Synthesis and biological evaluation of benzodiazepines containing a pentafluorosulfanyl group


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Synthesis and biological evaluation of benzodiazepines containing a pentafluorosulfanyl group.

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\textsc{ABSTRACT}

The widely used pentafluorosulfanyl group (SF\textsubscript{5}) was deployed as a bioisosteric replacement for a chloro-group in the benzodiazepine diazepam (Valium\textsuperscript{TM}). Reaction of 2-amino-5-pentafluorosulfanyl-benzophenone with chloroacetyl chloride followed by hexamethylenetetramine, in the presence of ammonia, led to 7-sulfurpentfluoro-5-phenyl-1H-benzo[1,4]diazepin-2(3H)-one (2e). The latter was able to undergo a Pd-catalysed ortho-arylation, demonstrating that these highly fluorinated benzodiazepines can be further modified to form more complicated scaffolds. The replacement of Cl by the SF\textsubscript{5} group, led to a loss of potency for potentiating GABA\textsubscript{A} receptor activation, most likely because of a lost ligand interaction with His102 in the GABA\textsubscript{A} receptor \textalpha subunit.

Dedicated to an inspirational and humble pioneer, Prof Jonathan Williams, a colleague and mentor in chemistry.

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1. Introduction

The pentafluorosulfanyl group is often employed in medicinal chemistry as a bioisosteric “super trifluoromethyl” group. Possessing high thermal stability, low toxicity, electron withdrawing effects and high lipophilicity, it has been used in a number of drug discovery projects.\textsuperscript{11-14}

Since their discovery in the late 1950s, benzodiazepines (BZDs) which act as positive allosteric modulators on \textgreek{gamma}-subunit containing synaptic GABA\textsubscript{A} receptors (GABA\textsubscript{A},R\textsubscript{S})\textsuperscript{2,3}, have been widely employed to treat a wide spectrum of disorders such as anxiety, insomnia, seizures and alcohol withdrawal.\textsuperscript{3,5,8,9,11-13}

Structure activity relationships show, inter alia, that electron withdrawing groups at the \textgreek{gamma}-position are important for improved receptor affinity (Fig. 1).\textsuperscript{1,3,12,17}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig1.png}
\caption{Selected clinically-used BZDs.}
\end{figure}

With research outputs in both benzodiazepine\textsuperscript{10,17} and SF\textsubscript{5} chemistry\textsuperscript{18}, there was a natural inclination for us to combine these interests in the design of SF\textsubscript{5}-containing BZDs. We, therefore, aimed to synthesise analogues 2a - 2c (Scheme 1) related to the much-prescribed drug, diazepam (Valium\textsuperscript{TM}) in order to evaluate the effect of changing a Cl for a SF\textsubscript{5} group on biological activity.

2. Results and Discussion

We opted for a one-pot microwave route to synthesise SF\textsubscript{5}-substituted BZD analogues.\textsuperscript{10-21} Commercially available 2-amino-5-pentafluorosulfanyl-benzophenone 1c was coupled under microwave irradiation with Boc-Gly-OH, and DCC as the coupling agent, in toluene at 150 °C for 30 min, followed by Boc-deprotection with TFA.\textsuperscript{17,21} However, the attempt was unsuccessful and one speculation for the failure was the poor nucleophilicity of the aniline. To validate this hypothesis, we attempted the same reaction with 2-amino-5-nitrobenzophenone as the nitro group has an electronic effect fairly close to that of the SF\textsubscript{5} group (\emph{t}\nnoreq=0.68\textsuperscript{20} for SF\textsubscript{5}, and \emph{t}\nnoreq=0.78\textsuperscript{21} for NO\textsubscript{2}). The result was as postulated, unsuccessful.

