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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_5) was deployed as a bioisosteric replacement for a chloro-group in the benzodiazepine diazepam (Valium™). 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 SF_5 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.

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

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 $GABA_A$ receptors ($GABA_A$Rs) [7,8], have been widely employed to treat a wide spectrum of disorders such as anxiety, insomnia, seizures and alcohol withdrawal [9–12]. Structure activity relationships show, *inter alia*, that electron withdrawing groups at the 7-position are important for improved receptor affinity (Fig. 1) [13–15].

With research outputs in both benzodiazepine [16,17] and SF_5 chemistry [18], there was a natural inclination for us to combine these interests in the design of SF_5-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 SF_5 group on biological activity.

2. Results and discussion

We opted for a one-pot microwave route to synthesise SF_5-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 SF_5 group

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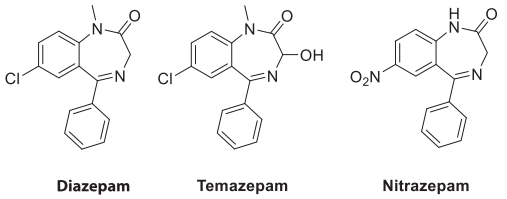
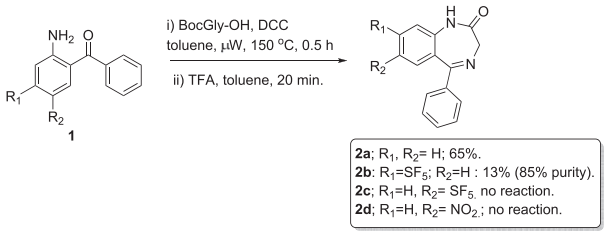


Fig. 1. Selected clinically-used BZDs.



Scheme 1. Synthesis of BZDs by microwave techniques.

($\sigma_p = 0.68$ [5] for SF_5 and $\sigma_p = 0.78$ [23] for NO_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 synthesized 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**.

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 [24–26] 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 ^{1}H 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.

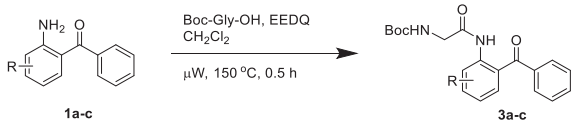
Compound **2c** was crystallised by a diffusion method using dichloromethane/hexane and obtained as colourless crystals and this confirmed both the regiochemistry of the SF_5-substituent and the formation of the BZD core (CCDC Deposition Number 2064966) (Scheme 2) [27].

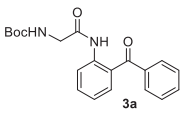
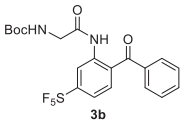
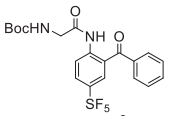
A standard *N*-methylation of **2c** using sodium hydride and methyl iodide yielded the desired SF_5-BZD **5c** in modest yield (see Scheme 3).

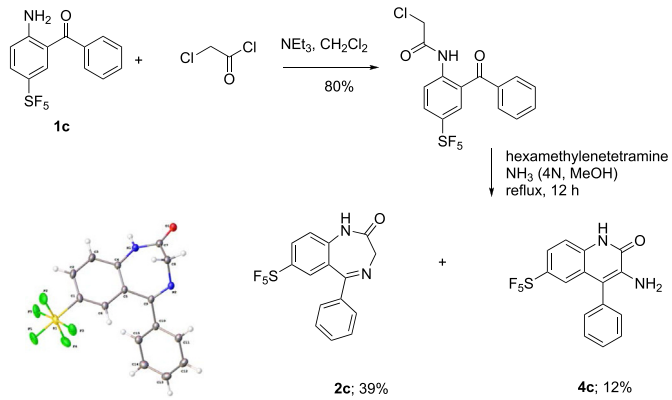
An unoptimised attempt at Pd-catalysed C–H activation with iodonium salts [28], using our previously described conditions, involving microwave chemistry afforded the expected ortho-aryated product **6c** (see Scheme 4). This illustrates that catalytic C–H activation chemistry is now amenable to the synthesis of polyfluorinated BZDs and **6c** was now available for biological assay.

