hz or 15 Hz could initially not be distinguished. In order to assign the chemical shifts and the corresponding coupling constants to carbons C-1 and C-6, we have recorded a ^{13}C NMR spectrum of 2-fluorobenzamide (**9**, Figure 2).^[19] An X-ray crystal structure of **9** revealed the orientation of the NH_2 moiety to the fluorine substituent, while in other *ortho*-halobenzamides the oxygen pointed in the direction of the halogen.^[20] As in **9**, a preferred intramolecular $\text{F}\cdots\text{H}-\text{N}$ interaction was also postulated for our compounds **3**. We have now considered the $^2J(\text{C}-1,\text{F})$ coupling constant in **9** of 14.2 Hz as a reference for the $^2J(\text{C}-6,\text{F})$ coupling constants in compounds **3**. Accordingly, we could assign the ^{13}C NMR signals with $^2J(\text{C},\text{F})$ values of around 15 Hz to C-6 (δ 120 ppm) and those with 22 Hz to C-1 (δ 128 ppm). In 3-fluorobenzamide (**10**, Figure 2), the $^2J(\text{C}-2,\text{F})$ coupling constant of 22.6 Hz was determined. Thus, the magnitude of the heteronuclear spin-spin coupling depended not only on the distance between the carbon and fluorine nuclei, but also on the orientation of their orbitals and intramolecular interactions.

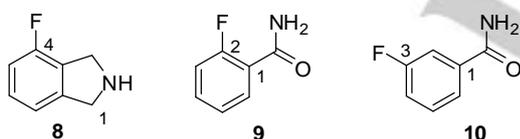


Figure 2. Reference compounds.

Tetrafluorophthalamic acids **4** exhibited $^2J(\text{C},\text{F})$ values for the heteronuclear coupling of C-6 but also of C-1 were between 13 and 19 Hz, indicating a different pattern of intramolecular interactions in comparison to the corresponding salts **3**. The assignment of the ^{13}C NMR signals was based on the chemical shifts and in relation to compounds **3** and **5** (see below); hence C-6 resonates at 123 ppm, C-1 at 118 ppm. A single crystal X-ray analysis of **4a** as a representative compound was carried out (Figure 3). As in case of 2-fluorobenzamide (**9**), the NH is oriented towards the 5-fluorine substituent to form an intramolecular $\text{H}\cdots\text{F}$ hydrogen bond. It produces, together with an intermolecular contact to a carbonyl oxygen, a bifurcated hydrogen bond network and accounts for a dihedral angle of 37° between the tetrafluorinated benzene ring and the plane through the carboxamide moiety. The carboxylate plane is twisted

relative to the aromatic plane by 70° . A sandwich π - π stacking is created with alternating orientation of the substituents and a 3.7 Å centroid-distance of the benzene rings.

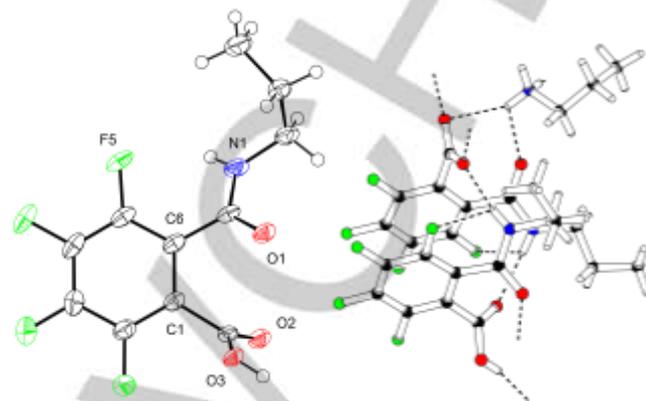


Figure 3. Left. Molecular plot of 2,3,4,5-tetrafluoro-6-(propylcarbamoyl)benzoic acid (**4a**) showing the displacement ellipsoids at the 50% probability level for the non-H atoms. Right. Packing diagram of **4a**. The $\text{N}-\text{H}\cdots\text{F}$ and $\text{N}-\text{H}\cdots\text{O}$ contacts are shown as broken lines.^[21]

The regioselectivity of the nucleophilic substitution was confirmed by the chemical shifts in the ^{13}C NMR spectra. Compounds **6** and **7** exhibited $^2J(\text{C}-1,\text{F})$ values of ~ 13 Hz, similar to the coupling constants of the carboxamide-substituted aromatic carbons in **3** and **4**. The unsubstituted aromatic carbons of **5**, **6** and **7** showed $^2J(\text{C}-6,\text{F})$ constants in the usual range of 20-23 Hz. An X-ray refraction of **7e** (Figure 4) revealed a sandwich π - π stacking (4.5 Å centroid-distance) and that the residues are pointing in the same direction. The amidic and every other anilinic NH moiety make intramolecular $\text{N}-\text{H}\cdots\text{F}$ bonds. Due to further intermolecular $\text{N}-\text{H}\cdots\text{O}$ contact, a bifurcated H-bond motif is formed. The geometrical analysis demonstrated a chair conformation of the cyclohexane ring.

To determine the antiangiogenic activity of the synthesized compounds, the rat aortic ring assay was performed and the compounds were administered at a concentration of 50 μM (Table 1). 5-Amino-1-[[3,5-dichloro-4-(4-chlorobenzoyl)phenyl]-methyl]-1*H*-1,2,3-triazole-4-carboxamide (CAI), a synthetic, cytostatic inhibitor of non-voltage-operated calcium channels and calcium channel-mediated signaling pathways, as well as *O*-chloroacetyl-carbamoyl fumagillol (TNP-470), a methionine aminopeptidase-2 and neovascularization inhibitor, were used as positive controls. Moreover, data of two particularly potent representatives of tetrafluorophthalimido and benzamido barbituric acids (types **III** and **IV**) were included in Table 1.

In comparison to the DMSO-treated ring explants, most of the test compounds of chemotypes **2**, **4** and **5** showed moderate inhibitory activities, with the *N*-isopropyl-tetrafluorophthalimide **2b** as the most potent derivative. Thus, the isopropyl residue was advantageous for antiangiogenic activity. However, the strong activity of tetrafluorophthalimides with a barbituric acid moiety as the *N*-substituent, as reported previously,^[9] was not reached with compound **2b**. The corresponding derivatives of

chemotypes **4** and **5** bearing the isopropyl moiety, *i.e.* **4b** and **5b**, showed lower activity, compared to **2b**. The antiangiogenic potential of tetrafluorobenzamides has already been elucidated,^[9] and was confirmed for chemotype **5** in this study. Tetrafluorophthalamic acids have first been shown as antiangiogenic agents herein. The exchange of hydrogen by a carboxyl group did not have a strong impact on angiogenesis inhibition, as can be seen by a comparison of representatives **5a-f** with the analogues **4a-f**. Chemotypes **4** and **5** are considered to be more appropriate for further structural optimization because these ring-opened derivatives are more conformationally flexible than phthalimides of type **2**. Moreover, phthalimides **2** are inferior in stability because of the easy opening of the five-membered ring.

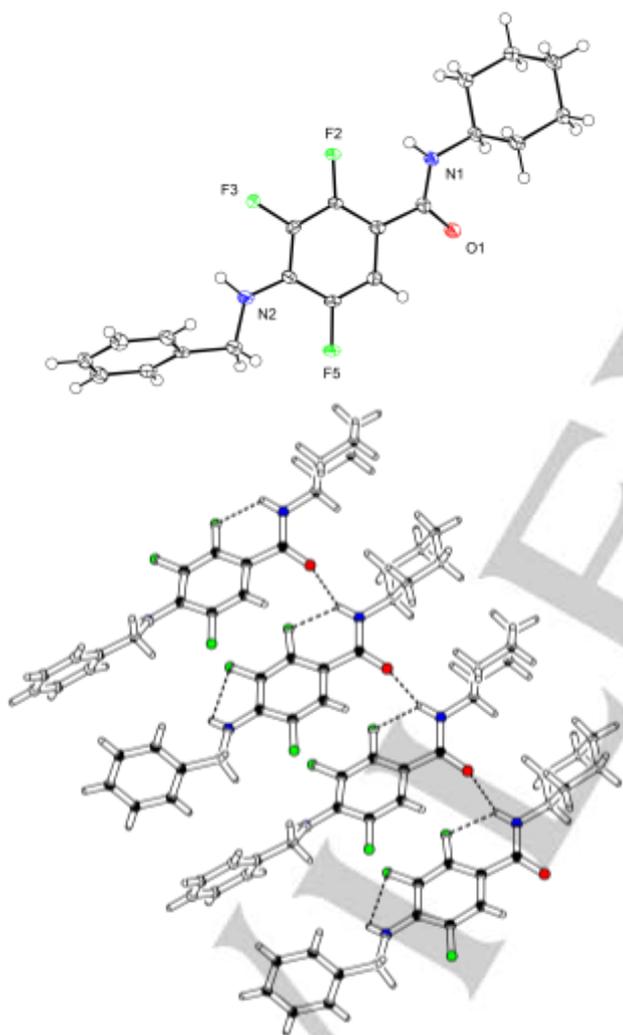


Figure 4. Top. Molecular plot of 4-(benzylamino)-*N*-cyclohexyl-2,3,5-trifluorobenzamide (**7e**) showing the displacement ellipsoids at the 50% probability level for the non-H atoms. Bottom. Packing diagram of **7e**. The N–H...F and N–H...O contacts are shown as broken lines.^[21]

We selected two tetrafluorobenzamides, **5b** and **5d**, and introduced a further point of diversity, *i.e.* an amino substituent at position 4. The antiangiogenesis data in response to trifluorobenzamides **6** and **7** at an inhibitor concentration of 50 μ M are presented in Table 2. Among the *N*-isopropylcarboxamides **6**, the cyclohexylamino derivative **6d** exhibited an improved activity (80% inhibition), when compared to its precursor **5b** (58% inhibition). In case of **5d** (35% inhibition), the exchange of fluorine by all four different amino groups led to slightly improved inhibitors **7** (> 50% inhibition). Thus, trifluorobenzamides have been identified as a new chemotype of antiangiogenic agents.

