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Exploring subtype selectivity and metabolic stability of a novel series of ligands for the benzodiazepine binding site of the $GABA_A$ receptor

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ABSTRACT

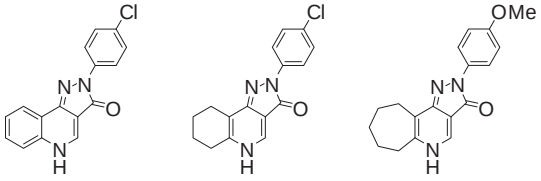
A novel series of agonists at the benzodiazepine binding site of the $GABA_A$ receptor was prepared by functionalizing a known template. Adding substituents to the pyrazolone-oxygen of CGS-9896 led to a number of compounds with selectivities for either α2- or α1-containing $GABA_A$ receptor subtypes offering an entry into indications such as anxiety and insomnia. In this communication, structure–activity relationship and efforts to increase in vitro stabilities are discussed.

Benzodiazepines (BZs) as positive modulators of γ-amino butyric acid type A ($GABA_A$) receptors have been for years the most widely used drugs for disorders of the nervous system such as anxiety and insomnia. Despite their fast onset of action and relatively high efficacy, they are no longer first-line treatment choice for most types of anxiety spectrum disorders because of their adverse side effect profile.[1]

Benzodiazepines bind to $GABA_A$ receptors at the interface of α and γ subunits that form together with β subunits most of the central pentameric receptor channels. Out of the six known α subunits, α1, α2, α3 and α5 are part of receptors carrying the benzodiazepine (BZ) binding site.

Recent molecular genetic studies have led to the hypothesis that subtype-specific $GABA_A$ receptor modulators can alleviate anxiety without unwanted side-effects such as sedation, memory impairment or abuse liability.[2–4] Mice with mutations in the α1 subunit that render those receptors insensitive to BZs showed no sedation upon treatment with diazepam, whereas the drug was no longer anxiolytic in mice carrying a benzodiazepine-insensitive α2 subunit. CGS-17876A, a BZ-site ligand with anxiolytic but much less sedative activity in rodents was shown *a posteriori* to have low efficacy at α1-carrying $GABA_A$ receptors.[5] In fact, the structurally related compound CGS-20625 was evaluated in clinical trials[6] based on its interesting anxio-selective profile in pre-clinical tests.[7] This evidence encouraged us to identify novel compounds with improved α2 selectivity profiles for the treatment of anxiety disorders.

We decided to use CGS-9896 (**1**) as starting point for derivatization efforts to identify subtype selective compounds. The oxygen-atom of the dihydro-pyrazolone moiety was used as attachment point for further substituents.



CGS-9896 (**1**) CGS-17867A CGS-20625

Compounds were routinely screened for their in vitro efficacy at α1- and α2-containing $GABA_A$ receptor subtypes. The assay was performed at recombinant $GABA_A$ receptors expressed in CHO cells using a membrane potential sensitive fluorometric read-out.[8] The compound-induced potentiation of the response to a sub-maximal GABA concentration was measured and data are reported for a single defined concentration of test compounds. The intended target profile was partial to full agonism at α2 and/or α3 and antagonism at α1. For selected compounds, affinity for α1- and α2-containing recombinant $GABA_A$ receptors was determined using [^{3}H]-flumazenil as radioligand[9] to confirm binding to the BZ binding site.

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Initially, we pursued a medicinal chemistry program around benzyl-substituted analogs. They were prepared by alkylation of CGS-9896[10] under basic conditions in a microwave reactor (Scheme 1).

This straightforward route was used for the initial SAR work even though the isolated yield was usually rather low. After chromatographic removal of N-alkylated side products, the pure O-alkylated isomers were typically isolated in yields between 10% and 30%.

Table 1 summarizes results of a set of representative compounds. The 2-chloro-benzyl substituent was initially kept constant in order to explore the influence of core modifications[10,11] in this novel series of modulators. Variations of substituents on the phenyl pyrazolone moiety (cf. **2**–**4**) showed mixed results. The corresponding compounds were very weak agonists at α2 and some behaved as antagonists at α1. The 2-fluoro analog **3** had highest affinity but only low efficacy.

Next, the influence of changes in the quinoline core was investigated by looking at modifications which had previously been reported for members of the CGS-9896 family.[5] Partial saturation of **2** was well tolerated and led to the equipotent compound **5** with functional preference for α2. Expanding the ring-size (**6**) led to a full agonist at both subtypes whereas incorporation of polar oxygen (**7**) yielded a weak but α2-selective agonist. Finally, incorporation of a *gem*-dimethyl substituents in the 8-positon of the core (**8**) yielded compound with preference for α1.

