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Synthesis and biological evaluation of benzodiazepines containing a pentafluorosulfanyl group

Arathy Jose^a, Raysa Khan Tareque^a, Martin Mortensen^b, Remi Legay^c, Simon J. Coles^d, Graham J. Tizzard^d, Barnaby Greenland^a, Trevor G. Smart^b, Mark C. Bagley^a, John Spencer^{a,*}

^aChemistry Department, School of Life Sciences, Falmer, Brighton, BN1 9QJ, UK.

^bDepartment of Neuroscience, Physiology & Pharmacology, Division of Biosciences, University College London, Gower Street, London, WC1E 6BT, UK.

^cENSICAEN, 6 boulevard du Maréchal Juin, F-14050 Caen, Cedex, France.

^dNational Crystallography Service, School of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK

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ABSTRACT

The widely used pentafluorosulfanyl group (SF₅) was deployed as a bioisosteric replacement for a chloro- group in the benzodiazepine diazepam (Valium™). The replacement of Cl by the SF₅ group, led to a loss of potency for potentiating GABA_A receptor activation most likely because of a lost ligand interaction with His102 in the GABA_A receptor α subunit.

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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, electron withdrawing effects and high lipophilicity, it has enjoyed success 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 GABA_A receptors (GABA_ARs), have been widely employed to treat a wide spectrum of disorders such as anxiety, insomnia, seizures and alcohol withdrawal.^{[7][8–10]} Structure activity relationships show, *inter alia*, that electron withdrawing groups at the 7-position are important for improved receptor affinity (Fig. 1).^{[11,12][13]}

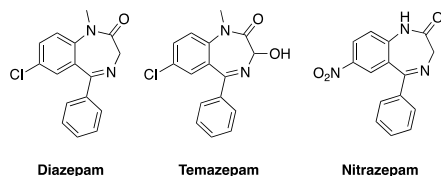
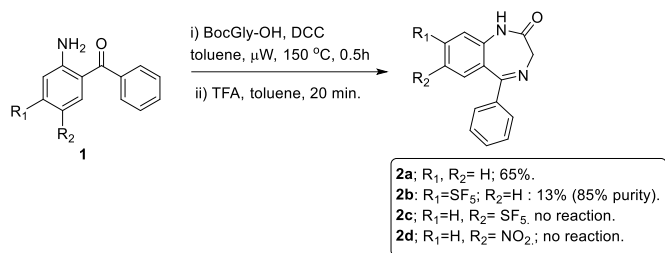


Fig.1. Selected clinically-used BZDs.

With research outputs in both benzodiazepine^[14,15] and SF₅ chemistry^[16], 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 (Valium™) in order to evaluate the effect of changing a Cl for a SF₅ group on biological activity.

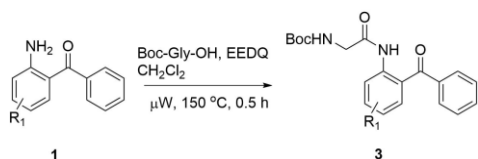
2. Results and Discussion

We opted for a one-pot microwave route to synthesise SF₅-substituted BZD analogues.^[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.^{[15][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 SF₅ group ($\sigma_p = 0.68$ for SF₅ and $\sigma_p = 0.78$ 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 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**.

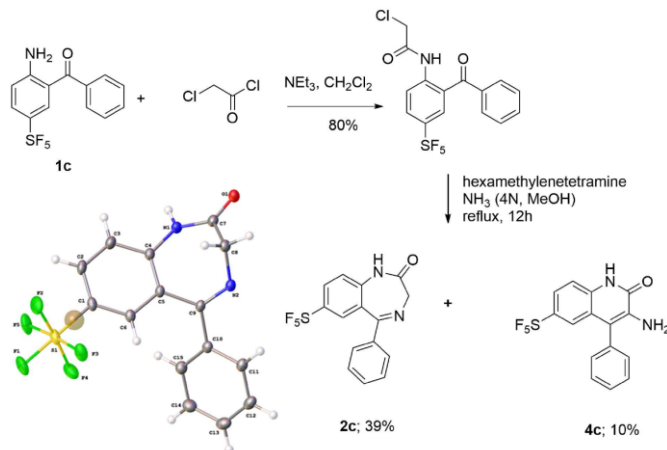


Entry	Compound	Yield (%)
1		86
2		10
3		-

Table 1. Boc-Gly-OH coupling reactions.

As the microwave-mediated attempts towards the SF_5 -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^[21,22] [23] 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.

