EXPERT OPINION

Benzodiazepine receptor ligands: a patent review (2006 – 2012)

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Introduction: Ligands at the benzodiazepine site of the $GABA_A$ receptor ($GABA_A$-R) act by modulating the effect of GABAA (γ-aminobutyric acid). The selective modulator effects of such ligands are related to the α-subunits type (i.e., α1, α2, α3, and α5), being shown that the α1 subunit is associated with sedative, anticonvulsant and amnesic effects; whereas the α2 and α3 subunits mediate anxiolytic and myorelaxant effects. Recently it was shown the involvement of α5 subunit in pain relief, which is involved in cognitive processes of learning and memory.

Areas covered: This review covers patents, published from January 2006 to October 2012, on ligands for the benzodiazepine binding site of the $GABA_A$-Rs. Patents filed from different companies and research groups report many series of compounds that may be used in the treatment or prevention of a large variety of neurodegenerative diseases.

Expert opinion: Most patents highlighted that various memory deficits, related to Alzheimer's disease, Down syndrome, mood disorders, schizophrenia, and age-related cognitive impairment may be treated using α5-selective ligands. Other aspects related to the use of allosteric modulators of the α7-nAchR and/or α5-$GABA_A$-R (dual approach) for alleviating the impaired cognition and the use of α2-selective ligands for pain relief are highlighted, being particularly intriguing as new therapeutic approaches.

Keywords: benzodiazepine receptor ligands, cognitive impairment, GABAA subtype receptor modulators, neurodegenerative diseases, neuropathic pain

Expert Opin. Ther. Patents [Early Online]

1. Introduction

The $GABA_A$ receptors ($GABA_A$-Rs) are heteropentamer members of the Cys-loop ligand-gated ion channel (LGIC) superfamily permeable to chlorine ions. They are composed of two α-, two β-, and one γ-subunits (despite the 19 total subunits, i.e., α1 – 6, β1 – 3, γ1 – 3, δ, ε, τ, π and ρ1 – 3, that could arrange in enormous number of theoretical pentameric combinations). Recently, Olsen and Sieghart [1] reported the criteria to establish which $GABA_A$ subtype receptors are unequivocally identified in the brain and a list of three categories of native receptor subtypes was suggested, as "identified," "existence with probability" and "tentative." Only 9 – 11 different subtypes were identified in the brain up until now [1], but the list is continuously being updated with new information. However the distribution of the "identified" $GABA_A$-Rs in the brain predicts that the combination α1βγ2 is the most abundant type (60%), being located in the cortex, hippocampal neurons, thalamus, and cerebellum. The combination α2βγ2 and α3βγ2 are moderately abundant (10 – 20%) and they are found in the hippocampal formation, hypothalamus, and amygdale. Finally, the combination α5βγ2 is expressed in the hippocampus (pyramidal cells), amygdale, and hypothalamus (5%) [2]. The identity of the β subunits in these combinations is lacking and often cannot be determined, because

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Article highlights.

- Most of the analyzed patents claim that $GABA_A$ α5 inverse agonists or α5 agonists may be useful for memory therapy associated with neurodegenerative diseases.
- An intriguing aspect that was revealed regards the use of α2/α3-selective ligands for the prevention or suppression of neuropathic pain in addition to their anxiolytic effects.
- The use of a dual approach (allosteric modulators of α7-nAchR and/or α5-$GABA_A$-R) is claimed as a therapeutically relevant modality to ameliorate CNS disorders.
- The chemical scaffolds of the claimed drugs show a high degree of heterogeneity and an enhanced flexibility.
- The label strategy is another topic reported in some patents with the aim to obtain a higher metabolic stability or useful diagnostics tools for imaging methods.

This box summarizes key points contained in the article.

of the co-precipitation of all three subunits in immunoprecipitation or electrophysiological studies [3]. However, even if the subunit β1 is the least common, the β2 is the most abundant and widespread, whereas the β3 is more discrete. All β isoforms can exist in functional receptors, and usually as one type per pentamer [3]. Subunits α4 and α3 are present in combination with β2/3 isoforms and with the subunit δ and represent a minor population of receptors in the striatum and cerebellum [4].

The benzodiazepine site on $GABA_A$-R (alternatively named $GABA_A$/Bz site, $GABA_A$ subtype receptor, or α_n-containing $GABA_A$-R basing on the contained α subunit) is one among the numerous "binding sites" in the $GABA_A$-R. In fact barbiturates, anesthetics, picrotoxin, neurosteroids, alcohol, and Zn^{2+} can bind and directly activate or modulate the receptor function [4]. In particular, benzodiazepine (Bz) and non-benzodiazepine ligands bind the receptor in a site that lies at the interface between the α/γ subunit. These ligands act by modulating the effect of GABA (γ-aminobutyric acid), by increasing the GABA-evoked chlorine current (agonist, positive allosteric modulators [PAM]) or reducing (inverse agonist, negative allosteric modulators [NAM]) or not influencing the chlorine flux (antagonist). The ability of Bz ligands to modulate GABA-mediated channel activity depends on the presence of the γ-subunit in the pentamer, whereas their pharmacological effects are related to the type of the α-subunits [5].

