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Imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepines as potent and highly selective $GABA_A$ α5 inverse agonists with potential for the treatment of cognitive dysfunction

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ABSTRACT

In a search for $GABA_A$ α5 ligands that combine high subtype binding selectivity with a marked inverse agonism imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepines were identified as a promising class. A short tandem reaction allowed rapid access to this chemical series, thereby facilitating rapid SAR generation which guided the optimization process. Two compounds (**10e** and **11f**) were found to be active in an in vivo paradigm for cognitive improvement.

γ-Aminobutyric acid (GABA) is one of the main neurotransmitters in human and rodent brain. It exerts its effects by interaction with $GABA_A$, $GABA_B$ and $GABA_C$ receptors. Among these, $GABA_A$ receptors have a proven record as drugable targets, for example, benzodiazepines, barbiturates and neurosteroids interact at this receptor.[1] $GABA_A$ receptors are functionally characterized as ligand-gated chloride channels with a GABA recognition site. Structurally, they are defined as pentameric assemblies of different subunits.[2] Most mammalian $GABA_A$ receptors consist of α1, α2, α3 or α5 in conjunction with β2 or β3 and γ2 -subunits.[3] These subtypes also contain a recognition site for prototypical benzodiazepines = BZ (exemplified by diazepam **1**, Fig. 1), which allosterically modulate the functional effect of GABA. Based on their modulatory effects, BZ-site ligands are categorized as either agonists (positive modulators, facilitating Cl^--flux), antagonists ('neutral' modulators, normalizing Cl^--flux at basal level) or inverse agonists (negative modulators, reducing Cl^--flux). For example, **1** does not discriminate between α1, α2, α3 and α5 containing $GABA_A$ subtypes; functionally, it increases Cl^--flux. Compounds with this profile are defined as non-selective agonists. A number of drugs of this type are marketed as sedatives, anxiolytics and/or anticonvulsants. Reported side effects are memory impairment and dependence.[4] Another marketed drug, Flumazenil **2**, is characterized as a subtype non-selective antagonist. It is clinically used in the treatment of BZ-agonist overdosing.[5]

The therapeutic potential of subtype non-selective inverse agonists (exemplified by FG7142, **3**) as cognition enhancers has been shown to be limited by severe side effects (anxiety, agitation).[6,7]

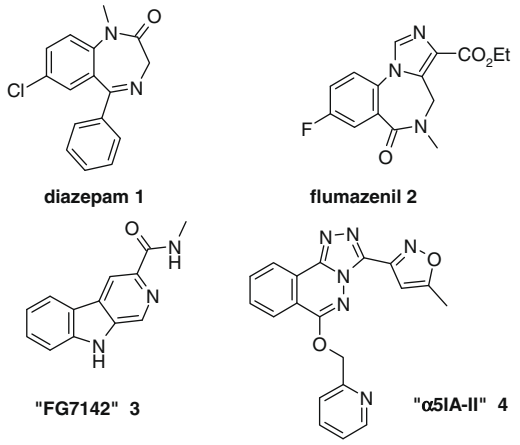

Figure 1. Reference compounds: BZ-ligands with different profiles.

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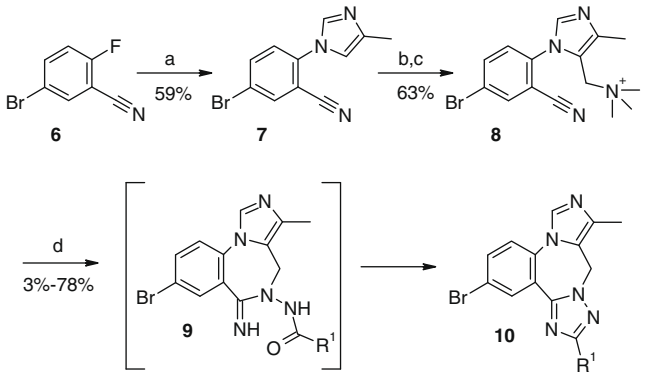
With knowledge generated by the use of genetically modified mice meanwhile evidence has accumulated that a selective $GABA_A$ α5 inverse agonists can be expected to be devoid of these side effects.[7] Two conceptionally different approaches have been followed to design compounds with this profile: Selective $GABA_A$ α5 inverse agonism may be achieved either by a high binding selectivity combined with the desired modulatory effect at the $GABA_A$ α5 receptor or by functional selectivity (i.e., inverse agonism at the $GABA_A$ α5 receptor with low efficacy at the other $GABA_A$ receptor subtypes). Following the first approach, researchers at Merck in 2003 described a series of compounds that combined the desired efficacy with a significant $GABA_A$ α5 binding selectivity (up to 30-fold).[8] Subsequently, this group focused on functionally selective compounds active in animal models of cognitive function (e.g., α5IA-II, **4**).[9,10] A related compound α5IA was tested in humans and shown to reverse alcohol induced memory impairment without causing significant side effects.[7]