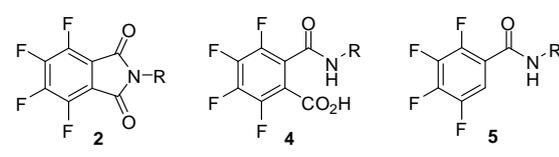
The antiangiogenic activity of the two best compounds in the rat aortic ring model was complemented by a preliminary *in vitro* human umbilical vein endothelial cell (HUVEC) cytotoxicity assay, in which **2b** and **6d**, at a concentration of 10 μ M, inhibited the survival of HUVECs 74% and 78%, respectively.

Conclusions

In this study, different molecular entities of fluoroaromatic compounds were synthesized and subjected to detailed spectroscopic and crystallographic analyses. Such compounds are potentially bioactive.

We have determined the antiangiogenic activity of tetrafluorophthalimides with ordinary *N*-alkyl residues. Although these compounds **2** were less potent than tetrafluorophthalimides with a barbituric acid moiety as *N*-substituent, they provide a starting point for tailored structural modifications and a comparative analysis of the resulting chemotypes. With respect to antiangiogenic activity, ring opening as realized in **4-7** was tolerated. In particular, we found that one fluorine substituent can be replaced by an amino group leading to **6/7** without an essential loss of bioactivity. Thus, the repertoire of structural frameworks to develop angiogenesis inhibitors was expanded. Further biological studies are ongoing to understand the clinical utility of selected compounds from this cohort.

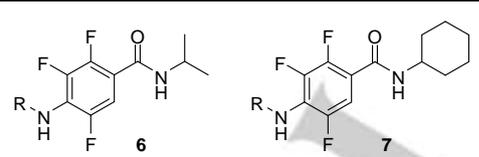
Future structural modifications are intended as follows. The carboxamide moiety of **6/7** can be revised and re-equipped with a barbituric acid unit. The scopes and limitations of the aromatic amino substitution need to be further explored. It can also be envisaged to utilize this substitution for the introduction of linkers which bear a second functionality. Thus, our study might pave the way for the design of bifunctional molecules with fluorescent or biotin labels as tool compounds for studying angiogenesis.

Table 1. Inhibitory effects of tetrafluoro derivatives **2**, **4** and **5** in the rat aortic ring assay of angiogenesis.


Substitution pattern	R	Inhibition [%] ^[a]		
		2	4	5
a	<i>n</i> -Pr	59	55	n.i. ^[b]
b	<i>i</i> -Pr	81	n.i.	58
c	<i>i</i> -Bu	67	50	31
d	cyclohexyl	59	30	35
e	Bn	10	50	31
f	<i>tert</i> -Bu	n.d. ^[c]	46	44

Positive controls ^[d]	Inhibition [%] ^[a]		
	CAI ^[e]	TNP-470	III ^[f] IV ^[f] R ¹ = Et, R ² = <i>i</i> -Pr
	73	47	>95 90

[a] Compounds were administered as a single dose at a concentration of 50 μ M and incubated for 5 days. Outgrowth was compared to microvessel outgrowth from rings treated with the control (0.5% DMSO). Control outgrowth is classed at 100% outgrowth. [b] n.i.: no inhibitory activity. [c] n.d.: not determined. [d] Incubation was conducted for 4 days. [e] A concentration of 30 μ M was used. [f] Data from ref. [9].

Table 2. Inhibitory effects of trifluoro derivatives **6** and **7** in the rat aortic ring assay of angiogenesis.


Substitution pattern	R	Inhibition [%] ^[a]	
		6	7
a	<i>n</i> -Pr	51	54
b	<i>i</i> -Pr	32	67
c	<i>i</i> -Bu	52	51
d	cyclohexyl	80	54
e	Bn	n.a. ^[b]	n.i. ^[c]

[a] Compounds were administered as a single dose at a concentration of 50 μ M and incubated for 5 days. Outgrowth was compared to microvessel outgrowth from rings treated with the control (0.5% DMSO). Control outgrowth is classed at 100% outgrowth. [b] n.a.: not applicable. [c] n.i.: no inhibitory activity.

Experimental Section

Melting points were determined on a Gallenkamp capillary melting point apparatus or on a Rapido Boetius apparatus, and are uncorrected. The purity of the obtained substances and the composition of the reaction mixtures were controlled by thin-layer chromatography (TLC) using Merck aluminum silica gel plates with 60 F₂₅₄ indicator. Preparative column chromatography was performed using Merck silica gel 60 (63–200 mesh). Petroleum ether used was a mixture of alkanes boiling between 40–60 °C, according to the supplier's declaration. NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (¹H NMR) and 125 MHz (¹³C NMR) in DMSO-*d*₆. Chemical shifts (δ) are reported in ppm. Spin multiplicities are indicated by the following symbols: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), sext (sextet), sept (septet), oct (octet), non (nonet), m (multiplet). All multiplets related with J(C, F) couplings in ¹³C NMR spectra are centered. Mass spectra were recorded on an MS-50 A.E.I. spectrometer under electron impact ionization (EI) at 70 eV. Elemental analyses were performed with a Vario EL apparatus.

Chemistry

Synthesis of tetrafluorophthalimides 2a-f: General Procedure. A mixture of the primary amine (1.5 mmol) and tetrafluorophthalic anhydride (**1**, 0.40 g, 1.8 mmol) in glacial AcOH (11 mL) was stirred under reflux for 3 h. The resulting solution was then evaporated to dryness under reduced pressure. The oily residue was recrystallized from EtOH.

4,5,6,7-Tetrafluoro-2-propylisoindoline-1,3-dione (2a) was prepared from **1** and propylamine (89 mg, 0.12 mL); yield 49% (0.19 g); mp 106–108 °C; ¹H NMR (DMSO-*d*₆) δ 0.86 (t, *J* = 7.4 Hz, 3H, CH₃), 1.59 (sext, *J* = 7.3 Hz, 2H, CH₂CH₃) 3.50 (t, *J* = 7.1 Hz, 2H, NCH₂); ¹³C NMR (DMSO-*d*₆) δ 11.22 (CH₃), 21.12 (CH₂CH₃), 39.91 (NCH₂), 114.30 (m, ³J(C,F) = 9.4 Hz, C-3a, C-7a), 142.50 (m, ¹J(C,F) = 264.2 Hz, C-4, C-7), 144.09 (m, ¹J(C,F) = 260.9 Hz, C-5, C-6), 162.86 (CO). Anal. calcd for C₁₁H₇F₄NO₂: C 50.59, H 2.70, N 5.36, found: C 50.65, H 2.61, N 5.40.

4,5,6,7-Tetrafluoro-2-isopropylisoindoline-1,3-dione (2b)^[22] was prepared from **1** and isopropylamine (89 mg, 0.13 mL); yield 51% (0.20 g); mp 148–151 °C; ¹H NMR (DMSO-*d*₆) δ 1.38 (d, *J* = 7.0 Hz, 6H, CH₃), 4.34 (sept, *J* = 6.9 Hz, 1H, CH); ¹³C NMR (DMSO-*d*₆) δ 19.61 (CH₃), 43.38 (CH), 114.09 (m, ³J(C,F) = 9.9 Hz, C-3a, C-7a), 142.46 (m, ¹J(C,F) = 264.1 Hz, C-4, C-7), 144.02 (m, ¹J(C,F) = 258.7 Hz, C-5, C-6), 162.62 (CO). Anal. calcd for C₁₁H₇F₄NO₂: C 50.59, H 2.70, N 5.36, found: C 50.59, H 2.90, N 5.36.

4,5,6,7-Tetrafluoro-2-isobutylisoindoline-1,3-dione (2c) was prepared from **1** and isobutylamine (110 mg, 0.15 mL); yield 99% (0.41 g); mp 136–138 °C; ¹H NMR (DMSO-*d*₆) δ 0.87 (d, *J* = 6.7 Hz, 6H, CH₃), 1.91–2.02 (m, 1H, CH), 3.35 (d, *J* = 7.3 Hz, 2H, CH₂); ¹³C NMR (DMSO-*d*₆) δ 20.02 (CH₃), 27.40 (CH), 45.57 (CH₂), 114.11 (m, ³J(C,F) = 7.4 Hz, C-3a, C-7a), 142.55 (m, ¹J(C,F) = 263.8 Hz, C-4, C-7), 144.16 (m, ¹J(C,F) = 262.5 Hz, C-5, C-6), 163.06 (CO). Anal. calcd for C₁₂H₉F₄NO₂: C 52.37, H 3.30, N 5.09, found: C 52.63, H 3.21, N 5.09.