Although position-8 on the BZD ring was not a region of interest in terms of biological activity, we were curious about the electronic effect a pentafluorosulfanyl group would have. Again, we used the microwave approach for the attempted synthesis of 2b (Scheme 1). The reaction was moderately successful with 2b formed in 13\% yield with only a purity of 88\% by LCMS. The unsubstituted benzodiazepine 2a was synthesised in 65\% yield.
Unperturbed in this approach, we next attempted the microwave mediated route, utilizing 1 and Boc-Gly-OH but with EEDQ as the coupling agent (Table 1). Moreover, the coupling reaction mixture was worked up and the anticipated intermediate was isolated and purified before continuing to the next step, viz. Boc-group deprotection. This would enable us to establish whether this initial coupling step was responsible, or the cyclisation step, for the poor overall yield. We found that the coupling step was very low yielding for the reaction of 1b and the reaction was also, disappointingly, again, unsuccessful for 1c.

Scheme 1. Synthesis of BZDs by microwave techniques.

Table 1. Boc-Gly-OH coupling reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>-</td>
</tr>
</tbody>
</table>

As the microwave-mediated attempts towards the SF$_2$-BZD derivatives were unsuccessful, yet worked on a standard 1,4-BZD core (entry 1), we sought a route towards the desired products using other protocols. A method using hexamethyleneenetramine[$^{24,25}$] with ammonia as aminating reagent, was reported to be successful, even for starting materials with electron-withdrawing substituents. Accordingly, this was our next method of choice.

Hence, 1c was acylated using chloroacetyl chloride then aminated with hexamethyleneenetramine in the presence of ammonia (Scheme 2). Analysis of the crude mixture, gratifyingly, showed the presence of the expected product as well as a similar by-product, which we tentatively assigned the structure 4c, notably by the similarity of its 1H NMR spectrum to that of its 4-chloro-derivative. The two products could be separated after a normal phase and a reverse phase column chromatographic purification.

Scheme 2. Multi-step synthesis of a SF$_2$-substituted BZD.

Compound 2c was crystallised by a diffusion method using dichloromethane/hexane and obtained as colourless crystals and this confirmed both the regiochemistry of the SF$_2$-substituent and the formation of the BZD core (Scheme 2).[$^{27}$] A standard N-methylation of 2c using sodium hydride and methyl iodide yielded the desired SF$_2$-BZD 5c in modest yield (Scheme 3).

Scheme 3. N-Methylation of a SF$_2$-BZD.

An unoptimised attempt at Pd-catalysed C-H activation with iodonium salts,[26] using our previously described conditions, involving microwave chemistry afforded the expected ortho-arylated product 6c. This illustrates that catalytic C-H activation chemistry is now amenable to the synthesis of polyfluorinated BZDs and 6c was now available for biological assay (Scheme 4).

Scheme 4. Ortho-arylation of a BZD.

The compounds were docked into the cryo-EM structure (PDB ID: 6HUP) of the αH3y2L GABAA receptor at the interfascial benzodiazepine binding site between the principal (+) γ and complementary (-) γ subunit using Schrödinger Glide.[27] We evaluated their apparent binding affinity using the Glide score, which predicts possible binding of the ligands in the benzodiazepine binding site of the receptor and produces a set of initial ligand conformations. Different ligand poses can then be generated and ranked. Scoring is related to the strength of interaction between the ligand and the protein which is expressed as binding free energy,[28] Therefore, more negative values represent tighter binders. Glide is primarily concerned with generating accurate poses for each protein-ligand complex and identifying poses with appreciable binding affinity. However, the task of accurately estimating protein-ligand binding affinities is beyond the capabilities of docking scoring functions and, hence Glide scores are not always congruent with experimental data.[29]

A Glide score was determined for compounds 2c, 5c and 6c and was compared against diazepam and the metabolite, nordiazepam (Table 2). The SF$_2$-substituted nordiazepam analogue, 2c, however, gave a better Glide score than diazepam suggesting it may bind more strongly in the binding site. The Glide score of the ortho C-H activated analogue 6c was very poor in comparison.

Table 2. Glide score of Diazepam versus SF$_2$-substituted BZDs.

