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Synthesis and biological evaluation of benzodiazepines containing a pentafluorosulfanyl group

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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}). 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 a subunit.

1. Introduction

The pentafluorosulfanyl group is often employed in medicinal chemistry as a bioisosteric “super trifluoromethyl” group. Possessing high thermal stability, electron withdrawing effects and high lipophilicity, it has enjoyed success in a number of drug discovery projects.\textsuperscript{1-4} Since their discovery in the late 1950s, benzodiazepines (BZDs) which act as positive allosteric modulators on γ subunit containing synaptic GABA\textsubscript{A} receptors (GABA\textsubscript{A}Rs), have been widely employed to treat a wide spectrum of disorders such as anxiety, insomnia, seizures and alcohol withdrawal.\textsuperscript{7-10} Structure activity relationships show, inter alia, that electron withdrawing groups at the 7-position are important for improved receptor affinity (Fig. 1).\textsuperscript{11-13}

![Fig.1. Selected clinically-used BZDs.](image)

With research outputs in both benzodiazepine\textsuperscript{14,15} and SF\textsubscript{5} chemistry\textsuperscript{16}, 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{17-19} 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{18}\textsuperscript{20} 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 (\(\sigma_p = 0.68\) for SF\textsubscript{5} and \(\sigma_p = 0.78\) 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 lead to at this position. 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 85% yield.
Scheme 1. Synthesis of BZDs by microwave techniques.

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 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.

Table 1. Boc-Gly-OH coupling reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</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 hexamethylenetetramine 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 hexamethylenetetramine 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 its $^1$H 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 white crystals and this confirmed both the regiochemistry of the SF$_2$-substituent and the formation of the BZD core (Scheme 2). [24]

We next wished to N-methylate the nordiazepam analogue 2c. A standard N-methylation route using sodium hydride and methyl iodide yielded the desired SF$_2$-BZD 5c in modest yield.

Scheme Error! No text of specified style in document. N-methylation to form a N-methylated SF$_2$-BZD.

An unoptimised attempt at Pd-catalysed C-H activation using iodonium salts, [25] 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, although expected to be inactive from our previous SAR studies, was now available for biological assay.

Scheme Error! No text of specified style in document. Ortho-arylation of a BZD.

The compounds were docked into the cryo-EM structure (PDB ID: 6HUP) of the α/βγ2L GABA$_a$ receptor at the interface benzodiazepine binding site between the principal (+) α and complementary (-) γ subunit using Schrödinger Glide. [26] We evaluated their apparent binding affinity using the Glide score, which finds all the possible binding locations and positions 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. [27]

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. [28]

A Glide score was determined for compounds 2c, 5c and 6c and was compared against diazepam and the metabolite, nordiazepam (Table 1). The SF$_2$-substituted nordiazepam analogue, 2c, however gave a better Glide score than diazepam suggesting it may bind stronger in the binding site. The Glide score of the ortho C-H activated analogue 6c was very poor in comparison.
Poses of diazepam, 2c, 5c and 6c respectively with the best Glide score docked in the α1β3γ2L receptor are shown in Figure 2. The dashed lines indicate hydrogen bonds and π-π interactions. The chlorine atom interacts with the critical αHis102 side chain (Ref). The distance between the chlorine atom and the nitrogen on the αHis102 was measured as 2.89 Å. The images show that there is no interaction between SF5 and the amino acid side chains. A direct comparison of 5c and diazepam can be made. We calculated the distance between SF5 and αHis102 to be 5.34 Å. This was calculated between the closest fluorine of SF5 to αHis102. This distance is almost double the distance between chlorine and αHis102 for Diazepam (2.89 Å). This could explain the lack of interaction between SF5 and the αHis102. This increased distance and lack of interaction also applies to 2c and 6c as well.

Figure 2: a) Diazepam (pink) and SF5-diazepam (5c, teal) in complex with α1β3γ2L receptor. b) Nordiazepam (blue) and SF5-nordiazepam (2c, green) in complex with α1β3γ2L receptor. c) 6c (red) in complex with α1β3γ2L receptor. Yellow dashed lines indicate hydrogen bonds. Pi-π interactions are indicated by blue dashed lines.

To access functionality of the BDZ ligands, we used whole-cell patch-clamp recording from human embryonic kidney cells expressing recombinant α1β2γ2L GABAA Rs. The analogues, 2c, 5c and 6c were compared to diazepam for their ability to potentiate 2μM GABA-induced currents (~EC50). The three SF5-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 2). 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[28], and could reflect increased desensitization of GABAA Rs. From these data, it is clear that substituting Cl on the benzo ring for SF5 has a deleterious effect primarily on BDZ potency and to a large extent, also on their 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 BDZ modulation at GABAA receptors. Indeed, mutation of His for Arg at this location, and found in BDZ-insensitive α4 and α6 receptors, completely abolishes BDZ modulation of GABAA receptor activation (Wieland et al 1992).