4,5,6,7-Tetrafluoro-2-cyclohexylisoindoline-1,3-dione (2d)^[22] was prepared from **1** and cyclohexylamine (150 mg, 0.17 mL); yield 80% (0.36 g); mp 155–158 °C; ¹H NMR (DMSO-*d*₆) δ 1.10–2.04 (m, 10H, CH₂), 3.93 (tt, *J* = 12.5, 3.9 Hz, 1H, CH); ¹³C NMR (DMSO-*d*₆) δ 24.94 (C-4'), 25.48 (C-3', C-5'), 29.22 (C-2', C-6'), 51.12 (C-1'), 113.98 (m, ³J(C,F) = 7.7 Hz, C-3a, C-7a), 142.49 (m, ¹J(C,F) = 264.4 Hz, C-4, C-7), 144.06 (m,

$^1\text{J}(\text{C},\text{F}) = 262.5$ Hz, C-5, C-6), 162.64 (CO). Anal. calcd for $\text{C}_{14}\text{H}_{11}\text{F}_4\text{NO}_2$: C 55.82, H 3.68, N 4.65, found: C 55.58, H 3.76, N 4.72.

4,5,6,7-Tetrafluoro-2-benzylisoindoline-1,3-dione (2e) was prepared from **1** and benzylamine (161 mg, 0.16 mL); yield 93% (0.43 g); mp 177-179 °C; lit. mp 179-181 °C; ^{13}C NMR (DMSO- d_6) δ 4.74 (s, 2H, CH_2), 7.26-7.35 (m, 5H, Ar-H); ^{13}C NMR (DMSO- d_6) δ 41.65 (CH_2), 114.19 (m, $^3\text{J}(\text{C},\text{F}) = 8.3$ Hz, C-3a, C-7a), 127.73, 128.75 (C-2', C-6', C-3', C-5'), 127.81 (C-4'), 135.92 (C-1'), 142.70 (m, $^1\text{J}(\text{C},\text{F}) = 267.0$ Hz, C-4, C-7), 144.27 (m, $^1\text{J}(\text{C},\text{F}) = 263.6$ Hz, C-5, C-6), 162.69 (CO). Anal. calcd for $\text{C}_{15}\text{H}_7\text{F}_4\text{NO}_2$: C 58.26, H 2.28, N 4.53, found: C 58.19, H 2.32, N 4.56.

4,5,6,7-Tetrafluoro-2-tert-butylisoindoline-1,3-dione (2f) was prepared from **1** and *tert*-butylamine (110 mg, 0.16 mL); yield 11% (45 mg); mp 174-176 °C; ^1H NMR (DMSO- d_6) δ 1.59 (s, 9H, CH_3); ^{13}C NMR (DMSO- d_6) δ 28.41 (CH_3), 59.47 ($\text{C}(\text{CH}_3)_3$), 114.01 (m, $^3\text{J}(\text{C},\text{F}) = 7.2$ Hz, C-3a, C-7a), 142.36 (m, $^1\text{J}(\text{C},\text{F}) = 264.9$ Hz, C-4, C-7), 144.06 (m, $^1\text{J}(\text{C},\text{F}) = 262.5$ Hz, C-5, C-6), 163.49 (CO). Anal. calcd for $\text{C}_{12}\text{H}_9\text{F}_4\text{NO}_2$: C 52.37, H 3.30, N 5.09, found: C 52.36, H 3.12, N 5.04.

Synthesis of ammonium tetrafluorophthalamates 3a-f: General Procedure. A mixture of the primary amine (15 mmol) and tetrafluorophthalic anhydride (**1**, 1.65 g, 7.5 mmol) in dry toluene (500 mL) was stirred under reflux for 3 h. The mixture was allowed to cool down to room temperature, the resulting solid was filtered off, washed with *n*-hexanes (2 x 20 mL) and dried under reduced pressure.

Propylammonium 2,3,4,5-tetrafluoro-6-(propylcarbamoyl)benzoate (3a) was prepared from **1** and propylamine (0.89 g, 1.24 mL); yield 65% (1.65 g); mp 160-164 °C; ^1H NMR (DMSO- d_6) δ 0.87 (t, $J = 7.6$ Hz, 3H, CH_3), 0.87 (t, 7.3 Hz, 2H, CH_2), 1.46 (sext, $J = 7.3$ Hz, 2H, CH_2CH_3), 1.53 (sext, $J = 7.6$ Hz, 2H, CH_2CH_3), 2.69 (t, $J = 7.6$ Hz, 2H, NCH_2), 3.08-3.12 (m, 2H, NCH_2), 8.05 (br s, 3H, NH_3), 8.36 (t, $J = 5.7$ Hz, 1H, NH); ^{13}C NMR (DMSO- d_6) δ 11.01 (CH_3), 11.48 (CH_3), 20.62 (CH_2CH_3), 22.19 (CH_2CH_3), 40.53 (NCH_2), 41.02 (NCH_2), 119.69 (m, $^2\text{J}(\text{C},\text{F}) = 15.4$ Hz, C-6), 127.96 (m, $^2\text{J}(\text{C},\text{F}) = 21.6$ Hz, C-1), 137.75 (m, $^1\text{J}(\text{C},\text{F}) = 239.6$ Hz), 139.67 (m, $^1\text{J}(\text{C},\text{F}) = 241.6$ Hz), 142.57 (m, $^1\text{J}(\text{C},\text{F}) = 241.1$ Hz), 143.60 (m, $^1\text{J}(\text{C},\text{F}) = 245.6$ Hz, C-2, C-3, C-4, C-5), 161.51 (CO), 163.11 (CO). Anal. calcd for $\text{C}_{14}\text{H}_{18}\text{F}_4\text{N}_2\text{O}_3 \times 0.25 \text{H}_2\text{O}$: C 49.05, H 5.44, N 8.17, found: C 48.77, H 5.89, N 8.20.

Isopropylammonium 2,3,4,5-tetrafluoro-6-(isopropylcarbamoyl)benzoate (3b) was prepared from **1** and isopropylamine (0.89 g, 1.29 mL); yield 88% (2.24 g); mp 178-180 °C; ^1H NMR (DMSO- d_6) δ 0.87 (d, $J = 6.7$ Hz, 6H, CH_3), 1.16 (d, $J = 6.6$ Hz, 6H, CH_3), 3.25 (sept, $J = 6.6$ Hz, 1H, CH), 3.87-3.97 (m, 1H, CH); 8.03 (s, 3H, NH_3), 8.24 (d, $J = 7.6$ Hz, 1H, NH); ^{13}C NMR (DMSO- d_6) δ 20.56 (CH_3), 22.16 (CH_3), 41.21 (CH), 42.96 (CH), 119.77 (m, $^2\text{J}(\text{C},\text{F}) = 15.9$ Hz, C-6), 127.43 (m, $^2\text{J}(\text{C},\text{F}) = 21.3$ Hz, C-1), 137.87 (m, $^1\text{J}(\text{C},\text{F}) = 229.2$ Hz), 139.73 (m, $^1\text{J}(\text{C},\text{F}) = 233.6$ Hz), 142.68 (m, $^1\text{J}(\text{C},\text{F}) = 242.9$ Hz), 143.66 (m, $^1\text{J}(\text{C},\text{F}) = 244.5$ Hz, C-2, C-3, C-4, C-5), 160.59 (CO), 163.06 (CO). Anal. calcd for $\text{C}_{14}\text{H}_{18}\text{F}_4\text{N}_2\text{O}_3 \times 0.1 \text{H}_2\text{O}$: C 49.44, H 5.39, N 8.24, found: C 49.20, H 5.78, N 8.27.

Isobutylammonium 2,3,4,5-tetrafluoro-6-(isobutylcarbamoyl)benzoate (3c) was prepared from **1** and isobutylamine (1.10 g, 1.50 mL); yield 33% (0.92 g); mp 168-170 °C; ^1H NMR (DMSO- d_6) δ 0.87 (d, $J = 6.6$ Hz, 6H, CH_3), 0.89 (d, $J = 6.7$ Hz, 6H, CH_3), 1.71-1.87 (m, 2H, 2 x CH), 2.58 (d, $J = 7.0$ Hz, 2H, CH_2), 2.98 (t, $J = 6.0$ Hz, 2H, CH_2), 8.00 (br s, 3H, NH_3), 8.37 (t, $J = 6.0$ Hz, 1H, NH); ^{13}C NMR (DMSO- d_6) δ 19.87 (CH_3), 20.27 (CH_3), 26.59 (CH), 28.06 (CH), 45.87 (CH_2), 46.78 (CH_2), 119.53 (m, $^2\text{J}(\text{C},\text{F}) = 15.1$ Hz, C-6), 128.12 (m, $^2\text{J}(\text{C},\text{F}) = 22.1$ Hz, C-1), 137.69 (m, $^1\text{J}(\text{C},\text{F}) = 242.5$ Hz), 139.64 (m, $^1\text{J}(\text{C},\text{F}) = 244.1$ Hz), 142.48

(m, $^1\text{J}(\text{C},\text{F}) = 240.6$ Hz), 143.69 (m, $^1\text{J}(\text{C},\text{F}) = 247.4$ Hz, C-2, C-3, C-4, C-5), 161.53 (CO), 163.00 (CO). Anal. calcd for $\text{C}_{16}\text{H}_{22}\text{F}_4\text{N}_2\text{O}_3 \times \text{H}_2\text{O}$: C 50.00, H 6.29, N 7.29, found: C 49.82, H 5.98, N 7.30.