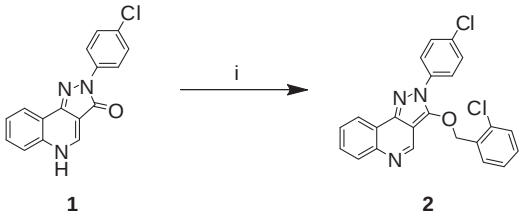
Based on these results, compound **5** combining both functional preferences for α2 and adequate potency was chosen as a basis for further investigations with the main goal to improve potency and selectivity.

Then, the influence of substituents on the benzyl group with respect to potency and subtype selectivity was investigated (Table 2). Placing the chloro-substituent on different positions was detrimental for selectivity. But replacing the 2-chloro with the 2-fluoro substituent gave a compound (**11**) with a very promising functional selectivity for the α2 subtype. Also the trifluoro-methyl derivative **12** was very potent and reached a similar maximal efficacy as midazolam, the standard benzodiazepine used in the assay. Unfortunately, this potent compound was completely unselective.

All these modifications usually had limited impact on the affinity for the BZ binding site. Only the 4-fluoro analog **13** showed an increased affinity for the α2 subtype but without any selectivity in the functional assay. Replacing the phenyl by a cyclopropyl substituent (**14**) was tolerated only on α1.

In parallel to the ongoing derivatization program, compound **5** was chosen for further profiling. It was anticipated to test whether the weak α2 selectivity in vitro would translate into a measurable effect in vivo. Unfortunately, the properties of compound **5** precluded it from testing in anxiety models. Especially, the clearance was very high (in vitro: CL_{int} rat: 128.3 μl/min∗mg). Therefore, a detailed in vitro metabolism study with rat liver microsomes[12] to identify major pathways and block weak spots in next generation compounds was performed.

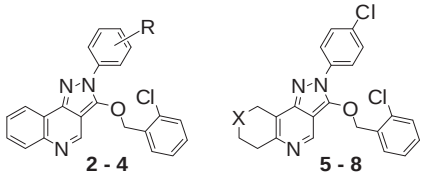
This study identified two main degradation pathways for compound **5** (Fig. 1). Extensive hydroxylation of the aliphatic part of the core was observed (pathway A) but the exact positions of



1 **2**

Scheme 1. Reagents and conditions: (i) K_2CO_3, acetone, 1-bromomethyl-2-chlorobenzene, microwave, 80 °C, 30 min, 20% yield.

Table 1
Efficacy and affinity for selected CGS-9896 analogs at α1- and α2-containing $GABA_A$ receptor subtypes

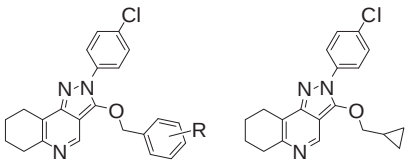


2 - 4 **5 - 8**

Compds	R	X	% Efficacy[a]		Affinity[b] (nM)	
			α1	α2	IC_{50} α1	IC_{50} α2
1			38	47	1	4
2	4-Cl		13	22	64	265
3	2-F		−8	17	1	2
4	3,4-(OCH_2O)		−15	19	50	155
5		CH_2	25	50	49	271
6		$(CH_2)_2$	114	96	242	605
7		O	4	32	n.d.	461
8		CMe_2	48	23	67	213

[a] Determined for single compound concentration (3 μM [except **1**: 1 μM]; $n = 2$) and normalized to the maximal effect of midazolam (300 nM).
[b] n.d. = not determined.

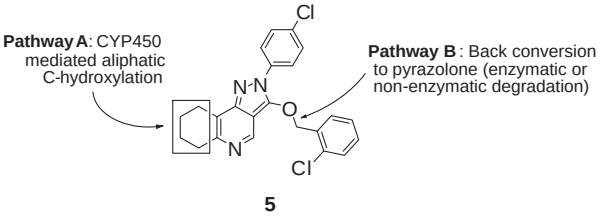
Table 2
Affinity and efficacy at α1 and α2 $GABA_A$ receptor subtypes for differently substituted analogs of **5**



5, 9-13 **14**

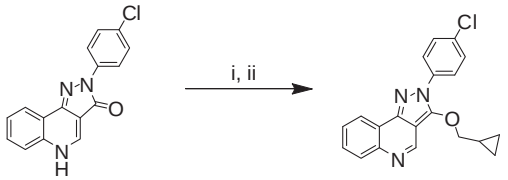
Entry	R	% Efficacy[a]		Affinity[b] (nM)	
		α1	α2	IC_{50} α1	IC_{50} α2
5	2-Cl	25	50	49	271
9	3-Cl	38	50	n.d.	n.d.
10	4-Cl	20	42	157	177
11	2-F	31	83	64	154
12	2-CF_3	84	94	n.d.	n.d.
13	4-F	24	41	50	57
14	—	37	36	74	472

[a] Determined at 3 μM compound concentration ($n = 2$) and normalized to the maximal effect of midazolam (300 nM).
[b] n.d. = not determined.



5

Figure 1. The main pathways A and B which led to high in vitro clearance of compound **5**.



