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2,5-Dihydropyrazolo[4,3-*c*]pyridin-3-ones: functionally selective benzodiazepine binding site ligands on the $GABA_A$ receptor

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Abstract—2,5-Dihydropyrazolo[4,3-*c*]pyridin-3-ones are $GABA_A$ receptor benzodiazepine binding site ligands with functional selectivity for the α3 subtype over the α1 subtype. SAR studies to optimise this functional selectivity are described.

The major inhibitory neurotransmitter in the central nervous system is γ-aminobutyric acid (GABA). GABA receptors are subdivided into three pharmacological classes of which $GABA_A$[1,2] and $GABA_C$ are ligand gated ion channels, whilst $GABA_B$ receptors are coupled to G-proteins. The binding of GABA to $GABA_A$ ion channels causes them to open, allowing chloride flow into the neuron, which hyperpolarises the membrane and inhibits further neuronal activity.

Allosteric modulatory sites on $GABA_A$ receptors exist for various ligands, including benzodiazepines (BZs), barbiturates, ethanol, avermectins, picrotoxin, zinc cations and loreclezole, of which the BZ site has attracted most attention. Positive allosteric modulators (agonists) at the BZ site enhance GABAergic inhibition, whilst negative allosteric modulators (inverse agonists) reduce inhibition. Antagonists at the BZ site are also known, which elicit no functional response.

Full agonists acting at the BZ site have long been used as anxiolytics, but exhibit side effects such as cognitive impairment, sedation, ataxia and potentiation of ethanol. Chronic use can result in tolerance, dependence and withdrawal symptoms. The search for improved anxiolytics has been spurred on by the recent identification of subtypes of $GABA_A$ receptors (α1–6, β1–3, γ1–3, δ, ε, π and θ). The BZ site is formed in receptors containing β, γ2 and either α1, α2, α3 or α5 subunits. It is thought that subtype selective ligands will discriminate between the behaviours mediated by $GABA_A$ channels. Studies in transgenic mice have demonstrated that sedation is mediated through the α1-containing $GABA_A$ receptors.[3,4] Our laboratory has reported the discovery of triazolo[4,3-*b*]pyridazines as functionally selective α2/3 partial agonists,[5] which act as non-sedating anxiolytics in animal models.[6] In this communication, we report that 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-ones are also functionally selective α2/3 ligands, and describe the medicinal chemistry that led to their optimisation.

In 1987 researchers from Ciba–Geigy described CGS 17867A, a tetrahydropyrazoloquinoline, which was a potent inhibitor of [^{3}H]flunitrazepam binding to the central BZ site ($IC_{50} = 1.0$ nM).[7] In animal models, CGS 17867A showed anxiolytic activity, but without many of the limiting effects observed for classical BZs. We decided to screen this compound for in vitro activity against the α1, α2 and α3 $GABA_A$ subtypes. Affinity was measured at cloned human α1β3γ2, α2β3γ2 and α3β3γ2 $GABA_A$ receptors stably expressed in L(tk$^-$) cells by displacement of [^{3}H]Ro15-1788 binding.[8] Efficacy was determined at the same receptors transiently expressed in *Xenopus* oocytes by measurement of the modulatory

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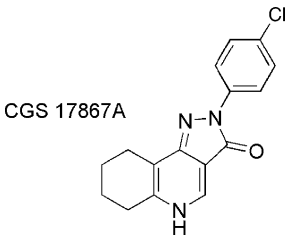
Table 1. In vitro data for CGS 17867A

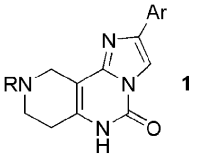


$GABA_A$ subtype	K_i (nM)[a]	Oocyte efficacy (%)[b]	Patch-clamp efficacy (%)[a]
α1	0.19 (±0.07)	12 (±4) 30	17 (±2)
α2	0.77 (±0.28)	61 (±9) 100	43 (±14)
α3	1.03 (±0.28)	93 (±10) 100	70 (±3)

[a] Values are means (±SEM) for $n = 3$–7.
[b] Values are means (±SEM) for $n = 3$–7, concentration of test compound (nM).