<table>
<thead>
<tr>
<th>Entry</th>
<th>1,4-BZD</th>
<th>Glide score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>diazepam</td>
<td>-4.74</td>
</tr>
<tr>
<td>2</td>
<td>nordiazepam</td>
<td>-5.36</td>
</tr>
<tr>
<td>3</td>
<td>2c</td>
<td>-4.97</td>
</tr>
<tr>
<td>4</td>
<td>5c</td>
<td>-4.74</td>
</tr>
<tr>
<td>5</td>
<td>6c</td>
<td>-3.98</td>
</tr>
</tbody>
</table>

Pposes of diazepam, 2c, 5c and 6c respectively with the best Glide score docked in the αH3y2L GABAA receptor are shown in Figure 2. The dashed lines indicate hydrogen bonds and π-π interactions. The chloride atom interacts with the critical His110 side chain.[30,31] The distance between the chloride atom and the nitrogen on the His110 was measured as 2.89 Å. The images show that there is no interaction between SF$_2$ and the amino acid side chains. A direct comparison of 5c and diazepam can be made. We calculated the distance between SF$_2$ and His110 to be 5.34 Å. This was calculated between the closest fluorine of SF$_2$ to His110. This distance is almost double the distance between chlorine and His110 (2.89 Å). This could explain the lack of interaction between SF$_2$ and the His110. This increased distance and lack of interaction also applies to 2c and 6c as well.

To access functionality of the BZD ligands, we used whole-cell patch-clamp recording from human embryonic kidney cells expressing recombinant α1β2γ2L GABA$_A$Rs. The analogues, 2c, 5c and 6c were compared to diazepam for their ability to potentiate 2μM GABA-induced currents ($\pm$EC$_{50}$). The three SF$_2$-diazepam analogues showed much lower potencies than diazepam (shifted 60- (2c), 70- (6c), and 190-fold lower (5c)). The relative extent of potentiation was very low for 6c, ~half that of diazepam for 5c, or near equivalent with diazepam for 2c (Fig. 3; Table 3). For 6c, the efficacy level of the potentiation was reduced at the highest concentration of 100 μM (Fig. 3). Such inhibition has been reported before for benzodiazepines like diazepam and flurazepam[32], and could reflect increased desensitization of GABA$_A$Rs.
From these data, it is clear that substituting Cl on the benzo ring for SF, has a deleterious effect primarily on BZD potency and to a large extent, also on relative efficacy at GABAA receptors. This is likely to be due to disruption of the Cl – αH102 interaction, which is known to be critical for BZD modulation at GABAA receptors. Indeed, mutation of His for Arg at this position affects the biological activity catalysed C

Selected SF-substituted 1,4-BZDs have been synthesized, one by a Pd-catalysed C-H activation method, and evaluated in silico and in vitro for their biological activity. For all compounds, which are direct analogues of diazepam, where a Cl has been replaced by a SF, reduced GABA potency and for 5c and 6c a reduced efficacy were evident.

4. Experimental

4.1. Organic chemistry

All commercially purchased materials and solvents were used without further purification unless specified otherwise. NMR spectra were recorded on a Varian VNMR 600 (1H 600 MHz, 13C 126 MHz) and VNMR 400 (1H 376 MHz, 1H 61 MHz and 13P 162 MHz) spectrometer and prepared in deuterated solvents such as Chloroform-d and DMSO-d6. H and 13C chemical shifts were recorded in parts per million (ppm). Multiplicity of 1H-NMR peaks are indicated by s – singlet, d – doublet, dd – doublets of doublets, t – triplet, p – pseudo triplet, q – quartet, m – multiplet and coupling constants are given in Hz. Electrospray ionisation – high resolution mass spectra (ESI-HRMS) were obtained using a Bruker Daltonics Apex III where Apollo ESI was used as the ESI source. All analyses were conducted by Dr A. K. Abdul-Sada. The molecular ion peaks [M]+ were recorded in mass to charge (m/z) ratio. LC-MS spectra were acquired using Shimadzu LC-MS 2020, on a Gemini 5 μ C18 110 Å column. X-ray analysis was performed at the UK National Crystallography Services, Southampton. Purifications were performed by flash chromatography on silica gel columns or C18 columns using a Combi flash RF 75 PSI, ISCO unit.