The compounds were docked into the cryo-EM structure (PDB ID: 6HUP) of the $\alpha1\beta3\gamma2L$ $GABA_A$ 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

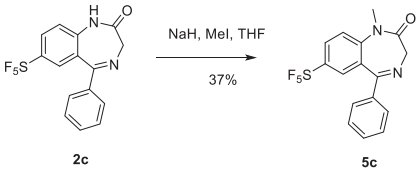
Table 1
Boc-Gly-OH coupling reactions.



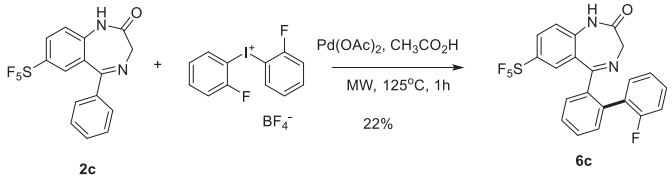
Entry	Product	Yield (%)
1	 3a	86
2	 3b	10
3	 3c	–



Scheme 2. Multi-step synthesis of a SF_5 substituted BZD.



Scheme 3. N-Methylation of a SF_5-BZD.



Scheme 4. Ortho-arylation of a BZD.

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 SF_5-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.

Poses of diazepam, **2c**, **5c** and **6c** respectively with the best Glide score docked in the α1β3γ2L receptor are shown in Fig. 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 SF_5 and the amino acid side chains. A direct comparison of **5c** and diazepam can be made. We calculated the distance between SF_5 and αHis102 to be 5.34 Å. This was calculated between the closest fluorine of SF_5 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 SF_5 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 $GABA_A$Rs. The analogues, **2c**, **5c** and **6c** were compared to diazepam for their ability to potentiate 2 μM GABA-induced currents (~$EC_{6.5}$). The three SF_5-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 $GABA_A$Rs.

From these data, it is clear that substituting Cl on the benzo ring for SF_5 has a deleterious effect primarily on BZD potency and to a

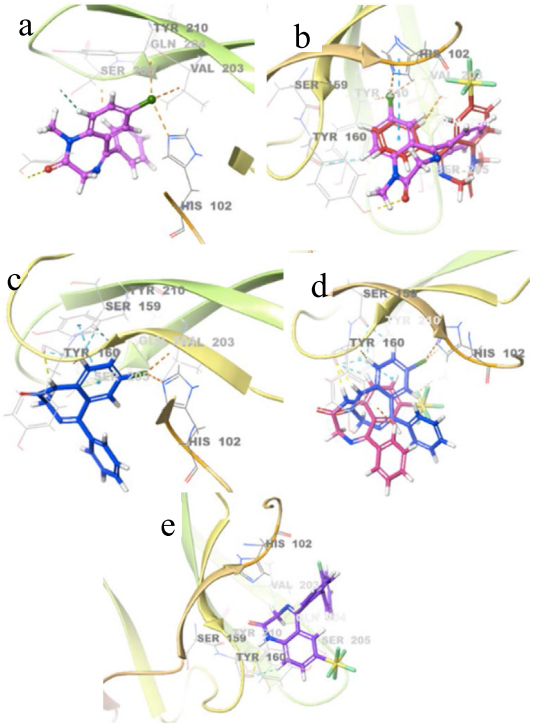


Fig. 2. Benzodiazepines in complex with α1β3γ2L receptor a) Brown dashed lines show hydrophobic clashes between Diazepam's (purple) chlorine and amino acid residues, His102, Val203, and Tyr210. b) Diazepam (purple) overlapped with SF_5-diazapam (**5c**, red). c) Interactions between nordiazepam (blue) and the benzodiazepine binding pocket and d) superposition of nordiazepam and SF_5-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. SF_5-diazapam and SF_5-nordiazapam do not interact with the His102 which is a key interaction between Diazepam and the binding pocket.