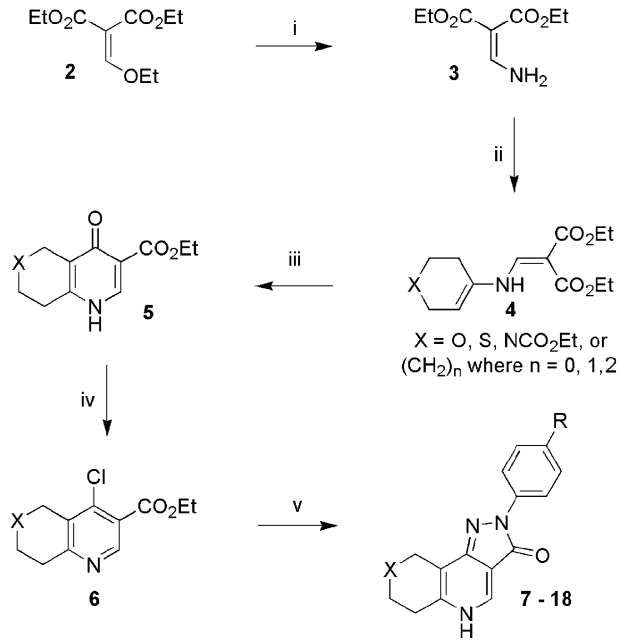
effect on the GABA EC_{20} ion current using two electrode voltage-clamp electrophysiology;[9] these experiments were performed using a single concentration of the test compound. Efficacy was also measured by whole-cell patch-clamp recording from L(tk^-) cells expressing the receptors as above;[10] full concentration-effect curves were generated using this method. The results are shown in Table 1.

As expected, CGS 17867A had high affinity for all the subtypes measured, but most intriguingly it was found to have functional selectivity, with significant efficacy at α3 and α2, but much reduced efficacy at α1. The reduced sedation observed in animals dosed with CGS 17867A is attributable with hindsight to the low efficacy of this compound at the α1 subtype, with the anxiolytic behaviour being mediated through α2/α3. 7,8,9,10-Tetrahydroimidazo[1,2-*c*]pyrido[3,4-*e*]-pyrimidin-5(6*H*)-ones **1** have recently been reported as functionally selective ligands for the $GABA_A$ receptor BZ site,[11] but this is the first time that the structurally related 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-ones have been disclosed as having a similar profile.



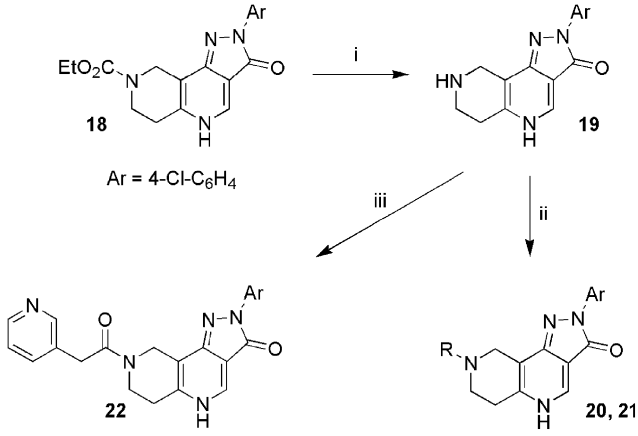
Using CGS 17867A as a lead, we pursued a medicinal chemistry programme to optimise the series for α3 functional selectivity. The desired efficacy profile was to have partial to full agonism at α3, but antagonism at α1. In vitro screens were set up as described above; oocyte data was used as the primary efficacy screen, with whole-cell patch-clamp recording used as a more robust secondary assay for selected compounds.

Studies concentrated initially on varying the chlorine substituent on the phenyl ring, and on making changes to the saturated ring fused to the 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-one core. The general chemistry employed is shown in Scheme 1. Diethyl ethoxymethylenemalonate **2** was converted to its amino analogue **3** by treatment with ethanolic ammonia, and then **3** was condensed with ketones under Dean–Stark conditions to afford enamines **4**. Pyrolysis of **4** yielded the pyridones **5**, which were converted into 4-chloropyridines **6** with phosphorus oxychloride. The target compounds **7**–**18** were then prepared by condensation of intermediates **6** with various aryl hydrazines.



Scheme 1. (i) NH_3, EtOH, 100%; (ii) ketone, cat. 4-toluenesulfonic acid, toluene, reflux; (iii) Dowtherm A, reflux; (iv) $POCl_3$, reflux; (v) 4-R–$C_6H_4NHNH_2$, *n*-BuOH, reflux.