With the aim to identify $GABA_A$ α5 subtype selective BZ-ligands endowed with significant inverse agonism, a high-throughput screening campaign of the Roche compound collection was performed. A subsequent lead generation process furnished imidazotriazolobenzodiazepine **5** as a promising candidate for further optimization. Compared with reference compound **4** (Table 1), it is characterized by a somewhat reduced $GABA_A$ α5 receptor binding affinity combined with a significant binding selectivity versus $GABA_A$ α1, α2 and α3 subtype receptors. As both compounds potently modulate Cl^--flux of the $GABA_A$ α5 receptor (by −41% and −42%, respectively), they are functionally characterized as inverse agonists.[11]

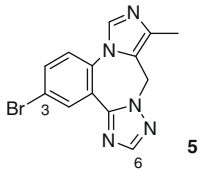
The known literature synthesis of compounds with an imidazotriazolobenzodiazepine skeleton (as in **5**) relies on a long linear sequence which turned out to be unpractical for rapid SAR exploration.[12] Since we sought for a synthetic sequence that allowed a late stage introduction of the 6-substituent, we developed an alternative approach that commenced with a nucleophilic aromatic substitution of 5-bromo-2-fluoro-benzonitrile **6** by 4-methylimidazole (Scheme 1). The mixture of regioisomeric products was recrystallizated to afford **7** as a pure regioisomer. Reaction with Eschenmoser's salt then introduced regioselectively the dimethylaminomethyl-function. The desired leaving group on the heterocycle could cleanly be obtained by reaction with methyl iodide (**8**). Substituting this with a wide range of acylhydrazides at 120 °C led to intermediates **9** (identified by MS, not isolated), which under prolonged heating condensed to the desired triazolo-annelated imidazobenzodiazepine **10**.[13] Using differently substituted 2-fluorobenzonitriles, analogous products could be obtained (Table 3).

First we studied (Table 2) the influence of substituents at the triazole (position 6). Formally adding a methyl to **5** (leading to **10a**) reduced $GABA_A$ α5 receptor affinity threefold. However, also affinity at the $GABA_A$ α1, α2 and α3 receptors was reduced by a factor 10, leading overall to a marked selectivity increase. Steric bulk directly linked to the heterocycle (as in **10b**) led to an even further reduction of $GABA_A$ α5 receptor affinity, rendering the compound uninteresting. Introduction of a small ether functionality (**10c**) restored $GABA_A$ α5 receptor affinity, with an overall selectivity profile comparable to the simple methyl substituted **10a**. A marked $GABA_A$ α5 receptor affinity increase combined with a significant $GABA_A$ α1, α2 and α3 receptor selectivity (almost 2 log units) was observed for the methylene linked cyclopropane **10d**. Compared to **5**, however, each of these compounds were functionally not very efficacious, they were only weak inverse agonists (efficacy >−25%). With the aim to combine high binding selectivity with a reasonable intrinsic efficacy, a large number of substituents were screened. Methylene linked pyrrolidinone **10e** had a selectivity profile comparable to **10d** with a threefold higher efficacy. Generally, methylene linked nonaromatic as well as aromatic heterocycles were found to be highly binding selective. Notably, isoxazole **10f** (selectivity vs $GABA_A$ α1, α2 and α3 receptors >100) was one of the most interesting compounds, as it also was categorized as a partial inverse agonist (efficacy <−25%). Oxadiazole **10g** was the most binding selective compound. However, selectivity was achieved only at the expense of functional activity (weak inverse agonism). Overall, appropriately designed substituents positioned at C-6 gave access to high binding selectivity. Substituents at this position were tolerated at the α5, but not at $GABA_A$ α1, α2 and α3 receptors. The structure-efficacy-relationship was much less well understood; purely lipophilic interactions seemed to reduce inverse agonism.



Scheme 1. Reagents and conditions: (a) 4-methylimidazole, K_2CO_3, DMSO, 90 °C; (b) Eschenmoser's salt, DMF, 100 °C; (c) methyl iodide, CH_2Cl_2, 4 °C; (d) R^1–$CONHNH_2$, DMF, 120 °C.