7-(Pentafluoro-4'-sulfanyl)-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine-2-one (2c). Triethylamine (0.188 g, 1.86 mmol) was added to a solution of 2-benzoyl-4-(pentafluoro-4'-sulfanyl)aniline (0.300 g, 0.93 mmol) in dichloromethane (1 mL) and the mixture was stirred for 1 hour at room temperature. After an hour the mixture was cooled in an ice bath, and chloroacetyl chloride (0.210 g, 1.86 mmol) dissolved in dichloromethane (1 mL) and cooled in an ice bath was added dropwise to the reaction mixture. The reaction was stirred overnight at room temperature. The reaction mixture was refuxed overnight. After an hour the latter was concentrated in vacuo and dissolved in toluene (5 mL), p-Toluene sulfonic acid (6 mg, 0.03 mmol), was added to the solution and the mixture was refluxed for 1 hour. The crude was concentrated in vacuo and purified over a column of silica (hexane:EtOAc; 3:7), followed by a reverse phased column (C18, acetonitrile:water:1, 3) to obtain the pure product as a colourless solid (83 mg, 31%). 1H NMR (600 MHz, Chloroform-d) δ 9.40 (s, 1H, NH), 7.87 (dd, J = 8.9, 2.6 Hz, 1H, ArH), 7.74 (d, J = 2.6 Hz, 1H, ArH), 7.53 – 7.50 (m, 2H, 2ArH), 7.50 – 7.47 (m, 1H, ArH), 7.41 (pt, 2H, 2ArH), 7.25 (d, J = 8.9 Hz, 1H, ArH), 4.37 (s, 2H, CH2); 13C NMR (600 MHz, Chloroform-d) δ 172.1 (C=O), 169.8 (C=N), 148.1 (t, J, CF= 18.9 Hz, Ar-CF=), 141.0 (Arc), 138.2 (ArC), 131.1 (Arc), 129.6 (2ArC), 129.5 (m, ArC), 129.1 – 129.01 (m, ArC), 128.5 (2ArC), 126.8 (ArC), 121.5 (ArC), 56.7 (CH3); 19F NMR (376 MHz, Chloroform-d) δ 83.51 (q, J = 150.5 Hz), 63.32 (d, J = 150.5 Hz); LCMS Purity (UV) = 96%, R 18.11 min; HRMS - ESI (m/z) found 385.0404, calc. for [C8H7F3N2OS]+: 385.0404; IR (neat) νmax/cm−1: 3089 (N-H), 1688 (C=O), 1610 (C=O), 824 (S=O); mp = 158 – 159 °C.

Table 3. Mean maximum potentiation and potency values for diazepam and SF3-substituted BZDs for modulating GABAA receptors.

<table>
<thead>
<tr>
<th>Diazepam analogues, μM</th>
<th>Maximum Potentiation</th>
<th>Potency pEC50 ± SEM (EC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>133 ± 15%</td>
<td>7.52 ± 0.07 (30 nM)</td>
</tr>
<tr>
<td>2c</td>
<td>138 ± 19%</td>
<td>5.78 ± 0.04 (1.7 μM)</td>
</tr>
<tr>
<td>5c</td>
<td>77 ± 20%</td>
<td>5.25 ± 0.12 (5.7 μM)</td>
</tr>
<tr>
<td>6c</td>
<td>28 ± 2.5%</td>
<td>5.70 ± 0.08 (2.0 μM)</td>
</tr>
</tbody>
</table>

Fig. 2. Benzodiazepines in complex with α1β3γL receptor a) Brown dashed lines show hydrophobic clashes between Diazepam’s (purple) chlorine and amino acid residues, His102, Val203, and Tyr210. b) Diazepam (purple) overlapped with SF3-diazapam (5c, red). (blue) and the benzodiazepine binding pocket and d) superposition of nordiazapam and SF3-nordiazapam (2c, pink). e) 6c (Violet) in complex with α1β3γL receptor shows no interaction with His102. Aromatic hydrogen bonds are indicated by blue dashed lines.Yellow dashed lines indicate hydrogen bonds. Pi-pi interactions are indicated by blue dashed lines. SF3-diazapam and SF3-nordiazapam do not interact with the His102 which is a key interaction between Diazepam and the binding pocket.
mg, 12%, ~90% purity by LCMS after several more attempted purifications. 3H NMR (600 MHz, CDCl3) δ 10.25 (s, 1H), 7.65 – 7.61 (m, 3H), 7.56 – 7.53 (m, 1H), 7.39 – 7.35 (m, 3H), 7.31 (d, J = 7.5 Hz, 1H), 4.69 (s, 2H).