For the compounds incorporating a fused piperidine ring, Scheme 2 shows how the piperidine nitrogen was elaborated. The ethoxycarbonyl protected compound **18** was converted to the secondary amine **19** by treatment with hydrogen bromide in acetic acid. This was then alkylated with alkyl halides in the presence of Hünig's

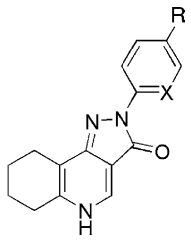


Scheme 2. (i) HBr–AcOH, 80%; (ii) RX (X = Cl, Br), DIPEA, DMF; (iii) 3-pyridine acetic acid, WSCDI, HOBt, DIPEA, DCM, 47%.

base, affording compounds **20** and **21**. The amide **22** was prepared by carbodiimide-mediated coupling with 3-pyridine acetic acid.

Table 2 shows the effect on affinity and efficacy of varying the 4-substituent on the phenyl ring. In general, affinity for the two subtypes was not greatly affected by changes at this position. Disappointingly, none of the substituents gave α3 efficacy as high as that of CGS 17867A. A fluorine substituent (**7**) reduced the α3 efficacy to 33%, although complete antagonism at α1 was observed in the oocytes. Increasing fluorination of a methoxy group to give difluoro- and trifluoromethoxy substituents gave no obvious trend in the α3 oocyte data, but the difluorinated analogue **10** was found to be an antagonist at α1 when measured in the patch-clamp assay. More compounds were prepared incorporating the difluoromethoxy group for this reason (see Table 3). Incorporating a cyclobutyloxy group (**11**) significantly increased the efficacy at α1, an effect possibly related to the size and lipophilicity of the substituent. Adding the smaller cyclopropyloxy group gave the best compound in this series (**12**) other than CGS 17867A, with the highest α3 efficacy recorded in both functional assays,

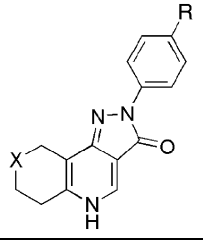
Table 2. Affinity and efficacy at α1 and α3 $GABA_A$ BZ receptor subtypes for 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-ones varying the 4-phenyl substituent

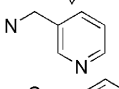
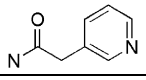


Compds	R	X	K_i α1/nM[a]	K_i α3/nM[a]	Oocyte efficacy α1 (%)[b]	Oocyte efficacy α3 (%)[b]	Patch-clamp efficacy α1 (%)[a]	Patch-clamp efficacy α3 (%)[a]
CGS 17867A	Cl	CH	0.19 (±0.07)	1.03 (±0.28)	12 (±4) 30	93 (±10) 100	17 (±2)	70 (±3)
7	F	CH	0.29 (±0.06)	0.65 (±0.13)	−2 (±4) 30	33 (±5) 30	—	—
8[12]	OMe	CH	0.31 (±0.07)	0.61 (±0.12)	15 (±4) 30	55 (±6) 30	—	—
9	OCF_3	CH	7.33 (±3.13)	4.70 (±1.40)	14 (±20) 300	35 (±2) 300	—	—
10	$OCHF_2$	CH	1.00 (±0.00)	2.25 (±0.65)	21 (±4) 100	56 (±7) 300	2.0 (±2)	34 (±6)
11	O–cyBu	CH	1.30 (±0.10)	2.55 (±0.20)	49 (±9) 100	36 (±10) 300	—	—
12	O–cyPr	CH	1.13 (±0.46)	2.19 (±0.57)	19 (±2) 100	62 (±9) 100	−4.3 (±4)	54 (±4)
13	H	N	0.57 (±0.03)	1.14 (±0.14)	−39 (±3) 100	−7 (±2) 100	—	—

[a] Values are means (±SEM) for $n = 3$–7.
[b] Values are means (±SEM) for $n = 3$–7, concentration of test compound (nM).