**Table 1.** Glide score of Diazepam versus SF5-substituted BDZs.

<table>
<thead>
<tr>
<th>Entry</th>
<th>1,4-BZD</th>
<th>Glide score</th>
</tr>
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<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>

**Table 2.** Mean maximum potentiation and potency values for diazepam and SF5-substituted BDZs for modulating GABAA receptors.

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

Fig. 3. Concentration-response curves for the potentiation of GABA-induced currents by diazepam (black), 2c (green), 5c (red), and 6c (blue) on recombinant α1β2γ2L GABAA Rs expressed in HEK293 cells. Data points (mean ± SEM; n = 5) are plotted as percentages of current potentiation above that induced by 2 μM GABA in the absence of modulator.
Conclusion

Selected SF$_2$-substituted 1,4-BZDs have been synthesised 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$_2$ group, reduced GABA potency and for 5c and 6c a reduced efficacy were evident.

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 VNMRS 600 (1 H 600 MHz, 13C 126 MHz) and VNMRS 400 (1 H 400 MHz, 13C 100 MHz) spectrometers, and prepared in deuterated solvents such as CDCl$_3$ and DMSO-$d_6$. 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, pt – pseudo triplet, q – quartet, m – multiplet and coupling constants are given in Hertz (Hz).

Electronspin 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 ratio (m/z) ratio. LC-MS spectra were acquired using Shimadzu LC-MS 2020, or Agilent HP1100 series. NMR columns were calibrated and purifications were performed by flash chromatography on silica gel (1H and 13C), and prepared in 10 ml microwave vial. The vial was degassed and purged with argon before adding palladium (II) acetate (7 mg, 0.0089 mmol, and 0.01equiv) and stirring at 125 °C in the microwave for 1 hour. Thereafter, the cooled reaction mixture was filtered through celite, washed with dichloromethane (50 ml) and concentrated in vacuo. The residue was dissolved in dichloromethane (15 ml), washed with sodium bicarbonate (20 ml) and the organic layer extracted with dichloromethane (20 ml x 3), dried over MgSO$_4$, filtered and concentrated in vacuo. The neutral oil was purified over a column of silica (hexane:EtOAc 7:3) to give the pure product.

4.2. Computational ligand docking

Docking was performed using the solved cryo-EM structure of the α5β2γ2L receptor in complex with GABA and Diazepam obtained from PDB (ID: 6HUP). The software used was Schrodinger Glide.

4.3. Cell culture and recombinant GABA$_A$R expression

HEK cells were maintained at 37°C, 95% CO$_2$, 5% O$_2$ in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% v/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 murine α1, β2, γ2L GABA$_A$R 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 GABA$_A$ receptor currents as described previously using an Axopatch 200B Axon Instruments amplifier. Patch pipettes (resistance 3-5MΩ) were filled with a solution containing (mM): 120 KCl, 1 MgCl$_2$, 11 EGTA, 30 KOH, 10 HEPES, 1 CaCl$_2$, and 2 adenosine triphosphate; pH 7.11. The cells were continuously perfused with Krebs recording solution containing (mM): 140 NaCl, 2.4 KCl, 1.8 MgCl$_2$, 2.5 CaCl$_2$, 11 Glucose and 7 HEPES; pH 7.4. Diazepam and SF$_2$ analogues were first dissolved in DMSO (stock), and for functional electrophysiology experiments subsequently diluted at least 1000-fold in Krebs solution. Drug solutions were applied to recording cells via a Y-tube application system. 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 (EC$_{50}$). The efficacy and potency for the potentiation by each ligand was established by fitting the GABA current response concentration relationship data points from each of the five individual experiments using the Hill equation, $I/I_{max} = e^{(x - EC_{50})/Hill's}$. The ligand potency, EC$_{50}$, represents the concentration of the ligand (IL) inducing 50% of the maximal potentiation current (in the presence of 2 μM GABA), and is expressed as μM.

Since concentration response EC$_{50}$ data are distributed on a logarithmic scale, we converted these to pEC$_{50}$ values (pEC$_{50}$ = -log(EC$_{50}$)) which are distributed on a linear scale. From pEC$_{50}$ values we calculated mean values ± sem, and to facilitate data interpretation we re-transformed these mean pEC$_{50}$ values into mean EC$_{50}$ values (Table 2). The relative
efficacy for GABA current potentiation was calculated as a mean percentage ± sem of the current induced by 2 μM GABA alone.

References.