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Arathy Jose, Raysa Khan Tareque, Martin Mortensen, Remi Legay, Simon J. Coles, Graham J. Tizzard, Barnaby W. Greenland, Trevor G. Smart, Mark C. Bagley\*, John Spencer\*



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|  | Tetrahedron |  |

Synthesis and biological evaluation of benzodiazepines containing a pentafluorosulfanyl group

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| ARTICLE INFO |  | ABSTRACT |
| Article history:  Received  Received in revised form  Accepted  Available online |  | The widely used pentafluorosulfanyl group (SF5) was deployed as a bioisosteric replacement for a chloro- group in the benzodiazepine diazepam (ValiumTM). Reaction of 2-amino-5-pentafluorosulfanyl-benzophenone with chloroacetyl chloride followed by hexamethylenetetramine, in the presence of ammonia, led to 7-sulfurpentafluoro-5-phenyl-1*H*-benzo[*1,4*]diazepin-2(*3H*)-one (2c). 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 SF5 group, led to a loss of potency for potentiating GABAA receptor activation, most likely because of a lost ligand interaction with His102 in the GABAA receptor α subunit.  Dedicated to an inspirational and humble pioneer, Prof Jonathan Williams, a colleague and mentor in chemistry.  2009 Elsevier Ltd. All rights reserved. |
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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. [1–6]

Since their discovery in the late 1950s, benzodiazepines (BZDs) which act as positive allosteric modulators on γ-subunit containing synaptic GABAA receptors (GABAARs)[7,8], have been widely employed to treat a wide spectrum of disorders such as anxiety, insomnia, seizures and alcohol withdrawal.[9][10–12] Structure activity relationships show, *inter alia*, that electron withdrawing groups at the 7-position are important for improved receptor affinity (Fig. 1).[13,14][15]



**Fig.1.** Selected clinically-used BZDs.

With research outputs in both benzodiazepine[16,17] and SF5 chemistry[18], there was a natural inclination for us to combine these interests in the design of SF5-containing BZDs. We, therefore, aimed to synthesise analogues **2a** - **2c** (Scheme 1) related to the much-prescribed drug, diazepam (ValiumTM) in order to evaluate the effect of changing a Cl for a SF5 group on biological activity.

1. **Results and Discussion**

We opted for a one-pot microwave route to synthesise SF5-substituted BZD analogues*.*[19–21] 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.[17][22] 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 SF5 group (σ*p*= 0.68[5] for SF5 and σ*p* = 0.78[23] for NO2). 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 65% 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, 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**.

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| Entry | Product | Yield (%) |
| 1 |  | 86 |
| 2 |  | 10 |
| 3 |  | - |

**Table 1**. Boc-Gly-OH coupling reactions.

As the microwave-mediated attempts towards the SF5-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[24,25] [26] 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 of its 1H NMR[26] 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 SF5 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 SF5-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 SF5-BZD **5c** in modest yield (Scheme 3).



**Scheme 3.** N-Methylation of a SF5-BZD.

An unoptimised attempt at Pd-catalysed C-H activation with iodonium salts,[28] using our previously described conditions, involving microwave chemistry afforded the expected ortho-aryated 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 α1β3γ2L GABAA receptor at the interfacial benzodiazepine binding site between the principal (+) α and complementary (-) γ subunit using Schrödinger Glide.[29] 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.[30] 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.[31]

A Glide score was determined for compounds **2c**, **5c** and **6c** and was compared against diazepam and the metabolite, nordiazepam (Table 2). The SF5-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.

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| Entry | 1,4-BZD | Glide score |
| 1 | diazepam | -4.74 |
| 2 | nordiazepam | -5.36 |
| 3 | **2c** | -4.97 |
| 4 | **5c** | -4.74 |
| 5 | **6c** | -3.98 |