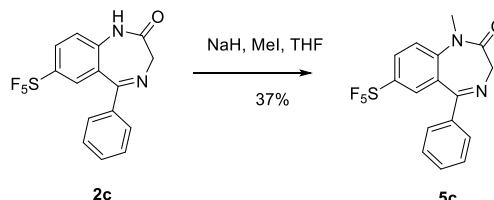
Hence, **1c** was acylated using chloroacetyl chloride then aminated with hexamethylenetetramine in the presence of ammonia (Scheme 2). Analysis of the crude mixture, gratifyingly, showed the presence of the expected product as well as a similar by-product, which we tentatively assigned the structure **4c**, notably by the similarity its 1H NMR^[23] 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_5 substituted BZD.

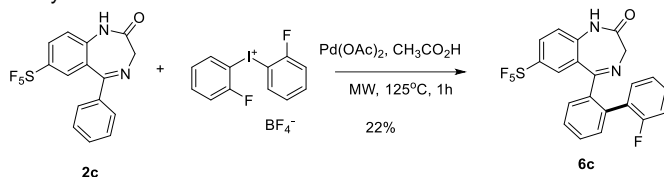
Compound **2c** was crystallised by a diffusion method using dichloromethane/hexane and obtained as white crystals and this confirmed both the regiochemistry of the SF_5 -substituent and the formation of the BZD core (Scheme 2).^[24]

We next wished to N-methylate the nordazepam analogue **2c**. A standard N-methylation route using sodium hydride and methyl iodide yielded the desired SF_5 -BZD **5c** in modest yield.



Scheme Error! No text of specified style in document.. N-methylation to form a N-methylated SF_5 -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 $\alpha 1\beta 3\gamma 2L$ GABA_A receptor at the interfacial 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_5 -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.

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 1. Glide score of Diazepam versus SF₅-substituted BZDs.

Poses of diazepam, **2c**, **5c** and **6c** respectively with the best Glide score docked in the $\alpha 1\beta 3\gamma 2L$ receptor are shown in Figure 2. The dashed lines indicate hydrogen bonds and π - π interactions. The chlorine atom interacts with the critical α His102 side chain (Ref). The distance between the chlorine atom and the nitrogen on the α His102 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 α His102 to be 5.34 Å. This was calculated between the closest fluorine of SF₅ to α His102. This distance is almost double the distance between chlorine and α His102 for Diazepam (2.89 Å). This could explain the lack of interaction between SF₅ and the α His102. This increased distance and lack of interaction also applies to **2c** and **6c** as well.

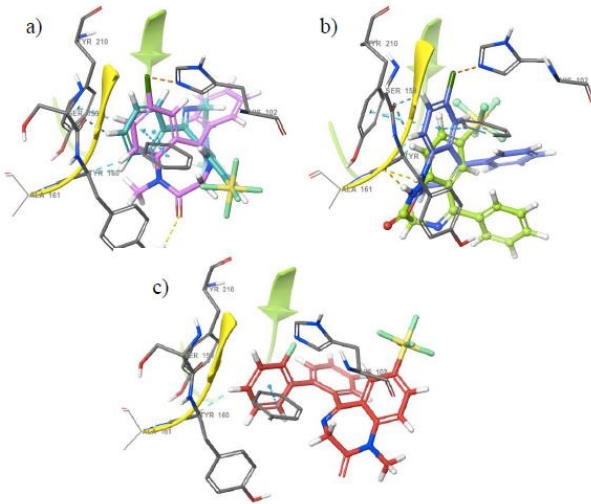
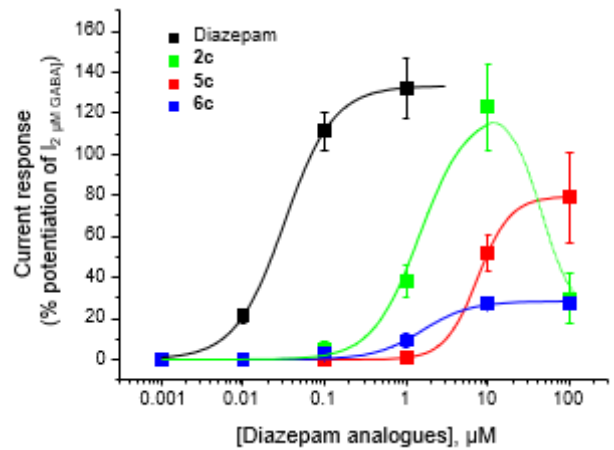