Cyclohexylammonium 2,3,4,5-tetrafluoro-6-(cyclohexylcarbamoyl)benzoate (3d) was prepared from **1** and cyclohexylamine (1.49 g, 1.72 mL); yield 91% (2.86 g); mp 212-214 °C; ^1H NMR (DMSO- d_6) δ 1.07-1.30 (m, 10H, CH_2), 1.51-1.89 (m, 10H, CH_2), 2.89-2.93 (m, 1H, CH), 3.59-3.66 (m, 1H, CH), 8.01 (3H, NH_3), 8.25 (d, $J = 7.9$ Hz, 1H, NH); ^{13}C NMR (DMSO- d_6) δ 23.96 (CH_2), 24.44 (CH_2), 24.72 (CH_2), 25.37 (CH_2), 30.55 (CH_2), 32.05 (CH_2), 48.15 (CH), 49.36 (CH), 119.30 (m, $^2\text{J}(\text{C},\text{F}) = 13.9$ Hz, C-6), 128.31 (m, $^2\text{J}(\text{C},\text{F}) = 22.8$ Hz, C-1), 137.66 (m, $^1\text{J}(\text{C},\text{F}) = 241.3$ Hz), 139.62 (m, $^1\text{J}(\text{C},\text{F}) = 249.1$ Hz), 142.41 (m, $^1\text{J}(\text{C},\text{F}) = 240.4$ Hz), 143.78 (m, $^1\text{J}(\text{C},\text{F}) = 245.5$ Hz, C-2, C-3, C-4, C-5), 160.65 (CO), 162.88 (CO). Anal. calcd for $\text{C}_{20}\text{H}_{26}\text{F}_4\text{N}_2\text{O}_3$: C 57.41, H 6.26, N 6.69, found: C 57.58, H 6.41, N 6.71.

Benzylammonium 2,3,4,5-tetrafluoro-6-(benzylcarbamoyl)benzoate (3e) was prepared from **1** and benzylamine (1.61 g, 1.64 mL); yield 61% (1.98 g); mp 181-183 °C; ^1H NMR (DMSO- d_6) δ 3.98 (s, 2H, CH_2), 4.40 (d, $J = 5.9$ Hz, 2H, CH_2), 7.20-7.45 (m, 10H, 2 x Ar-H), 8.34 (br s, 3H, NH_3), 8.94 (t, $J = 5.9$ Hz, 1H, NH); ^{13}C NMR (DMSO- d_6) δ 42.49 (CH_2), 42.71 (CH_2), 119.49 (m, $^2\text{J}(\text{C},\text{F}) = 15.4$ Hz, C-6), 126.77 (C-Ar), 127.26 (C-Ar), 128.26 (C-Ar), 128.31 (C-Ar), 128.63 (C-Ar), 128.87 (C-Ar), 134.91 (C-Ar), 139.00 (C-Ar), 137.76 (m, $^1\text{J}(\text{C},\text{F}) = 251.4$ Hz), 139.75 (m, $^1\text{J}(\text{C},\text{F}) = 245.4$ Hz), 142.70 (m, $^1\text{J}(\text{C},\text{F}) = 241.1$ Hz), 143.68 (m, $^1\text{J}(\text{C},\text{F}) = 245.5$ Hz, C-2, C-3, C-4, C-5), 161.91 (CO), 163.00 (CO). One carbon signal (C-1) was not observed. Anal. calcd for $\text{C}_{22}\text{H}_{18}\text{F}_4\text{N}_2\text{O}_3$: C 58.41, H 4.46, N 6.19, found: C 58.47, H 4.60, N 6.19.

tert-Butylammonium 2,3,4,5-tetrafluoro-6-(tert-butylcarbamoyl)benzoate (3f) was prepared from **1** and *tert*-butylamine (1.10 g, 1.58 mL); yield 81% (2.22 g); mp 178-180 °C; ^1H NMR (DMSO- d_6) δ 1.24 (s, 9H, CH_3), 1.29 (s, 9H, CH_3), 7.96 (s, 1H, NH), 8.10 (br s, 3H, NH_3); ^{13}C NMR (DMSO- d_6) δ 27.34 (CH_3), 28.42 (CH_3), 50.80 ($\text{C}(\text{CH}_3)_3$), 51.00 ($\text{C}(\text{CH}_3)_3$), 119.91 (m, $^2\text{J}(\text{C},\text{F}) = 14.6$ Hz, C-6), 127.97 (m, $^2\text{J}(\text{C},\text{F}) = 22.1$ Hz, C-1), 137.67 (m, $^1\text{J}(\text{C},\text{F}) = 236.5$ Hz), 139.57 (m, $^1\text{J}(\text{C},\text{F}) = 239.3$ Hz), 142.39 (m, $^1\text{J}(\text{C},\text{F}) = 241.4$ Hz), 143.82 (m, $^1\text{J}(\text{C},\text{F}) = 247.0$ Hz, C-2, C-3, C-4, C-5), 160.94 (CO), 163.18 (CO). Anal. calcd for $\text{C}_{16}\text{H}_{22}\text{F}_4\text{N}_2\text{O}_3$: C 52.46, H 6.05, N 7.65, found C 52.03, H 6.80, N 7.17.

Synthesis of tetrafluorophthalamic acids 4a-f: General Procedure. A solution of the ammonium tetrafluorophthalamate (5 mmol) in H_2O was acidified with HCl (2 mol/L) to pH 1-2 and kept overnight at 4 °C. The colorless precipitate was filtered off, washed with H_2O and dried under reduced pressure.

2,3,4,5-Tetrafluoro-6-(propylcarbamoyl)benzoic acid (4a) was prepared from **3a** (1.69 g) in H_2O (30 mL); yield 73% (1.02 g); mp 132-134 °C; ^1H NMR (DMSO- d_6) δ 0.89 (t, $J = 7.3$ Hz, 3H, CH_3), 1.48 (sext, $J = 7.3$ Hz, 2H, CH_2CH_3), 3.14-3.18 (m, 2H, NCH_2), 8.66 (t, $J = 5.5$ Hz, 1H, NH), 14.02 (br s, 1H, CO_2H); ^{13}C NMR (DMSO- d_6) δ 11.42 (CH_3), 22.08 (CH_2CH_3), 41.19 (NCH_2), 117.86 (m, $^2\text{J}(\text{C},\text{F}) = 13.6$ Hz, C-1), 122.27 (m, $^2\text{J}(\text{C},\text{F}) = 15.4$ Hz, C-6), 140.24 (m, $^1\text{J}(\text{C},\text{F}) = 252.0$ Hz), 140.78 (m, $^1\text{J}(\text{C},\text{F}) = 254.4$ Hz), 143.63 (m, $^1\text{J}(\text{C},\text{F}) = 245.0$ Hz), 144.88 (m, $^1\text{J}(\text{C},\text{F}) = 250.7$ Hz, C-2, C-3, C-4, C-5), 160.10 (CO), 162.63 (CO). MS (EI) (m/z , %) 279 (M^+ , 13), 235 ($\text{M}^+ - \text{CO}_2$, 25), 221 ($\text{M}^+ - \text{C}_3\text{H}_8\text{N}$, 61), 177 ($\text{M}^+ - \text{C}_3\text{H}_8\text{N} - \text{CO}_2$, 100), 149 ($\text{M}^+ - \text{C}_3\text{H}_8\text{N} - \text{CO}_2 - \text{CO}$, 25). Anal. calcd for $\text{C}_{11}\text{H}_9\text{F}_4\text{NO}_3$: C 47.32, H 3.25, N 5.02, found: C 47.18, H 3.38, N 5.13.

2,3,4,5-Tetrafluoro-6-(isopropylcarbamoyl)benzoic acid (4b) was prepared from **3b** (1.69 g) in H_2O (36 mL); yield 81% (1.13 g); mp 144-146 °C; ^1H NMR (DMSO- d_6) δ 1.11 (d, $J = 6.6$ Hz, 6H, CH_3), 3.92-4.02

(m, 1H, CH), 8.55 (d, $J = 7.9$ Hz, 1H, NH), 14.00 (br s, 1H, CO₂H); ¹³C NMR (DMSO-*d*₆) δ 22.04 (CH₃), 41.53 (CH), 117.68 (m, ²J(C,F) = 14.6 Hz, C-1), 122.50 (m, ²J(C,F) = 15.9 Hz, C-6), 140.18 (m, ¹J(C,F) = 251.4 Hz), 140.80 (m, ¹J(C,F) = 255.2 Hz), 143.59 (m, ¹J(C,F) = 244.6 Hz), 144.93 (m, ¹J(C,F) = 251.4 Hz, C-2, C-3, C-4, C-5), 159.19 (CO), 162.60 (CO). MS (EI) (m/z , %) 279 (M⁺, 6), 235 (M⁺ - CO₂, 19), 221 (M⁺ - C₃H₈N, 38), 177 (M⁺ - C₃H₈N - CO₂, 100), 149 (M⁺ - C₃H₈N - CO₂ - CO, 25). Anal. calcd for C₁₁H₉F₄NO₃ × 0.1 H₂O: C 47.02, H 3.30, N 4.98, found: C 46.72, H 3.70, N 5.19.