**Table 2**. Glide score of Diazepam versus SF5-substituted BZDs.

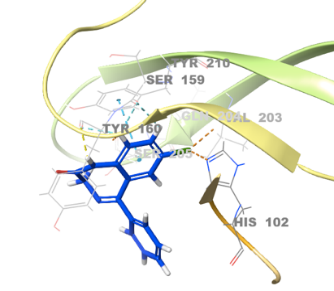
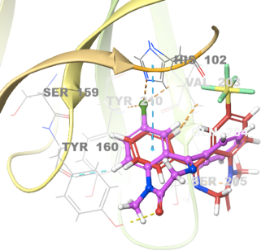
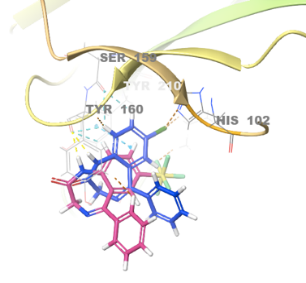
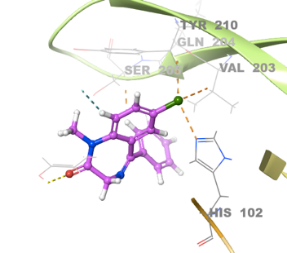
Poses of diazepam, **2c**, **5c** and **6c** respectively with the best Glide score docked in the α1β3γ2L receptor are shown in Figure 2. The dashed lines indicate hydrogen bonds and π-π interactions. The chlorine atom interacts with the critical αHis102 side chain.[32,33] The distance between the chlorine 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.

To access functionality of the BZD ligands, we used whole-cell patch-clamp recording from human embryonic kidney cells expressing recombinant α1β2γ2L GABAARs. The analogues, **2c**, **5c** and **6c** were compared to diazepam for their ability to potentiate 2μM GABA-induced currents (~EC6.5). 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 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[34], and could reflect increased desensitization of GABAARs.

c)

d)

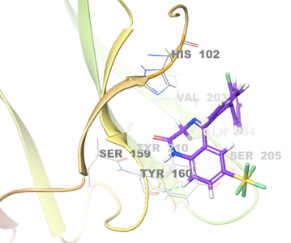


a)

b)

d)

c)



e)

**Fig. 2**. Benzodiazepines in complex with α1β3γ2L 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 SF5-diazapam (**5c**, red). c) Interactions between nordiazepam (blue) and the benzodiazepine binding pocket and d) superposition of nordiazepam and SF5-nordiazapam (**2c**, pink). e) **6c** (Violet) in complex with α1β3γ2L 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. SF5-diazapam and SF5-nordiazapam do not interact with the His102 which is a key interaction between Diazepam and the binding pocket.

From these data, it is clear that substituting Cl on the benzo ring for SF5 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 location, and found in BZD-insensitive α4 and α6 receptors, completely abolishes BZD modulation of GABAA receptor activation.[34]

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|  | Diazepam | 2c | 5c | 6c |
| Maximum Potentiation | 133 ± 15 % | 138 ± 19 % | 77 ± 20 % | 28 ± 2.5 % |
| Potency  pEC50 ± SEM (EC50) | 7.52 ± 0.07  (30 nM) | 5.78 ± 0.04  (1.7 μM) | 5.25 ± 0.12  (5.7 μM) | 5.70 ± 0.08  (2.0 μM) |

**Table 3**. Mean maximum potentiation and potency values for diazepam and SF5-substituted BZDs for modulating GABAA receptors.

Chart, line chart

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**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 GABAARs 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.

1. **Conclusion**

Selected SF5-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 SF5 group, reduced GABA potency and for **5c** and **6c** a reduced efficacy were evident.

1. **Experimental**
   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 (1H 600 MHz, 13C 126 MHz) and VNMRS 400 (19F 376 MHz, 2H 61 MHz and 31P 162 MHz) spectrometer and prepared in deuterated solvents such as Chloroform-d and DMSO-d6. 1H 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). Electronspray 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 m 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‐λ6‐sulfanyl)‐5‐phenyl‐2,3‐dihydro‐1H‐1,4‐benzodiazepin‐2‐one** (**2c**).Triethylamine (0.188 g, 1.86 mmol) was added to a solution of 2‐benzoyl‐4‐(pentafluoro‐**λ**6‐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 was monitored by TLC and the crude was concentrated on *vacuo* and purified by flash chromatography (petroleum ether: ethyl acetate; 7:3) to obtain pure N‐[2‐benzoyl‐4‐(pentafluoro‐**λ**6‐sulfanyl)phenyl]‐2‐chloroacetamide as a colourless solid (301 mg, 81%). Ammonium carbonate (0.360 g, 3.75 mmol) was suspended in a solution of ammonia (2M) in ethanol (5 mL) and stirred. Hexamethylenetetramine (0.531 g, 3.75 mmol) was added and refluxed. After 5 minutes of refluxing, a solution of *N*-(2-benzoyl-4-sulfur penta-fluoro phenyl)-2-chloroacetamide in dichloromethane (3 mL) was added and the reaction mixture was refluxed overnight. After cooling, 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, 1*JF,C* = 18.9 Hz, ArC-SF5), 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 (CH2); 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%, tR 18.11 min; HRMS - ESI (m/z) found 385.0404, calc. for [C15H11F5N2OS] [+Na]+: 385.0404; IR (neat) νmax /cm-1: 3089 (N-H), 1688 (C=O), 1610 (C=N), 824 (S-F); mp = 158 – 159 °C.