Figure 2: a) Diazepam (pink) and SF₅-diazepam (**5c**, teal) in complex with $\alpha 1\beta 3\gamma 2L$ receptor. b) Nordiazepam (blue) and SF₅-nordiazepam (**2c**, green) in complex with $\alpha 1\beta 3\gamma 2L$ receptor. c) **6c** (red) in complex with $\alpha 1\beta 3\gamma 2L$ receptor. Yellow dashed lines indicate hydrogen bonds. Pi-pi 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 $\alpha 1\beta 2\gamma 2L$ GABA_ARs. The analogues, **2c**, **5c** and **6c** were compared to diazepam for their ability to potentiate 2 μ M GABA-induced currents (\sim EC_{6.5}). 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**, \sim 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 μ M (Fig. 3). Such inhibition has been reported before for benzodiazepines like diazepam and flurazepam^[29], and could reflect increased desensitization of GABA_ARs. 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 GABA_A receptors. This is likely to be due to disruption of the Cl – α H102 interaction, which is known to be critical for BDZ modulation at GABA_A receptors. Indeed, mutation of His for Arg at this location, and found in BDZ-insensitive $\alpha 4$ and $\alpha 6$ receptors, completely abolishes BDZ modulation of GABA_A receptor activation (Wieland et al 1992).

Fig. 3. Concentration-response curves for the potentiation of GABA-induced currents by diazepam (black), **2c** (green), **5c** (red), and **6c** (blue) on recombinant $\alpha 1\beta 2\gamma 2L$ GABA_ARs expressed in HEK293 cells.



	Diazepam	2c	5c	6c
Maximum Potentiation	133 ± 15 %	138 ± 19 %	77 ± 20 %	28 ± 2.5 %
Potency pEC ₅₀ ± SEM (EC ₅₀)	7.52 ± 0.07 (30 nM)	5.78 ± 0.04 (1.7 μ M)	5.25 ± 0.12 (5.7 μ M)	5.70 ± 0.08 (2.0 μ M)

Table 2. Mean maximum potentiation and potency values for diazepam and SF₅-substituted BZDs for modulating GABA_A receptors.

Data points (mean ± SEM; n = 5) are plotted as percentages of current potentiation above that induced by 2 μ M GABA in the absence of modulator.

3. Conclusion

Selected SF₅-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 SF₅ group, 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 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 CDCl₃ 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). 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 m C18 110 Å. column. All X-ray analyses were 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. *In silico* studies

7-Sulfurpentafluoro-5-phenyl-1H-benzo[1,4]diazepin-2(3H)-one (2c).

Triethylamine (0.188 g, 1.86 mmol) was added to a solution of (2-amino-5-sulfur pentafluorophenyl) (phenyl) methanone (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 *in vacuo* and purified by flash chromatography (petroleum ether: ethyl acetate; 7:3) to obtain pure *N*-(2-benzoyl-4-sulfur penta-fluoro phenyl)-2-chloroacetamide as a white 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 white 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*J*_{F,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) ν_{max}/cm⁻¹: 3088.7 (N-H), 1687.4 (C=O), 1609.7 (C=N), 823.6 (S-F); m.p = 158 – 159 °C.

7-Sulfurpentafluoro-1-methyl-5-phenyl-1H-benzo[1,4]diazepin-2(3H)-one (5c). Sodium hydride (0.023 g, 0.94 mmol) was added to a solution of 7-sulfur pentafluoro-5-phenyl-1H-benzo[1,4]diazepin-2(3H)-one (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 white 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) ν_{max}/cm⁻¹: 1682.1 (C=O), 1611.1 (C=N), 834.9 (S-F); m.p = 240 – 242 °C.

7-pentafluorosulfanyl-5-(2,2'-difluorobiphenyl-2-yl)-1,3-dihydro-2H-1,4-benzodiazepin-2-on (6c). 7-Sulfurpentafluoro-5-phenyl-1H-benzo[1,4]diazepin-2(3H)-one (86mg, 0.24mmol), 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 white 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 [C₂₁H₁₅F₈N₂OS][H]⁺:457.0809; IR (neat) ν_{max}/cm⁻¹: 3063.6 (N-H), 1703.9 (C=O), 1615.9 (C=N), 1336.1 (C-F), 835.5 (S-F); m.p = 190 – 191 °C.

4.2. 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 GABA_AR 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 GABA_AR₁ 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^[30] 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 (EC_{6.5}). 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, $I / I_{\max \text{ pot}} = (1 / (1 + (EC_{50} / [L])^n))$. 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 2). The relative

efficacy for GABA current potentiation was calculated as a mean percentage \pm sem of the current induced by 2 μ M GABA alone.

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