Table 1
Binding and efficacy profile of **4** and **5**

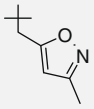
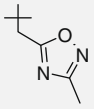


5

Compd	Affinity K_i (nM) GABA A αxβ3γ2 receptors[a]				Efficacy GABA A αxβ3γ2 receptors[a,b] (%)			
	α_5	α_1	α_2	α_3	α_5	α_1	α_2	α_3
4[c]	1.5	1.5	1.5	1.5	−41	−9	11	10
5	6	136	186	132	−42	−16	nt	nt

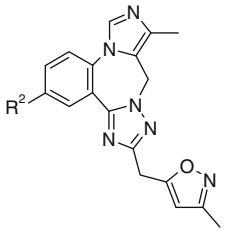
[a] Cloned rat or human receptor subunits were expressed in insect SF9 cells, human HEK293 cells or *Xenopus laevis* oocyts for ^{3}H-flumazenil $\alpha_1\alpha_2\alpha_3$ and α_5 binding or electrophysiology.
[b] Efficacy is determined as the percentage change of a submaximal (EC_{10}) response to GABA.
[c] Compound synthesized and tested at F. Hoffmann-La Roche Ltd. The results are in good agreement with those reported.[10]

Table 2
Binding and efficacy profile of variously substituted compounds **10**

Compd	R^1	Affinity K_i (nM) $GABA_A$ αxβ3γ2[d]				Efficacy $GABA_A$ α5β3γ2 receptors[d] (%)
		α5	α1	α2	α3	
10a	Me	20	1421	3160	1624	−17
10b	*t*-Bu	146	3160	1068	1206	nt
10c	CH_2–OMe	13	619	693	592	−3
10d	CH_2–Cyclopropyl	5	538	407	407	−12
10e		7	624	669	515	−34
10f		0.6	188	105	73	−27
10g		0.9	469	317	277	−18

[d] see Table 1.

Table 3
Binding and efficacy profile of analogues of **10f**



Compd	R^2	Affinity $GABA_A$ α5β3γ2[d] K_i (nM)	Selectivity: $\frac{K_i(nM)\alpha x\beta 3\gamma 2}{K_i(nM)\alpha 5\beta 3\gamma 2}$			Efficacy $GABA_A$ α5β3γ2 receptors[d] (%)
			versus α1	versus α2	versus α3	
10f	Br	0.6	314	174	122	−27
11f	Cl	0.6	466	251	223	−18
12f	OCF_3	11	68	83	111	−20
13f	H	1.6	57	20	16	nt
14f	Me	0.8	620	332	360	−16
15f	OMe	2.2	307	193	222	−13
16f	OH[e]	1.2	99	nt	nt	−7

[d] See Table 1.
[e] Synthesized by BBr_3 mediated hydrolysis of **15f**.

Table 4
Property profiles of **10e**, **10f** and **11f**

	10e	**10f**	**11f**
Solubility (μg/mL)[f]	>550	7	14
log $D_{7.4}$	1.5	2.6	2.3
Permeation coefficient (10^{-6} cm s^{-1}) (PAMPA)	1.5	3.3	3.1
Cl (mL/min/mg protein)[g] (rat/human)	16/3	10/0	6/0

[f] LYSA, for measurement details, see Ref. 15.
[g] From incubations with liver microsomes.

Table 5
Pharmacokinetic parameters of **10e**, **10f** and **11f** (in rats)

	10e	**10f**	**11f**
Half life $t_{1/2}$ (h)	0.8	1.4	1.4
Cl (mL/min/kg)	30	39	9.5
Vss (L/kg)	1.3	4.6	1.8
F (%)	32	34	44
Brain/plasma	0.09	0.2	1.0

We then studied the influence of substituents at position 3 of the imidazo-triazolobenzodiazepine keeping constant the methylene linked methylisoxazole that conferred high binding selectivity (Table 3). Formally replacing Br by Cl led to **11f** which in terms of $GABA_A$ α5 receptor affinity and selectivity was slightly superior. The desired inverse agonism, however, was reduced. Breaking σ-symmetry by introduction of the trifluoromethoxy substituent (**12f**) was clearly detrimental:[14] $GABA_A$ α5 receptor affinity was reduced almost 20-fold. Whilst a simple hydrogen (**13f**) gave a single digit affinity at the $GABA_A$ α5 receptor, selectivity versus $GABA_A$ α1, α2 and notably α3 receptors was compromised. Interestingly, at least in terms of $GABA_A$ α5 receptor affinity and selectivity, the electronic nature of the 3-substituent does not play a role: electron-donating methyl (**14f**) and methoxy (**15f**) combine high $GABA_A$ α5 receptor affinity with selectivity. In this respect, even