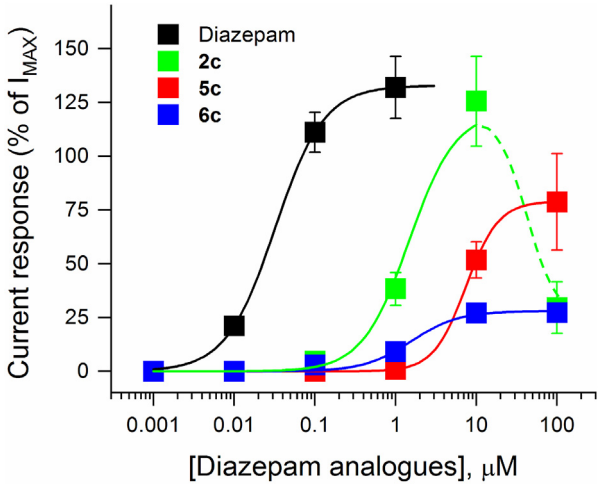


Fig. 3. Concentration-response curves for the potentiation of GABA-induced currents by diazepam (black), **2c** (green), **5c** (red), and **6c** (blue) on recombinant α1β2γ2L $GABA_A$Rs expressed in HEK293 cells. Data points (mean ± SEM; n = 5) are plotted as percentages of current potentiation above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

Table 2
Glide score of Diazepam versus SF_5-substituted BZDs.

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 3
Mean maximum potentiation and potency values for diazepam and SF_5-substituted BZDs for modulating $GABA_A$ receptors.

	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 μM)	5.25 ± 0.12 (5.7 μM)	5.70 ± 0.08 (2.0 μM)

large extent, also on relative efficacy at $GABA_A$ receptors. This is likely to be due to disruption of the Cl − αH102 interaction, which is known to be critical for BZD modulation at $GABA_A$ receptors. Indeed, mutation of His for Arg at this location, and found in BDZ-insensitive α4 and α6 receptors, completely abolishes BZD modulation of $GABA_A$ receptor activation [32,34].

3. Conclusion

Selected SF_5-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_5 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 (^{1}H 600 MHz, ^{13}C 126 MHz) and VNMRS 400 (^{19}F 376 MHz, ^{2}H 61 MHz and ^{31}P 162 MHz) spectrometer and prepared in deuterated solvents such as Chloroform-d and DMSO-d_6. ^{1}H and ^{13}C chemical shifts were recorded in parts per million (ppm). Multiplicity of ^{1}H 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-λ^6-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 h 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 (2 M) in ethanol (5 mL) and stirred. Hexamethylenetetramine (0.531 g, 3.75 mmol) was added and refluxed. After 5 min of refluxing, a solution of *N*-(2-benzoyl-4-sulfur pentafluoro 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 h. 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%). ^{1}H 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, CH_2); ^{13}C NMR (600 MHz, Chloroform-d) δ 172.1 (C═O), 169.8 (C═N), 148.1 (t, $^1J_{F,C}$ = 18.9 Hz, ArC-SF_5), 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 (CH_2); ^{19}F NMR (376 MHz, Chloroform-d) δ 83.51 (q, J = 150.5 Hz), 63.32 (d, J = 150.5 Hz); LCMS Purity (UV) = 96%, $_t$R 18.11 min; HRMS - ESI (m/z) found 385.0404, calc. for $[C_{15}H_{11}F_5N_2OS]$ $[+Na]^+$: 385.0404; IR (neat) ν_{max}/cm^{-1}: 3089 (N−H), 1688 (C═O), 1610 (C═N), 824 (S−F); mp = 158−159 °C.