2,3,4,5-Tetrafluoro-6-(isobutylcarbamoyl)benzoic acid (4c) was prepared from **3c** (1.83 g) in H₂O (100 mL); yield 58% (0.85 g); mp 148-150 °C; ¹H NMR (DMSO-*d*₆) δ 0.88 (d, $J = 6.7$ Hz, 6H, CH₃), 1.74-1.82 (m, 1H, CH), 3.02-3.04 (m, 2H, CH₂), 8.67 (t, $J = 5.8$ Hz, 1H, NH), 14.04 (br s, 1H, CO₂H); ¹³C NMR (DMSO-*d*₆) δ 20.18 (CH₃), 28.00 (CH), 46.94 (CH₂), 117.90 (m, ²J(C,F) = 14.6 Hz, C-1), 122.27 (m, ²J(C,F) = 19.1 Hz, C-6), 140.21 (m, ¹J(C,F) = 249.7 Hz), 140.74 (m, ¹J(C,F) = 253.4 Hz), 143.66 (m, ¹J(C,F) = 244.2 Hz), 144.82 (m, ¹J(C,F) = 250.6 Hz, C-2, C-3, C-4, C-5), 160.15 (CO), 162.65 (CO). MS (EI) (m/z , %) 293 (M⁺, 3), 249 (M⁺ - CO₂, 8), 221 (M⁺ - C₄H₁₀N, 36), 177 (M⁺ - C₄H₁₀N - CO₂, 100), 149 (M⁺ - C₄H₁₀N - CO₂ - CO, 18). Anal. calcd for C₁₂H₁₁F₄NO₃ × 0.1 H₂O: C 48.86, H 3.83, N 4.75, found: C 48.62, H 4.11, N 4.96.

2,3,4,5-Tetrafluoro-6-(cyclohexylcarbamoyl)benzoic acid (4d)^[23] was prepared from **3d** (2.09 g) in H₂O (700 mL); yield 85% (1.36 g); mp 174-178 °C; ¹H NMR (DMSO-*d*₆) δ 1.11-1.81 (m, 10H, CH₂), 3.64-3.71 (m, 1H, CH), 8.54 (d, $J = 7.9$ Hz, 1H, NH), 14.00 (br s, 1H, CO₂H); ¹³C NMR (DMSO-*d*₆) δ 24.43 (CH₂), 25.27 (CH₂), 32.00 (CH₂), 48.51 (CH), 117.61 (m, ²J(C,F) = 13.6 Hz, C-1), 122.54 (m, ²J(C,F) = 18.6 Hz, C-6), 140.15 (m, ¹J(C,F) = 252.7 Hz), 140.79 (m, ¹J(C,F) = 255.3 Hz), 143.58 (m, ¹J(C,F) = 245.2 Hz), 144.92 (m, ¹J(C,F) = 251.3 Hz, C-2, C-3, C-4, C-5), 159.19 (CO), 162.54 (CO). MS (EI) (m/z , %) 319 (M⁺, 5), 275 (M⁺ - CO₂, 18), 221 (M⁺ - C₆H₁₂N, 23), 177 (M⁺ - C₆H₁₂N - CO₂, 100), 149 (M⁺ - C₆H₁₂N - CO₂ - CO, 21). Anal. calcd for C₁₄H₁₃F₄NO₃: C 52.67, H 4.10, N 4.39, found: C 52.56, H 4.15, N 4.43.

2,3,4,5-Tetrafluoro-6-(benzylcarbamoyl)benzoic acid (4e) was prepared from **3e** (2.17 g) in H₂O (450 mL); yield 68% (1.11 g); mp 153-156 °C; ¹H NMR (DMSO-*d*₆) δ 4.45 (d, $J = 5.9$ Hz, 2H, CH₂), 7.24-7.35 (m, 5H, Ar-H), 9.21 (t, $J = 5.9$ Hz, 1H, NH), 14.14 (br s, 1H, CO₂H); ¹³C NMR (DMSO-*d*₆) δ 42.94 (CH₂), 117.94 (m, ²J(C,F) = 14.4 Hz, C-1), 121.89 (m, ²J(C,F) = 17.4 Hz, C-6), 127.08 (C-Ar), 127.39 (C-Ar), 128.43 (C-Ar), 138.58 (C-Ar), 140.40 (m, ¹J(C,F) = 255.3 Hz), 140.83 (m, ¹J(C,F) = 253.5 Hz), 143.82 (m, ¹J(C,F) = 245.7 Hz), 144.94 (m, ¹J(C,F) = 249.6 Hz, C-2, C-3, C-4, C-5), 160.40 (CO), 162.71 (CO). MS (EI) (m/z , %) 328 (M+H⁺, 9), 283 (M⁺ - CO₂, 100), 177 (M⁺ - C₇H₈N - CO₂, 95), 149 (M⁺ - C₇H₈N - CO₂ - CO, 27). Anal. calcd for C₁₅H₉F₄NO₃ × 0.7 H₂O: C 53.01, H 3.08, N 4.12, found: C 53.02, H 2.94, N 4.27.

2,3,4,5-Tetrafluoro-6-(tert-butylcarbamoyl)benzoic acid (4f) was prepared from **3f** (1.83 g) in H₂O (115 mL); yield 52% (0.76 g); mp 148-151 °C; ¹H NMR (DMSO-*d*₆) δ 1.31 (s, 9H, CH₃), 8.28 (s, 1H, NH), 14.02 (br s, 1H, CO₂H); ¹³C NMR (DMSO-*d*₆) δ 28.35 (CH₃), 51.43 (C(CH₃)₃), 117.56 (m, ²J(C,F) = 12.9 Hz, C-1), 123.17 (m, ²J(C,F) = 17.1 Hz, C-6), 139.98 (m, ¹J(C,F) = 251.8 Hz), 140.71 (m, ¹J(C,F) = 255.2 Hz), 143.61 (m, ¹J(C,F) = 245.7 Hz), 144.83 (m, ¹J(C,F) = 251.1 Hz, C-2, C-3, C-4, C-5), 159.44 (CO), 162.69 (CO). MS (EI) (m/z , %) 293 (M⁺, 10), 249 (M⁺ - CO₂, 8), 221 (M⁺ - C₄H₁₀N, 44), 177 (M⁺ - C₄H₁₀N - CO₂, 100), 149 (M⁺ - C₄H₁₀N - CO₂ - CO, 21). Anal. calcd for C₁₂H₁₁F₄NO₃: C 49.15, H 3.78, N 4.78, found: C 48.83, H 4.06, N 4.98.

Synthesis of tetrafluorobenzamides 5a-f: General Procedure. A mixture of the tetrafluorophthalamic acid (1.5 mmol) and dry DMSO (1 mL) was heated to 153 °C for 4 h. After cooling to room temperature,

H₂O was added (10 mL). The oil formed in the reaction mixture was removed and the aqueous solution was kept for 3 d at room temperature. The precipitate was filtered off, dried under reduced pressure and further purified as indicated.

2,3,4,5-Tetrafluoro-*N*-propylbenzamide (5a) was prepared from **4a** (0.42 g) and purified by column chromatography using petroleum ether/EtOAc (4:1); yield 26% (92 mg); mp 46-50 °C; ¹H NMR (DMSO-*d*₆) δ 0.89 (t, $J = 7.4$ Hz, 3H, CH₃), 1.48-1.55 (m, 2H, CH₂CH₃), 3.20 (q, $J = 6.5$ Hz, 2H, NCH₂), 7.53-7.59 (m, 1H, 6-H), 8.49 (br s, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ 11.43 (CH₃), 22.19 (CH₂CH₃), 41.25 (NCH₂), 111.70 (m, ²J(C,F) = 20.3 Hz, C-6), 121.20 (m, C-1), 140.00 (m, ¹J(C,F) = 247.6 Hz), 140.77 (m, ¹J(C,F) = 252.2 Hz), 144.69 (m, ¹J(C,F) = 246.7 Hz), 146.18 (m, ¹J(C,F) = 245.5 Hz, C-2, C-3, C-4, C-5), 160.89 (CO). MS (EI) (m/z , %) 235 (M⁺, 19), 220 (M⁺ - CH₃, 7), 206 (M⁺ - C₂H₅, 11), 177 (M⁺ - C₃H₈N, 100), 149 (M⁺ - C₃H₈N - CO, 14). Anal. calcd for C₁₀H₉F₄NO: C 51.07, H 3.86, N 5.96, found: C 51.32, H 4.44, N 5.69.

2,3,4,5-Tetrafluoro-*N*-isopropylbenzamide (5b) was prepared from **4b** (0.42 g) and recrystallized from *n*-hexanes; yield 43% (0.15 g); mp 111-113 °C; ¹H NMR (DMSO-*d*₆) δ 1.14 (d, $J = 6.7$ Hz, 6H, CH₃), 3.97-4.06 (m, 1H, CH), 7.52-7.57 (m, 1H, 6-H), 8.36 (d, $J = 6.3$ Hz, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ 22.18 (CH₃), 41.62 (CH), 111.68 (m, ²J(C,F) = 20.1 Hz, C-6), 121.51 (m, C-1), 139.98 (m, ¹J(C,F) = 250.9 Hz), 140.68 (m, ¹J(C,F) = 252.0 Hz), 144.63 (m, ¹J(C,F) = 247.1 Hz), 146.16 (m, ¹J(C,F) = 245.0 Hz, C-2, C-3, C-4, C-5), 160.12 (CO). MS (EI) (m/z , %) 235 (M⁺, 19), 220 (M⁺ - CH₃, 21), 177 (M⁺ - C₃H₈N, 100). Anal. calcd for C₁₀H₉F₄NO: C 51.07, H 3.86, N 5.96, found: C 50.81, H 4.23, N 5.97.