1 14

Scheme 2. Reagents and conditions: (i) $POCl_3$, sealed tube, 135 °C, 30 min (97%); (ii) NaH 60% in mineral oil, cyclopropylmethanol, THF, reflux, 2.5 h (66%).

core-hydroxylation could not be identified. In addition, significant amounts of the parent pyrazolone were observed (pathway B). This back-conversion could be due to oxidative cleavage or simple chemical instability of the benzylic position of the side-chain under test conditions. Based on these results, we designed the next series of compounds with the aim to lower the in vitro clearance.

A novel synthesis was developed to gain easy access to a diverse set of side-chain analogs. The optimized route is exemplified for compound **14** in Scheme 2. Pyrazolone **1** was chlorinated with phosphoryl chloride followed by nucleophilic displacement with the sodium salt of the corresponding alcohol. This led to the desired ethers in good overall yields and proved to be general for a wide variety of alcohols.

A first series of compounds with modifications in the tetrahydroquinoline was prepared to address the metabolic stability of the core. Indeed, incorporation of an oxygen (**15**) or methyl-substituents (**16**) in the 8-position led to decreased core-hydroxylation. This effect was however partially offset by increased degradation through pathway B and efficacy at the target was considerably lower (Table 3).

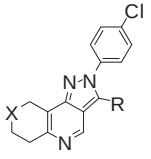
Next, we focused our efforts on the stabilization of the benzylic side chain. Replacement of benzyl by phenyl (**18**) led to a largely improved stability but at the expense of on-target activity. Here, the cyclopropyl-methyl group was an almost equipotent replacement for the 2-chloro-benzyl group with a nice functional selectivity for α2 (**17**). In addition, it showed significantly increased in vitro metabolic stability but core metabolism was still considered too high for in vivo testing.

Finally, the cyclopropyl replacement was applied to the quinoline core to combine both the optimal metabolic properties of both the core and the side chain. The imidazole substituted core was chosen based on a previous observation that it could influence the core stability beneficially (data not shown). And, indeed, cyclopropyl ether **19** showed an excellent in vitro metabolic stability with almost complete parent retention under assay conditions. Surprisingly, this stable analog showed α1 selectivity in the functional assay and from the binding assay α1 selectivity was also evident. In the binding assay **19** showed unusual displacement characteristics at both α1 and α2 with a Hill coefficient of only 0.5–0.6 which could be a hint for binding of multiple ligands.

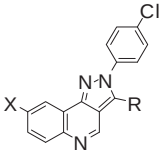
The high functional efficacy and binding selectivity of **19** for α1 offers an interesting starting point for further optimization of the affinity of this chemotype to identify candidates for the treatment of insomnia.

In conclusion, a novel series of agonists at the BZ binding site of the $GABA_A$ receptor based on modifications of a known template was described. The optimization work surprisingly yielded both α2 and α1 preferring agonists depending on the substitution patterns. Low metabolic stability of initial derivatives was improved by both core and side-chain modifications. Currently, further work is ongoing to identify compounds with low-nanomolar affinity, selectivity and stability for the treatment of either anxiety or insomnia.

Table 3
Affinity and efficacy at α1 and α2 $GABA_A$ BZ receptor subtypes and metabolic data for core and side-chain modified analogs



5, 15-18



19

Entry	X	R	Parent [%]+	Pathway **A**[a] (%) ∑ of metabolites	Pathway **B** (%)	Efficacy[b]		Affinity[c] (nM)	
						α1 (%)	α2 (%)	IC_{50} α1	IC_{50} α2
5	CH_2		4	82	11	25	50	46	271
15	O		41	41	16	4	32	n.d.	461
16	CMe_2		2	66	26	4	23	67	213
17	CH_2		35	55	10	18	64	94	185
18	CH_2		45	49	0	n.m.	n.m.	>3000	>3000
19			96	1	1	80	35	134	2114

[a] Relative peak areas assuming identical response factors in MS detection; further minor metabolites not reported (n.m. = not meaningful).
[b] Determined at 3 μM compound concentration ($n = 2$) and normalized to the maximal effect of midazolam (300 nM).
[c] n.d. = not determined.

Acknowledgments

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12. The in vitro metabolism was determined in rat liver microsomes (Gentest 452501, mixed gender). Incubations were conducted at 37 °C for 60 min using 0.3 mg/mL microsomal protein, NADPH regenerating system (isocitrate-dehydrogenase (1 U/mL), NADP (1 mmol/L), isocitrate (5 mmol/L), UDP-glucuronic acid (2.4 mM) and 5 µM of the respective parent compound. For detection of potential reactive intermediates 1.5 mM of glutathione was supplemented in a separate experiment. Metabolites were characterized by capillary HPLC/high resolution MS^n analysis. The data obtained are in accordance with elemental composition, MS/MS fragmentation and number of exchangeable hydrogen atoms (replacement of H_2O by D_2O in the mobile phase).