The proven clinical efficacy of the classical benzodiazepines (anxiolytic, anticonvulsant, myorelaxant, and hypnotic) is associated with several side effects such as sedation, memory deficit, dependence, abuse liability and withdrawal syndrome upon cessation of therapy [6]. Thus, the achievement of selective pharmacological effects is a challenge for researchers active in this field and a variety of approaches have been used to better understand the role of the different subunits in the physiology and pathophysiology of CNS [5]. The attempt to synthesize novel compounds to define the functions of $GABA_A$-R subtypes met with limited success. The advent of the molecular genetics has delineated new perspectives to define the relationships between the α isoform and the pharmacological effects of the Bz site ligands [3]. The generation of transgenic mouse (knock-in mouse) with a point mutation at the His residue, with Arg at the α1-3,5 subunits (at position 101 in the α1- and α2-subunits, and in homologous residues in the α3- and α5-subunits, respectively) produced α_n-subtype receptors insensible to diazepam, and permitted to identify the pharmacological activity of the specific mutate subunit [6]. In this way, it was shown that the α1 subunit is associated with the sedative, anticonvulsant and amnesic effects of diazepam. The α2- and α3-subunits mediate the anxiolytic and myorelaxant effects of diazepam [7], whereas the α5 subunit is involved in cognitive processes of learning and memory [8]. These evidences support the hypothesis that subtype-selectivity can lead to pharmacological selectivity, allowing thus the obtaining of anxioselective drugs devoid of sedative effects, as well as procognitive drugs without anxiogenic or convulsant effects [8]. Further genetic studies have been developed mainly with the aim to individuate new roles of the α subunits and to find novel therapeutic opportunities. The obtained results showed that the α2 subunits are involved in schizophrenia [9,10], depression [11-13] and chronic neuropathic pain [14-21]; the α5 subunits are involved in cognitive deficits related to aging [22] and to the Down syndrome (DS) [23,24]. With the same aims, other strategies based on multi-targeted therapy [25,26] and labeled drugs [27,28] have been used.

For the purpose of the present review, we have considered World, US and EP Patents filed from Companies, Universities and individual authors between January 2006 and October 2012, related to $GABA_A$ benzodiazepine receptor ligands. The patents were selected from SciFinder data base of Chemical Abstract (C.A.) and from Espacenet and have been grouped according to α-subtype-selective ligands, $GABA_A$-R modulators, labeled and natural compounds. In case where *in vivo* data were not given, the compounds were chosen based on the *in vitro* data, selecting those compounds that possess the highest affinity value (I%, IC_{50} or K_i). Otherwise, a description of all pharmacological data (*vitro* and *vivo*) was made by the authors of the review.

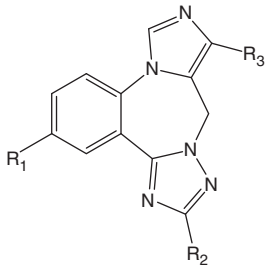
2. α-Subtype-selective ligands

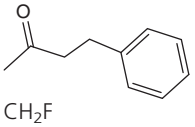
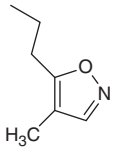
2.1 Selective α5 $GABA_A$ inverse agonists

2.1.1 New compounds

Hoffmann-La Roche has filed numerous patent applications in the field of α5-selective $GABA_A$ ligands. The compounds reported in these patents are useful for neurological disorders and in particular as cognitive enhancers or for therapeutic and/or prophylactic treatment of cognitive disorders such as AD. The affinity of these compounds at $GABA_A$-R subtype was measured by competition for [^{3}H]flumazenil binding to

Table 1. A list of the patents and representative compounds (Hoffmann-La Roche).



Patent	Compound*	R_1	R_2	R_3	K_i (nM)			
					α5	α1	α2	α3
US2006079507 [29]	**33**	Br	H		3.8	108.6	159.8	132.7
US2006084642 [30]	**6**	Br	H	CH_2F	8.7	215.0	261.2	107.5
US2006128691 [31]	**3**	Cl	H	CH_3	3.0	18.5	218.9	265.2
WO2006045429 [32]	**3**	OCH_3	H	Cl	0.8	12.2	17.2	11.7
WO2007042421 [33]	**123**	Br		CH_3	0.6	188.1	-	-

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.