Table 3. Affinity and efficacy at α1 and α3 $GABA_A$ BZ receptor subtypes for 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-ones varying the saturated ring



Compds	R	X	K_i α1/nM[a]	K_i α3/nM[a]	Oocyte efficacy α1(%)[b]	Oocyte efficacy α3(%)[b]
CGS 17867A	Cl	CH_2	0.19 (±0.07)	1.03 (±0.28)	12 (±4) 30	93 (±10) 100
14	Cl	$(CH_2)_0$	0.44 (±0.01)	1.12 (±0.36)	19 (±1) 10,000	51 (±3) 3000
15	Cl	$(CH_2)_2$	1.06 (±0.41)	4.27 (±1.62)	23 (±3) 100	87 (±7) 300
16	$OCHF_2$	O	1.55 (±0.05)	6.40 (±1.20)	11 (±2) 300	32 (±3) 1000
17	$OCHF_2$	S	0.38 (±0.09)	0.89 (±0.21)	9 (±2) 30	25 (±5) 100
20	Cl		0.45 (±0.00)	7.45 (±1.39)	111 (±14) 30	123 (±4) 1000
21	Cl		0.18 (±0.04)	0.56 (±0.05)	131 (±5) 100	199 (±21) 100
22	Cl		3.15 (±0.15)	5.40 (±0.20)	49 (±3) 300	2.3 (±9) 1000

[a] Values are means (±SEM) for $n = 3$–7.
[b] Values are means (±SEM) for $n = 3$–7, concentration of test compound (nM).

combined with complete antagonism as measured in the patch-clamp assay. Finally, introduction of a 2-pyridyl group (**13**) led to a dramatic reduction in efficacy at both subtypes, resulting in inverse agonism.

Table 3 shows the SAR for compounds with changes made to the saturated ring of CGS 17867A. Contraction to a five-membered ring (**14**) afforded a high affinity ligand for both subtypes, but exhibited reduced efficacy at α3. Expansion to a seven-membered ring (**15**) significantly increased the efficacy at α1 compared to CGS 17867A. Incorporating an oxygen (**16**) or sulfur atom (**17**) into the saturated ring led to much reduced efficacy at α3, thus removing most of the functional selectivity. Good affinity was maintained when amines were introduced to the ring (**20**, **21**), indicating a potentially useful space that could be further explored in this region of the molecule. However, functional selectivity had been lost for these compounds, with full agonism observed at both subtypes. Incorporating an amide (**22**) gave acceptable affinity, but the efficacy profile was now the reverse of that required, with agonism at α1 and antagonism at α3.

From the in vitro data, compound **12** looked to have the best efficacy profile in the patch-clamp assay, combining antagonism at α1 with partial agonism at α3. The compound was therefore selected for further profiling in vivo. Compound **12** was found to have excellent bioavailability of 91% in the rat, compared to only 21% (in our hands) for CGS 17867A. The improved bioavailability of our compound may be attributed to its reduced clearance (19 mL/min/kg for **12** compared to 30 mL/min/kg for CGS 17867A). Occupancy at $GABA_A$ receptors in the mouse brain was determined by inhibition of in vivo [^{3}H]Ro15-1788 binding.[13] Dosed i.p. at 10 mg/kg in 70% aqueous PEG, the occupancy for **12** after 30 min was 87% in the spinal cord (rich in α2/3 subtypes) and 96% in the cerebellum (rich in α1 subtypes). This compared favourably with CGS 17867A, for which the occupancy values were 81% and 95% in the spinal cord and cerebellum, respectively, when dosed as above. However, in the rat, occupancy for **12** determined 30 min after oral dosing at 10 mg/kg was only 18% (measured for the whole brain), which was considered insufficient to elicit an anxiolytic effect in vivo. For this reason, the compound was not progressed further into behavioural studies.

In conclusion, we have determined that CGS 17867A is a functionally selective BZ binding site ligand on the $GABA_A$ receptor, and this may explain its atypical behavioural profile in vivo compared to classical BZs. Taking CGS 17867A as a starting point, a series of 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-ones has been optimised for functional selectivity, leading to the synthesis of **12**. This compound has an excellent in vitro profile, combining good affinity at $GABA_A$ receptor subtypes with good partial agonism at α3 and complete antagonism at α1. The estimated receptor occupancy of **12** in the mouse is comparable to that of CGS 17867A, whilst its pharmacokinetic profile in the rat is superior. However, the low receptor occupancy of **12** in the rat precluded its further in vivo characterisation.

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