2,3,4,5-Tetrafluoro-*N*-isobutylbenzamide (5c) was prepared from **4c** (0.44 g); yield 80% (0.30 g); mp 88-90 °C; ¹H NMR (DMSO-*d*₆) δ 0.89 (d, $J = 6.6$ Hz, 6H, CH₃), 1.81 (non, $J = 6.7$ Hz, 1H, CH), 3.07 (t, $J = 6.5$ Hz, 2H, CH₂), 7.53-7.58 (m, 1H, 6-H), 8.50 (br s, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ 20.16 (CH₃), 28.06 (CH), 46.89 (CH₂), 111.67 (m, ²J(C,F) = 20.3 Hz, C-6), 121.36 (m, C-1), 139.99 (m, ¹J(C,F) = 248.3 Hz), 140.74 (m, ¹J(C,F) = 252.2 Hz), 144.64 (m, ¹J(C,F) = 247.8 Hz), 146.21 (m, ¹J(C,F) = 244.5 Hz, C-2, C-3, C-4, C-5), 161.02 (CO). MS (EI) (m/z , %) 249 (M⁺, 7), 234 (M⁺ - CH₃, 9), 177 (M⁺ - C₄H₁₀N, 100). Anal. calcd for C₁₁H₁₁F₄NO: C 53.02, H 4.45, N 5.62, found: C 52.27, H 4.75, N 5.68.

2,3,4,5-Tetrafluoro-*N*-cyclohexylbenzamide (5d) was prepared from **4d** (0.48 g) and recrystallized from *n*-hexanes; yield 56% (0.23 g); mp 124-126 °C; ¹H NMR (DMSO-*d*₆) δ 1.09-1.82 (m, 10H, CH₂), 3.67-3.74 (m, 1H, CH), 7.51-7.56 (m, 1H, 6-H), 8.35 (d, $J = 7.6$ Hz, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ 24.61 (CH₂), 25.25 (CH₂), 32.16 (CH₂), 48.70 (CH), 111.68 (m, ²J(C,F) = 20.3 Hz, C-6), 121.59 (m, C-1), 139.98 (m, ¹J(C,F) = 251.6 Hz), 140.66 (m, ¹J(C,F) = 250.6 Hz), 144.62 (m, ¹J(C,F) = 248.6 Hz), 146.15 (m, ¹J(C,F) = 244.0 Hz, C-2, C-3, C-4, C-5), 160.13 (CO). MS (EI) (m/z , %) 275 (M⁺, 19), 232 (M⁺ - C₃H₇, 18), 194 (M⁺ - C₆H₉, 64), 177 (M⁺ - C₆H₁₂N, 100). Anal. calcd for C₁₃H₁₃F₄NO: C 56.73, H 4.76, N 5.09, found: C 57.10, H 4.96, N 5.21.

2,3,4,5-Tetrafluoro-*N*-benzylbenzamide (5e) was prepared from **4e** (0.49 g) and recrystallized from *n*-hexanes; yield 47% (0.20 g); mp 96-99 °C; ¹H NMR (DMSO-*d*₆) δ 4.46 (d, $J = 6.0$ Hz, 2H, CH₂), 7.23-7.27 (m, 1H, 4'-H), 7.32-7.36 (m, 4H, 2'-H, 3'-H, 5'-H, 6'-H), 7.60-7.66 (m, 1H, 6-H), 9.06 (t, $J = 5.1$ Hz, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ 43.00 (CH₂), 111.84 (m, ²J(C,F) = 20.6 Hz, C-6), 120.76 (m, C-1), 127.08 (C-4'), 127.37, 128.48 (C-2', C-6', C-3', C-5'), 138.78 (C-1'), 140.08 (m, ¹J(C,F) = 249.4 Hz), 140.96 (m, ¹J(C,F) = 252.5 Hz), 144.88 (m, ¹J(C,F) = 247.7 Hz), 146.23 (m, ¹J(C,F) = 244.4 Hz, C-2, C-3, C-4, C-5), 161.08 (CO). MS (EI) (m/z , %) 283 (M⁺, 100), 177 (M⁺ - C₇H₈N, 80). Anal. calcd for C₁₄H₉F₄NO: C 59.37, H 3.20, N 4.95, found: C 58.99, H 3.80, N 5.25.

2,3,4,5-Tetrafluoro-*N*-tert-butylbenzamide (5f) was prepared from **4f** (0.44 g); yield 56% (0.21 g); mp 62-65-151 °C; ¹H NMR (DMSO-*d*₆) δ 1.34 (s, 9H, CH₃), 7.49-7.54 (m, 1H, 6-H), 8.06 (br s, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ 28.45 (CH₃), 51.51 (C(CH₃)₃), 111.62 (m, ²J(C,F) = 20.1 Hz, C-6), 122.44 (m, C-1), 139.84 (m, ¹J(C,F) = 246.3 Hz), 140.46 (m, ¹J(C,F) = 251.7 Hz), 144.48 (m, ¹J(C,F) = 246.5 Hz), 146.12 (m, ¹J(C,F) = 244.0 Hz, C-2, C-3, C-4, C-5), 160.10 (CO). MS (EI) (*m/z*, %) 234 (M⁺ - CH₃, 42), 177 (M⁺ - C₄H₁₀N, 100). Anal. calcd for C₁₁H₁₁F₄NO: C 53.02, H 4.45, N 5.62, found: C 52.61, H 4.73, N 5.51.

Synthesis of 4-amino-2,3,5-trifluorobenzamides 6-7. General Procedure A: A mixture of 1 mmol of the tetrafluorobenzamide **5b** (0.24 g) or **5d** (0.28 g) and the corresponding primary amine was heated for 4 h at an indicated oil bath temperature. Upon cooling and stirring, 2N HCl was added until a weak acidic pH was reached. A saturated aqueous solution of NaHCO₃ was then added to adjust a pH value of 7. The isolation and purification of the product was carried out as indicated.

Synthesis of 4-amino-2,3,5-trifluorobenzamides 7c and 7d. General Procedure B: A mixture of 0.5 mmol of 2,3,4,5-tetrafluoro-*N*-cyclohexylbenzamide **5d** (0.14 g) and the corresponding primary amine (1 mmol) in DMSO (3 mL) was reacted in a sealed microwave vessel for 10 min at 110 °C in a CEM Discovery reactor (max. 100 W). After addition of half-saturated brine (50 mL), it was extracted with ethyl acetate (2 × 25 mL), washed with 10% KHSO₄ (50 mL) and brine (50 mL), dried (Na₂SO₄) and the filtrate was evaporated to dryness. The crude product was purified by column chromatography using a gradient of petroleum ether/ethyl acetate (19:1 to 10:1).

2,3,5-Trifluoro-*N*-isopropyl-4-(propylamino)benzamide (6a) was prepared from **5b** and propylamine (7 mL) following General Procedure A at oil bath temperature of 55 °C. The aqueous mixture was kept overnight at 4 °C and the supernatant was decanted. The residue was dried in a desiccator and recrystallized from *n*-hexanes; yield 51% (0.14 g); mp 41-42 °C; ¹H NMR (DMSO-*d*₆) δ 0.86 (t, *J* = 7.3 Hz, 3H, CH₂CH₃), 1.12 (d, *J* = 6.7 Hz, 6H, CHCH₃), 1.49-1.54 (m, 2H, CH₂CH₃), 3.23-3.27 (m, 2H, NHCH₂), 3.98-4.02 (m, 1H, CH), 5.95-5.97 (m, 1H, NHCH₂), 7.11-7.16 (m, 1H, 6-H), 7.77 (d, *J* = 6.3 Hz, 1H, CONH); ¹³C NMR (DMSO-*d*₆) δ 11.11 (CH₂CH₃), 22.31 (CHCH₃), 23.54 (CH₂CH₃), 41.26 (CH), 46.14 (NHCH₂), 110.08 (m, ^{2,3}J(C,F) = 12.8, 6.9 Hz, C-1), 110.55 (m, ²J(C,F) = 23.3 Hz, C-6), 129.68 (m, C-4), 139.33 (m, ^{1,2,3}J(C,F) = 238.4, 17.2, 8.6 Hz, C-3), 145.97 (m, ^{1,2}J(C,F) = 242.9, 11.6 Hz, C-2), 146.53 (m, ^{1,3}J(C,F) = 235.1, 6.6 Hz, C-5), 161.14 (CO). MS (EI) (*m/z*, %) 274 (M⁺, 52), 245 (M⁺ - C₂H₅, 100), 216 (M⁺ - C₃H₈N, 64). Anal. calcd for C₁₃H₁₇F₃N₂O: C 56.93, H 6.25, N 10.21, found: C 56.93, H 6.38, N 10.38.

2,3,5-Trifluoro-*N*-isopropyl-4-(isopropylamino)benzamide (6b) was prepared from **5b** and isopropylamine (5 mL) following General Procedure A at oil bath temperature of 55 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was recrystallized from *n*-hexanes; yield 33% (91 mg); mp 56-59 °C; ¹H NMR (DMSO-*d*₆) δ 1.12 (d, *J* = 6.3 Hz, 6H, CH₃), 1.16 (d, *J* = 7.3 Hz, 6H, CH₃), 3.84-3.90 (m, 1H, CH), 3.97-4.04 (m, 1H, CH), 5.36 (d, *J* = 9.2 Hz, 1H, 4-NH), 7.13-7.17 (m, 1H, 6-H), 7.82 (d, *J* = 7.0 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 22.29 (CH₃), 23.39 (CH₃), 41.26 (CH), 46.19 (CH), 110.58 (m, ²J(C,F) = 23.6 Hz, C-6), 110.99 (m, ^{2,3}J(C,F) = 12.5, 7.2 Hz, C-1), 128.98 (m, C-4), 139.60 (m, ^{1,2,3}J(C,F) = 239.2, 17.1, 8.3 Hz, C-3), 145.88 (m, ^{1,2}J(C,F) = 241.9, 14.0 Hz, C-2), 146.79 (m, ^{1,3}J(C,F) = 235.6, 6.1 Hz, C-5), 161.09 (CO). MS (EI) (*m/z*, %) 274 (M⁺, 45), 259 (M⁺ - CH₃, 100), 216 (M⁺ - C₃H₈N, 20). Anal. calcd for C₁₃H₁₇F₃N₂O: C 56.93, H 6.25, N 10.21, found: C 56.94, H 6.48, N 9.80.