**3-Amino‐6‐(pentafluoro‐ -6‐sulfanyl)‐4‐phenyl‐1H‐quinolin‐2‐one** (**4c**) was isolated from the reversed phase column of **2c** as a colourless solid (33 mg, 12%, <90% purity by LCMS after several more attempted purifications) 1H NMR (600 MHz, CD3CN) δ 10.28 (s, 1H), 7.65 – 7.61 (m, 3H), 7.56 – 7.53 (m, 1H), 7.39 – 7.35 (m, 3H), 7.31 (d, *J* = 2.5 Hz, 1H), 4.69 (s, 2H).

***tert*-Butyl-*N*-({[2-benzoyl) phenyl]carbamoyl} methyl)carbamate** (**3a**).[20] 2-Aminobenzophenone (300.0 mg, 1.52 mmol), EEDQ (376.0 mg, 1.52 mmol), Boc-Gly-OH (268.0 mg, 1.52 mmol) and DCM ( 3 mL) were subjected to microwave irradiation at 150 ºC 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 5mL), dried over MgSO4, filtered and concentrated in vacuo. The crude was purified over a column of silica (hexane: EtOAc; 7:3) to obtain the title compound as a colourless solid (465 mg, 86%). 1H NMR (600 MHz, dmso-d6) δ 10.51 (s, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.46 (dd, *J* = 17.5, 7.7 Hz, 3H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.24 – 7.19 (m, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 4.08 (s, 2H), 2.47 (s, 9H). Known compound.

***tert*-Butyl-*N*-({[2-benzoyl-4-(pentafluoro-λ⁶-sulfanyl)phenyl]carbamoyl} methyl)carbamate** (**3b**). 2‐Benzoyl‐4‐(pentafluorosulfanyl)aniline (100.0 mg, 0.31 mmol), EEDQ (77.0 mg, 0.31 mmol), Boc-Gly-OH (55.0 mg, 0.31 mmol) and DCM ( 1 mL) were subjected to microwave irradiation at 150 ºC 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 5mL), dried over MgSO4, filtered and concentrated in vacuo. The crude was purified over a column of silica (Hexane: EtOAc; 7:3) to obtain the title compound as a colourless solid. (15 mg, 10%). 1H NMR (600 MHz, Chloroform-d) δ 11.12 (s, 1H, NH), 9.18 (s, 1H), 7.71 – 7.68 (m, 2H, ArH), 7.65 – 7.62 (m, 2H, ArH), 7.51 (d, *J* = 7.8 Hz, 2H, ArH), 7.49 – 7.46 (m, 2H, ArH), 3.99 (d, *J* = 6.0 Hz, 2H, ArH), 1.44 (s, 9H, (CH3)3). Insufficient material for 13C spectrum.

**1‐Methyl‐7‐(pentafluoro‐λ6‐sulfanyl)‐5‐phenyl‐2,3‐dihydro‐1H‐1,4‐benzodiazepin‐2‐one** (**5c**). Sodium hydride (0.023 g, 0.94 mmol) was added to a solution of **2c** (0.170 g, 0.47 mmol) in dry THF (1 mL) and the mixture was stirred for 1 hour. Methyl iodide (0.133g, 0.94 mmol) was added to the reaction mixture, which was stirred at room temperature overnight. Crude product was washed with water (3x15 mL), extracted with ethyl acetate (3x15 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 colourless solid as the title compound (66 mg, 37%). 1H NMR (600 MHz, Chloroform-d) δ 7.93 (dd, *J*= 9.1 Hz, 2.6 Hz, 1H, ArH), 7.73 (d, *J*= 2.6 Hz, 1H, ArH), 7.61 (m, 2H, ArH), 7.52 (m, 1H, ArH), 7.45(m, 3H, ArH), 4.90 (d, *J*= 11.0 Hz, 1H, CH), 3.79 (d, *J*= 11.0 Hz, 1H, CH), 3.44 (s, 3H, CH3); 13C NMR (600 MHz, Chloroform-d) δ 169.8 (C=O), 168.8 (C=N), 148.4 (C-SF5), 146.1(ArC), 137.7 (ArC), 131.1 (ArC), 129.4 (2ArC), 128.7 (4ArC), 128.6(ArC), 121.2 (ArC), 56.9 (CH2), 34.9 (CH3); 19F NMR (400 MHz, Chloroform-d) δ 83.10 (p, *J*= 150.4 Hz), 63.23 (d, *J*= 150.4 Hz); LCMS Purity (UV) = 96%, tR 19.52 min; HRMS -ESI(m/z) found 377.0749, calc. for [C16H13F5N2OS][+H]+:377.0742; IR (neat) νmax/cm-1: 1682 (C=O), 1611 (C=N), 835 (S-F); mp = 240 – 242 °C.