tert-Butyl-N-(2-benzozy-4-(pentfluorophenoxy)phenyl)carbamoyl)methylcarbamate (3a). 2-Aminobenzonitrile (300.0 mg, 1.52 mmol), EEDQ (376.0 mg, 1.52 mmol), Boc-Gly-OH (268.0 mg, 1.52 mmol) and DCN (3 mL) were subjected to microwave irradiation at 150 °C for 30 min at 200 W. After 30 minutes, the reaction mixture was diluted with DCM (5 mL) and washed with 10% HCl (3 x 5 mL). The organic layer was extracted with DCM (2 x 5 mL), dried over MgSO4, filtered and concentrated in vacuo. The crude was purified over a column of silica (hexane:EtOAc; 7:3) to obtain a white solid (87 mg, 42% yield). 1H NMR (400 MHz, Chloroform-d) δ 7.93 (d, J = 9.1 Hz, 2H, ArH), 7.58 – 7.53 (m, 1H, ArH), 7.52 (m, 1H, ArH), 7.50 (m, 1H, ArH), 7.40 (d, J = 11.0 Hz, 1H, CH3), 3.79 (d, J = 11.0 Hz, 1H, CH3), 3.44 (s, 3H, CH3). 13C NMR (100 MHz, Chloroform-d) δ 169.8 (C=O), 168.8 (C=O), 148.1 (C-5F), 146.1 (ArC=O), 137.7 (ArC), 131.1 (ArC), 129.4 (2ArC), 128.7 (4ArC), 128.6 (ArC), 121.2 (ArC), 56.9 (CH3), 34.9 (CH3). 

4.2. Computational ligand docking

Docking was performed using the solved cryo-EM structure of the α2βγ3 receptor complex in complex with GABA and Diazepam obtained from PDB (ID: 6HUP). The software used was Schrodinger Glide.

4.3. Cell culture and recombinant GABAβ expression

HEK cells were maintained at 37°C, 95% CO2/5% O2 in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% f/v fetal bovine serum and 100 U/ml penicillin/100 µg/ml streptomycin. Cells were transfected with cDNAs encoding enhanced green fluorescent protein (EGFP) and mu1, β2, γ2L GABAβR subunits in a 1:1:1:1 ratio using a standard calcium phosphate precipitation method.

4.4. Electrophysiology experiments

Whole-cell patch clamp recording from HEK cells was used to study GABAβ receptor currents as described previously [31] using an Axopatch 200B Axon Instruments amplifier. Patch pipettes (resistance 3–5 MΩ) were filled with a solution containing (mM): 140 NaCl, 1.2 MgCl2, 2.5 CaCl2, 11 Glucose and 5 HEPS; pH 7.4. Diazepam and SF1 analogues were first dissolved in DMSO (stock), and for functional electrophysiology experiments subsequently diluted at least 1:100-fold in Krebs solution. Drug solutions were applied to recording cells via a Y-tube application system [31]. The potentiating effects of diazepam, and analogues 2c, 5c and 6c were evaluated in the presence of 2 µM GABA which was equivalent to a current approximately 6.5% of the GABA maximum response (EC50). The efficacy and potency for the potentiation by each ligand was established by fitting curves to the GABA current response-concentration relationship data points from each of the five individual experiments using the Hill equation. JHill pot = 1 / (1 + (EC50/[L]). The ligand potency, EC50, represents the concentration of the ligand ([L]) inducing 50% of the maximum potentiation current (in the presence of 2 µM GABA), and n is the Hill slope. Since concentration response EC50 data are distributed on a logarithmic scale, we converted these to pEC50 values (pEC50 = -log(EC50)) which are distributed on a linear scale. From pEC50 values we calculated mean ± SEM, and to facilitate data interpretation we re-transformed these mean pEC50 values into mean EC50 values (Table 3). The relative efficacy for GABA current potentiation was calculated as a mean percentage ± SEM of the current induced by 2 µM GABA alone.

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References.