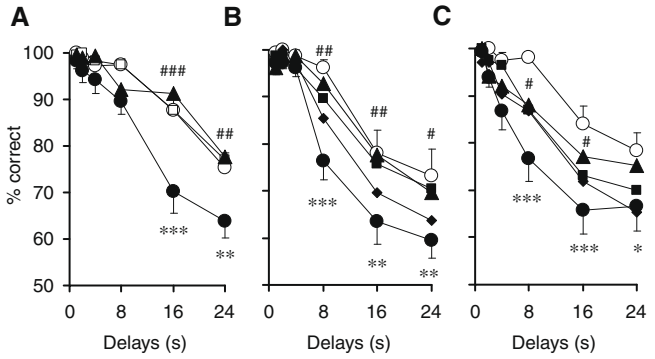


Figure 2. Effect of **4**, **10e** and **11f** on scopolamine (0.03 mg/kg s.c.) induced impairment of working memory, measured by choice accuracy across delay interval in the delayed match to position (*DMTP*) task. Key: ○ = vehicle; ● scopolamine. (**A**) Compound **4**: □ = 1.0 mg/kg ip; ♦ = 1.0 mg/kg ip versus scopolamine. (**B**) Compound **10e** versus scopolamine: ♦ = 0.3, ■ = 1.0, ♦ = 3.0 mg/kg ip. (**C**) Compound **11f** vs. scopolamine: ♦ = 0.1, ■ = 0.3, ♦ = 1.0 mg/kg po. Male Lister hooded rats (n=11–12) were used in each study. Data are expressed as mean ± SEM. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 versus vehicle; $^{\#}p$ <0.05, $^{\#\#}p$ <0.01, $^{\#\#\#}p$ <0.001 versus scopolamine.

a hydroxy (**16f**) substituent looks promising. In terms of efficacy, however, none of the substituents tested were as effective as the Br substituted structure **10f**.

Complimentary to the receptor parameters discussed above, other molecular properties were also determined systematically and were typically found to be in the desired range (Table 4). Whilst pyrolidinone-substituted **10e** combined low lipophilicity with a remarkable solubility, its methylisoxazole substituted analogue **10f** was found to be more lipophilic (by 1 log unit) with an acceptable solubility. Formally replacing Br in **10f** by Cl leading to **11f** expectedly reduced lipophilicity while increasing solubility. Permeation of all compounds was in the desired range, with a trend of facilitated transport for more lipophilic compounds. Finally, microsomal clearance (Cl) was low, with indications for modulation.

Based on their favorable drug-like properties, **10e**, **10f** and **11f** were selected for a pharmacokinetic study (Table 5). **10e** displayed a short half life in rats with medium clearance and volume of distribution (Vss). Bioavailability (F) was tolerable, however, brain penetration was suboptimal. The other Br substituted compound (**10f**) had a somewhat longer half life and a threefold higher volume of distribution. This was rationalized in terms of the increased lipophilicity (log D = 1.5 and 2.6, respectively). Interestingly, the Cl substituted analogue **11f** had an overall improved profile: notably brain penetration was in the desired range.

To answer the question, whether $GABA_A$ α5 receptor highly binding selective compounds with only partial or even weak inverse agonism may have a potential in the treatment of memory impairment, **10e** and **11f** had been selected for an in-depth evaluation on scopolamine-induced impairment of working memory in the delayed match to position (DMTP) task (Fig. 2).[16] Reference compound **4** administered at 1.0 mg/kg ip 30 min prior to testing, significantly reversed the working memory impairment induced by scopolamine (Fig. 2A). Compound **10e** significantly reversed the scopolamine-induced impairment in a dose-related manner, with full reversal achieved at 1.0–3.0 mg/kg ip 30 min post-administration (Fig. 2B). **10e** was not active following po administration. Compound **11f** significantly, but partially, attenuated the scopolamine-induced working memory impairment at 1.0 mg/kg po at 60 min post-administration (Fig. 2C).

Notably, in this experiment no behavioral impairment was observed. From these results it can be concluded that even weak inverse agonists at the $GABA_A$ α5 receptor may have potential as memory enhancing agents. In line with the referred literature we found that high binding selectivity versus $GABA_A$ α1, α2 and α3 receptors can prevent unwanted side effects.

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