2,3,5-Trifluoro-4-(isobutylamino)-*N*-isopropylbenzamide (6c) was prepared from **5b** and isobutylamine (8 mL) following General Procedure A at oil bath temperature of 70 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was purified by column chromatography using a gradient of petroleum ether/CH₂Cl₂ (1:1) to CH₂Cl₂; yield 26% (75 mg); mp 42-43 °C; ¹H NMR (DMSO-*d*₆) δ 0.86 (d, *J* = 6.6 Hz, 6H, CH₂CHCH₃), 1.12 (d, *J* = 6.6 Hz, 6H, NHCHCH₃), 1.73-1.81 (m, 1H, CH₂CH), 3.07-3.10 (m, 2H, CH₂), 3.97-4.03 (m, 1H, NHCH), 6.02 (t, *J* = 6.5 Hz, 1H, 4-NH), 7.11-7.16 (m, 1H, 6-H), 7.77 (d, *J* = 6.7 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 19.90 (CH₂CHCH₃), 22.30 (NHCHCH₃), 28.95 (CH₂CH), 41.26 (NHCH), 51.90 (CH₂), 110.08 (m, ^{2,3}J(C,F) = 12.5, 7.1 Hz, C-1), 110.56 (m, ²J(C,F) = 23.3 Hz, C-6), 129.74 (m, C-4), 139.33 (m, ^{1,2,3}J(C,F) = 238.5, 17.4, 8.4 Hz, C-3), 145.98 (m, ^{1,2}J(C,F) = 243.9, 12.6 Hz, C-2), 146.54 (m, ^{1,3}J(C,F) = 235.1, 7.3 Hz, C-5), 161.14 (CO). MS (EI) (*m/z*, %) 274 (M⁺, 30), 245 (M⁺ - C₃H₇, 100). Anal. calcd for C₁₄H₁₉F₃N₂O: C 58.32, H 6.64, N 9.72, found: C 57.81, H 6.69, N 9.37.

4-(Cyclohexylamino)-2,3,5-trifluoro-*N*-isopropylbenzamide (6d) was prepared from **5b** and cyclohexylamine (8 mL) following General Procedure A at oil bath temperature of 130 °C. The aqueous mixture was extracted with ethyl acetate (3 × 20 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was recrystallized from *n*-hexanes; yield 17% (53 mg); mp 83-85 °C; ¹H NMR (DMSO-*d*₆) δ 1.12 (d, *J* = 6.7 Hz, 6H, CH₃), 1.30-2.00 (m, 10H, CH₂), 3.41-3.52 (m, 1H, CH₂CH₂), 3.96-4.05 (m, 1H, CH₂CH₃), 5.35 (d, *J* = 9.2 Hz, 1H, 4-NH), 7.12-7.17 (m, 1H, 6-H), 7.80 (d, *J* = 6.3 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 22.30 (CH₃), 24.86, 25.31 (CHCH₂CH₂CH₂), 33.68 (CHCH₂), 41.29 (CHCH₃), 53.55 (CHCH₂), 110.59 (m, ²J(C,F) = 23.8 Hz, C-6), 110.90 (m, ^{2,3}J(C,F) = 12.6, 7.2 Hz, C-1), 128.80 (m, C-4), 139.61 (m, ^{1,2,3}J(C,F) = 239.0, 17.1, 8.4 Hz, C-3), 145.90 (m, ^{1,2,4}J(C,F) = 244.1, 12.8, 2.2 Hz, C-2), 146.81 (m, ^{1,3}J(C,F) = 235.8, 6.3 Hz, C-5), 161.11 (CO). MS (EI) (*m/z*, %) 314 (M⁺, 76), 271 (M⁺ - C₃H₇, 100), 174 (M⁺ - C₃H₈N - C₆H₁₁, 42). Anal. calcd for C₁₆H₂₁F₃N₂O: C 61.13, H 6.73, N 8.91, found: C 60.72, H 6.91, N 8.83.

***N*-Cyclohexyl-2,3,5-trifluoro-4-(propylamino)benzamide (7a)** was prepared from **5d** and propylamine (9 mL) following General Procedure A at oil bath temperature of 55 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was recrystallized from *n*-hexanes; yield 38% (0.12 g); mp 86-88 °C; ¹H NMR (DMSO-*d*₆) δ 0.86 (t, *J* = 7.4 Hz, 3H, CH₃), 1.11-1.79 (m, 10H, CHCH₂CH₂CH₂), 1.51 (sext, *J* = 7.3 Hz, 2H, CH₂CH₃), 3.24 (q, *J* = 6.9 Hz, 2H, NHCH₂), 3.67-3.69 (m, 1H, CHCH₂), 5.95 (br s, 1H, 4-NH), 7.10-7.14 (m, 1H, 6-H), 7.76 (d, *J* = 6.6 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 11.10 (CH₃), 23.52 (CH₂CH₃), 24.74, 25.30 (CHCH₂CH₂CH₂), 32.29 (CHCH₂), 46.14 (NHCH₂), 48.41 (CH), 110.15 (m, ^{2,3}J(C,F) = 12.4, 6.9 Hz, C-1), 110.54 (m, ²J(C,F) = 23.3 Hz, C-6), 129.65 (m, C-4), 139.32 (m, ^{1,2,3}J(C,F) = 238.4, 17.2, 8.6 Hz, C-3), 145.93 (m, ^{1,2}J(C,F) = 242.9, 11.6 Hz, C-2), 146.53 (m, ^{1,3}J(C,F) = 235.1, 6.6 Hz, C-5), 161.09 (CO). MS (EI) (*m/z*, %) 314 (M⁺, 80), 285 (M⁺ - C₂H₅, 34), 232 (M⁺ - C₆H₁₀, 100). Anal. calcd for C₁₆H₂₁F₃N₂O: C 61.13, H 6.73, N 8.91, found: C 61.00, H 6.96, N 8.80.

***N*-Cyclohexyl-2,3,5-trifluoro-4-(isopropylamino)benzamide (7b)** was prepared from **5d** and isopropylamine (6 mL) following General Procedure A at oil bath temperature of 35 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was purified by column chromatography using a gradient of petroleum ether/CH₂Cl₂ (1:1) to CH₂Cl₂; yield 30% (94 mg); mp 83-85 °C; ¹H NMR (DMSO-*d*₆) δ 1.16 (d, *J* = 6.7 Hz, 6H, CH₃), 1.24-1.82 (m, 10H,

CH₂), 3.66-3.68 (m, 1H, CH₂), 3.87 (oct, *J* = 6.8 Hz, CH₂CH₃), 5.35 (d, 1H, *J* = 9.1 Hz, 4-NH), 7.12-7.16 (m, 1H, 6-H), 7.80 (d, *J* = 7.0 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 23.39 (CH₃), 24.75, 25.32 (CHCH₂CH₂CH₂), 32.30 (CH₂CH₂), 46.21 (CHCH₃), 48.44 (CH₂CH₂), 110.39 (m, ^{2,3}*J*(C,F) = 12.5, 7.2 Hz, C-1), 110.60 (m, ²*J*(C,F) = 23.6 Hz, C-6), 128.95 (m, C-4), 139.64 (m, ^{1,2,3}*J*(C,F) = 239.2, 17.1, 8.3 Hz, C-3), 145.37 (m, ^{1,2}*J*(C,F) = 241.9, 14.0 Hz, C-2), 146.81 (m, ^{1,3}*J*(C,F) = 235.6, 6.1 Hz, C-5), 160.14 (CO). MS (EI) (*m/z*, %) 314 (M⁺, 100), 299 (M⁺ - CH₃, 75), 232 (M⁺ - C₆H₁₀). Anal. calcd for C₁₆H₂₁F₃N₂O: C 61.13, H 6.73, N 8.91, found: C 61.19, H 6.94, N 8.61.

N-Cyclohexyl-2,3,5-trifluoro-4-(isobutylamino)benzamide (7c) was prepared from **5d** and isobutylamine (9 mL) following General Procedure A at oil bath temperature of 80 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was recrystallized from *n*-hexanes; yield 37% (0.12 g); mp 102-105 °C; ¹H NMR (DMSO-*d*₆) δ 0.86 (d, *J* = 7.0 Hz, 6H, CH₃), 1.11-1.81 (m, 11H, CHCH₂CH₂CH₂, CHCH₃), 3.08 (t, *J* = 6.8 Hz, 2H, NHCH₂), 3.65-3.72 (m, 1H, NHCH), 6.02 (t, *J* = 6.5 Hz, 1H, 4-NH), 7.10-7.14 (m, 1H, 6-H), 7.76 (d, *J* = 6.6 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 19.89 (CH₃), 24.75, 25.32 (CHCH₂CH₂CH₂), 28.94 (CHCH₃), 32.29 (CH₂CH₂), 48.41 (NHCH), 51.90 (NHCH₂), 110.16 (m, ^{2,3}*J*(C,F) = 12.5, 7.1 Hz, C-1), 110.56 (m, ²*J*(C,F) = 23.3 Hz, C-6), 129.74 (m, C-4), 139.34 (m, ^{1,2,3}*J*(C,F) = 238.5, 17.4, 8.4 Hz, C-3), 145.95 (m, ^{1,2}*J*(C,F) = 243.9, 12.6 Hz, C-2), 146.54 (m, ^{1,3}*J*(C,F) = 235.1, 7.3 Hz, C-5), 161.12 (CO). MS (EI) (*m/z*, %) 328 (M⁺, 56), 285 (M⁺ - C₃H₇, 100), 246 (M⁺ - C₆H₁₀, 55). Anal. calcd for C₁₇H₂₃F₃N₂O: C 62.18, H 7.06, N 8.53, found: C 62.01, H 7.32, N 8.24. In a separate experiment, compound **5d** was reacted with isobutylamine under microwave conditions (General Procedure B). After column chromatography, the product **7c** was obtained in 45% yield.