**5‐{[1,1'‐Biphenyl]‐2‐yl}‐7‐(pentafluoro‐λ6‐sulfanyl)‐2,3‐dihydro‐1H‐1,4‐benzodiazepin‐2‐one (6c). 2c** (86 mg, 0.2 4mmol), bis(2‐fluorophenyl)iodonium tetrafluoroborate (145 mg, 0.36 mmol) and glacial acetic acid were combined in a 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 MgSO4, filtered and concentrated in vacuo. The bright red oil was purified over a column of silica (hexane:EtOAc; 1:4) to obtain a colourless solid as pure product (24 mg, 22%). 1H NMR (600 MHz, Chloroform-d) δ 9.49 (s,1H, NH), 7.82 –7.74 (m, 1H, ArH), 7.58 –7.53 (m, 2H, 2ArH), 7.50 (dd, *J*= 8.9, *J*=2.5 Hz, 1H, ArH), 7.34 –7.30 (m, 2H, 2ArH), 7.07 –7.01 (m, 1H, ArH), 6.92 –6.84 (m, 2H, ArH), 6.78 (d, *J* = 8.9 Hz, 1H, ArH), 6.75 (pt, *J* = 9.0 Hz, 1H), 4.32 (s, 2H, CH2);13C NMR (600 MHz, Chloroform-d) δ 171.1 (C=O), 170.9 (C=N), 158.7 (ArC-F, d, 1*J*F,C= 247.6 Hz), 148.6 –148.0(ArC-SF5), 139.5 (ArC), 138.9 (ArC), 135.7 (ArC), 131.3 (ArC), 131.0 (ArC),130.4 (ArC), 130.2 (ArC), 129.5 (ArC, d, 3*J*F,C= 7.9 Hz), 128.5 (2ArC), 128.2 (ArC), 127.8 (ArC, d, 2*J*F,C= 15.6 Hz), 127.6 (ArC), 123.7 (ArC), 120.5 (ArC), 115.0 (ArC, d, 2*J*F,C= 22.0 Hz), 56.5 (CH2); 19F NMR (376 MHz, Chloroform-d) δ 83.40 (p, *J*= 150.7 Hz, axial F), 63.07 (d, *J*= 150.7 Hz, equatorial F), -114.58 (F); LCMS Purity (UV) = 98%, tR: 19.85min; HRMS -ESI(m/z) found 457.0813, calc. for [C21H15F6N2OS][+H]+:457.0809; IR (neat) νmax/cm-1: 3064 (N-H), 1704 (C=O), 1616 (C=N), 1336 (C-F), 835.5 (S-F); mp = 190 – 191 °C.

* 1. *Computational ligand docking*

Docking was performed using the solved cryo-EM structure of the α1β3γ2L receptor in complex with GABA and Diazepam obtained from PDB (ID: 6HUP). The software used was Schrodinger Glide.

*4.3. Cell culture and recombinant GABAAR expression*

HEK cells were maintained at 37°C, 95% CO2 / 5% O2 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 GABAAR| 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 GABAA receptor currents as described previously[35] using an Axopatch 200B Axon Instruments amplifier. Patch pipettes (resistance 3-5MΩ) were filled with a solution containing (mM): 120 KCl, 1 MgCl2, 11 EGTA, 30 KOH, 10 HEPES, 1 CaCl2, and 2 adenosine triphosphate; pH 7.11. The cells were continuously perfused with Krebs recording solution containing (mM): 140 NaCl, 4.7 KCl, 1.2 MgCl2, 2.52 CaCl2, 11 Glucose and 5 HEPES; pH 7.4. Diazepam and SF5-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[30].

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 (EC6.5). 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, I / Imax pot = (1 / (1 + (EC50 / [L])n). The ligand potency, EC50, represents the concentration of the ligand ([L]) inducing 50% of the maximal 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 values ± 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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