3-Amino-6-(pentafluoro-λ^6-sulfanyl)-4-phenyl-1H-quinolin-2-one 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) ^{1}H NMR (600 MHz, CD_3CN) 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 °C for 30 min at 200 W. After 30 min, the reaction mixture was diluted with DCM (5 mL) and washed with 10% HCl (3 × 5 mL). The organic layer was extracted with DCM (2 x 5 mL), dried over $MgSO_4$, 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%). ^{1}H NMR (600 MHz, dmso-d_6) 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-λ^6-sulfanyl) phenyl]carbamoyl} methyl)carbamate** (**3b**). 2-benzoyl-4-(pentafluorosulfanyl)aniline methanone (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 °C for 30 min at 200 W. After 30 min, the reaction mixture was diluted with DCM (5 mL) and washed with 10% HCl (3 × 5 mL). The organic layer was extracted with DCM (2 x 5 mL), dried over $MgSO_4$, 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%). ^{1}H 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, $(CH_3)_3$). Insufficient material for ^{13}C spectrum.

1-methyl-7-(pentafluoro-λ^6-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 h. Methyl iodide

(0.133 g, 0.94 mmol) was added to the reaction mixture, which was stirred at room temperature overnight. Crude product was washed with water (3 × 15 mL), extracted with ethyl acetate (3 × 15 mL), dried over $MgSO_4$, 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%). ^{1}H 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, CH_3); ^{13}C NMR (600 MHz, Chloroform-d) δ 169.8 (C═O), 168.8 (C═N), 148.4 (C-SF_5), 146.1(ArC), 137.7 (ArC), 131.1 (ArC), 129.4 (2ArC), 128.7 (4ArC), 128.6(ArC), 121.2 (ArC), 56.9 (CH_2), 34.9 (CH_3); ^{19}F 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 $[C_{16}H_{13}F_5N_2OS][+H]^+$:377.0742; IR (neat) ν_{max}/cm^{-1}: 1682 (C═O), 1611 (C═N), 835 (S−F); mp = 240−242 °C.

5-{[1,1'-Biphenyl]-2-yl}-7-(pentafluoro-λ^6-sulfanyl)-2,3-dihydro-1H-1,4-benzodiazepin-2-one (6c). 2c (86 mg, 0.2 4 mmol), 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 h. 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 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%). ^{1}H 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, CH_2);^{13}C NMR (600 MHz, Chloroform-d) δ 171.1 (C═O), 170.9 (C═N), 158.7 (ArC-F, d, $^1J_{F,C}$ = 247.6 Hz), 148.6−148.0(ArC-SF_5), 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, $^3J_{F,C}$ = 7.9 Hz), 128.5 (2ArC), 128.2 (ArC), 127.8 (ArC, d, $^2J_{F,C}$ = 15.6 Hz), 127.6 (ArC), 123.7 (ArC), 120.5 (ArC), 115.0 (ArC, d, $^2J_{F,C}$ = 22.0 Hz), 56.5 (CH_2); ^{19}F 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 $[C_{21}H_{15}F_6N_2OS][+H]^+$:457.0809; IR (neat) ν_{max}/cm^{-1}: 3064 (N−H), 1704 (C═O), 1616 (C═N), 1336 (C−F), 835.5 (S−F); mp = 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% 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 $GABA_AR$ 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 [35] using an Axopatch 200B Axon Instruments amplifier. Patch pipettes (resistance 3−5 MΩ) 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, 4.7 KCl, 1.2 $MgCl_2$, 2.52 $CaCl_2$, 11 Glucose and 5 HEPES; pH 7.4. Diazepam and SF_5-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, $I/I_{max\ pot} = (1/(1 + (EC_{50}/[L])^n)$. The ligand potency, EC_{50}, 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 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 3). The relative efficacy for GABA current potentiation was calculated as a mean percentage ± sem of the current induced by 2 μM GABA alone.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this pape.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2021.132020.

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