N-Cyclohexyl-2,3,5-trifluoro-4-(cyclohexylamino)benzamide (7d) was prepared from **5d** and cyclohexylamine (6 mL) following General Procedure A at oil bath temperature of 130 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was recrystallized from *n*-hexanes; yield 27% (96 mg); mp 98-102 °C; ¹H NMR (DMSO-*d*₆) δ 1.09-1.87 (m, 20H, CH₂), 3.40-3.51 (m, 1H, CH), 3.62-3.70 (m, 1H, CH), 5.34 (d, *J* = 9.2 Hz, 1H, 4-NH), 7.11-7.15 (m, 1H, 6-H), 7.79 (d, *J* = 6.3 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 24.74, 24.85, 25.30, 25.30, (CHCH₂CH₂CH₂), 32.29, 33.67 (CH₂CH₂), 48.43 (CONHCH), 53.55 (4-NHCH), 110.58 (m, ²*J*(C,F) = 23.8 Hz, C-6), 111.00 (m, ^{2,3}*J*(C,F) = 12.6, 7.2 Hz, C-1), 128.77 (m, C-4), 139.59 (m, ^{1,2,3}*J*(C,F) = 239.0, 17.1, 8.4 Hz, C-3), 145.85 (m, ^{1,2,4}*J*(C,F) = 241.1, 12.8, 2.2 Hz, C-2), 146.81 (m, ^{1,3}*J*(C,F) = 235.8, 6.3 Hz, C-5), 161.07 (CO). MS (EI) (*m/z*, %) 354 (M⁺, 80), 272 (M⁺ - C₆H₁₀, 100). Anal. calcd for C₁₉H₂₅F₃N₂O: C 64.39, H 7.11, N 7.90, found: C 64.56, H 7.32, N 7.90. In a separate experiment, compound **5d** was reacted with cyclohexylamine under microwave conditions (General Procedure B). After column chromatography, the product **7d** was obtained in 43% yield.

4-(Benzylamino)-N-cyclohexyl-2,3,5-trifluorobenzamide (7e) was prepared from **5d** and benzylamine (9 mL) following General Procedure A at oil bath temperature of 130 °C. The aqueous mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic layers were dried (Na₂SO₄) and the filtrate was evaporated to dryness. The residue was recrystallized from *n*-hexanes; yield 45% (0.16 g); mp 133-136 °C; ¹H NMR (DMSO-*d*₆) δ 1.09-1.80 (m, 10H, CH₂), 3.62-3.70 (m, 1H, CH), 4.47 (d, *J* = 6.9 Hz, 2H, NHCH₂), 6.66-6.69 (m, 1H, 4-NH), 7.18-7.21 (m, 1H, 6-H), 7.27-7.31 (m, 5H, Ar-H), 7.79 (d, *J* = 6.6 Hz, 1H, CONH). ¹³C NMR (DMSO-*d*₆) δ 24.71, 25.31 (CHCH₂CH₂CH₂), 32.32 (CH₂CH₂), 47.63 (NHCH₂), 48.41 (NHCH), 110.48 (m, ²*J*(C,F) = 22.8 Hz, C-6), 111.25 (m, ²*J*(C,F) = 12.7 Hz, C-1), 126.84, 127.46, 128.34 (Ar-CH), 128.46 (m, C-4),

139.60 (m, ^{1,2,3}*J*(C,F) = 239.1, 16.9, 8.7 Hz, C-3), 140.43 (Ar-C), 145.70 (m, ^{1,2,4}*J*(C,F) = 244.1, 12.8, 1.8 Hz, C-2), 146.81 (m, ^{1,3}*J*(C,F) = 236.4, 6.8 Hz, C-5), 161.01 (CO). MS (EI) (*m/z*, %) 362 (M⁺, 46), 280 (M⁺ - C₆H₁₀, 48), 91 (C₇H₇⁺). Anal. calcd for C₂₀H₂₁F₃N₂O: C 66.29, H 5.84, N 7.73, found: C 66.55, H 5.91, N 7.44.

4-Fluoroisindoline (8). ¹³C NMR (DMSO-*d*₆) δ 47.32 (C-3), 50.38 (C-1), 114.99 (m, ²*J*(C,F) = 18.9 Hz, C-5), 119.45 (m, ⁴*J*(C,F) = 3.0 Hz, C-7), 121.85 (m, ²*J*(C,F) = 18.9 Hz, C-3a), 131.23 (m, ³*J*(C,F) = 6.9 Hz, C-6), 139.04 (m, ³*J*(C,F) = 4.7 Hz, C-7a), 157.29 (m, ¹*J*(C,F) = 247.6 Hz, C-4).

2-Fluorobenzamide (9). ¹³C NMR (DMSO-*d*₆) δ 116.23 (m, ²*J*(C,F) = 22.7 Hz, C-3), 123.99 (m, ¹*J*(C,F) = 14.2 Hz, C-1), 124.54 (m, ⁴*J*(C,F) = 3.4 Hz, C-5), 130.38 (m, ³*J*(C,F) = 2.9 Hz, C-6), 132.61 (m, ³*J*(C,F) = 8.6 Hz, C-4), 159.46 (m, ¹*J*(C,F) = 249.5 Hz, C-2), 165.38 (CO).

3-Fluorobenzamide (10). ¹³C NMR (DMSO-*d*₆) δ 114.39 (m, ²*J*(C,F) = 22.6 Hz, C-2), 118.24 (m, ²*J*(C,F) = 21.2 Hz, C-4), 123.78 (m, ⁴*J*(C,F) = 2.9 Hz, C-6), 130.51 (m, ³*J*(C,F) = 7.9 Hz, C-5), 136.89 (m, ³*J*(C,F) = 6.7 Hz, C-1), 162.13 (m, ¹*J*(C,F) = 244.3 Hz, C-3), 166.68 (m, ⁴*J*(C,F) = 2.5 Hz, CO).

Biological investigations

Rat aortic ring assay. To determine the extent of the antiangiogenic effects of the test compounds, the rat aortic ring assay was carried out as described elsewhere.^[8] Briefly, 24-well tissue culture plates were covered with 250 μL of Matrigel (Becton-Dickinson) and allowed to gel for 30 to 45 min at 37 °C and 5% CO₂. Sections of thoracic aorta were removed from 8- to 10-week-old male Sprague Dawley rats. Following excision of fibroadipose tissue, the aortic sections were cut into 1-mm-long cross-sections, placed on Matrigel-coated wells, and layered with additional Matrigel (250 μL). These were then allowed to set, after which the cross-sectional rings were covered with endothelial cell growth media (EGM-II) and incubated under 5% CO₂ at 37 °C overnight. EGM-II consists of endothelial cell basal medium (EBM-II) and endothelial cell growth factors. After 24 h, the medium was removed and replaced with EBM-II (1 mL) with the vehicle (0.5% DMSO), positive control TNP-470 (50 μM), or test compound (50 μM). This was replicated three times. The area of angiogenic sprouting was quantified using Adobe Photoshop.

Cytotoxicity assay. This assay was determined as described elsewhere.^[8] Briefly, HUVECs were treated with compounds at 10 μM. After 24 hours of incubation with compounds, 10 μL of CCK-8 solution (Dojindo, MD) was added to each well. The plates were left to incubate for 2 hours, and then read at a wavelength of 450 nm using a microplate reader.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed by the authors.

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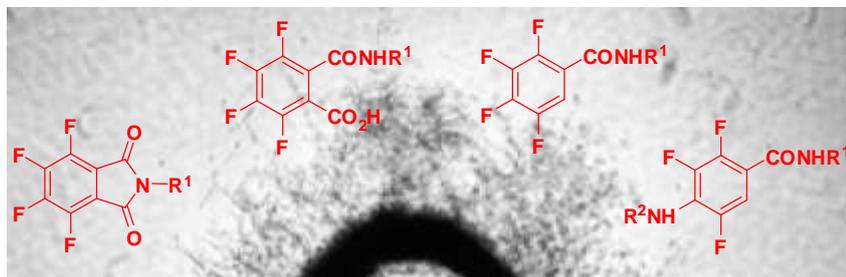
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FULL PAPER



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Synthesis, Structural Characterization and Antiangiogenic Activity of Polyfluorinated Benzamides

Two or one ring – four or three fluorines. A path of stepwise structural modifications led from tetrafluorophthalimides via tetrafluorophthalamic acids and tetrafluorobenzamides to 4-amino-2,3,5-trifluorobenzamides. The representatives of these chemotypes were investigated by means of spectroscopic and crystallographic methods and evaluated as inhibitors of angiogenesis.