HEK293 cells expressing rat (stably transfected) or human (transiently transfected) receptors of the composition α1β3γ2, α2β3γ2, α3β3γ2, α5β3γ2 [29-55]. In most cases, the compounds showed selectivity for the α5 subunit with respect to α1, α2 and α3 subunits (**Tables 1,2** and **3**; binding data to α1, α2 and α3 subunits are reported when available). This company claimed several chemical series related to imidazo[1,5-a][1,2,4]triazolo[4,3-d] [1,4]benzodiazepine (**Table 1**) [29-33], isoxazole (**Table 2**) [34-53], and triazole derivatives (**Table 3**) [54,55].

2.1.2 Known compounds

Many companies/institutions reported in their application known α5 inverse agonist ligands that, for the sake of clarity, are grouped in **Figure 1**. The intellectual property is concerned with the pharmacological, preclinic and clinical studies to evaluate the cognitive impairment caused by various diseases (psychiatric pathologies, DS, AS) or conditions (anesthesia, inflammation, etc.).

The Governing Council of the University of Toronto reports [56] methods for the prevention and or treatment of memory impairment (long- and short-term) arising as a consequence of excessive $GABA_A$-Rs activation. This increased activation can be caused by administration of an anesthetic or by an increase in interleukine-1β (IL-1β) expression and/or activity, induced by inflammation or surgery. This patent is concerned with the protocols for planning the pharmacological studies and reports several references for each considered aspect. Data obtained from these studies disclosed the therapeutic utility of the α5 inverse agonists. In particular, L655708 [57] reverses memory deficit after exposure to the anesthetic isoflurane and MRK-016 [58] reverses the memory deficit associated with inflammation, both in young and aging brain.

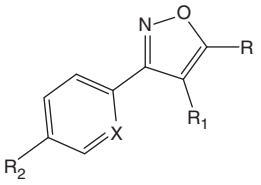
The invention of Cambridge Enterprise Ltd. [59]. reports to the treatment of impaired cognitive function in patients with psychiatric conditions. In treated patients, the use of flumazenil [60] improved cognitive performance, whereas healthy individuals treated in the same way showed a slightly impaired performance.

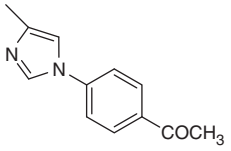
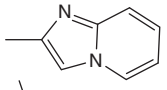
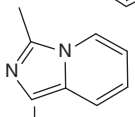
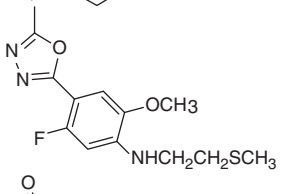
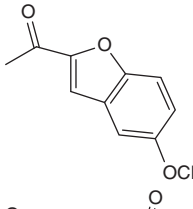
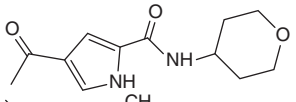
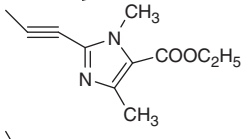
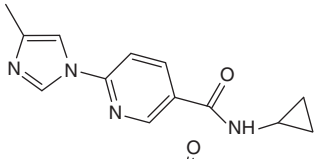
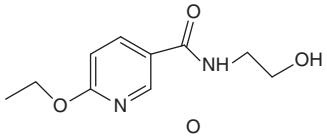
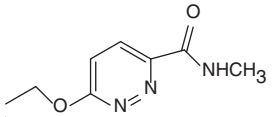
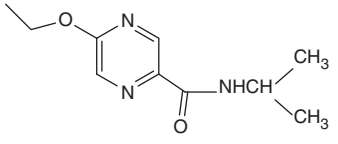
The Centre National De la Recherche Scientifique (CNRS) has deposited an application [61] in which the known compounds L655708, MRK-016, RO4938581 [62], α_5IA [8], having inverse agonist functional selectivity for $GABA_A$-Rs containing α_5 subunit, may possess superior efficacy to treat cognitive impairment associated with DS. This was evaluated by *in vitro* and *in vivo* tests on Ts65Dn mice, a murine model of DS.

The Board of Trustees of the Leland Stanford Junior University [63] reported methods for improving cognitive functions by administration of negative $GABA_A$-R modulators α_5IA, RO4938581, RO4882224 [62], and $GABA_A$-R antagonists, as bilobalide, ginkgolide B and picrotoxine. The methods provided in this application consisted in the administration of therapeutically effective doses of selected compounds so that the peak concentration occurred when the subject was asleep.

Wisys Technology Foundation in the Patent US7595395 [64] claimed pharmacological methods for the prevention and/or

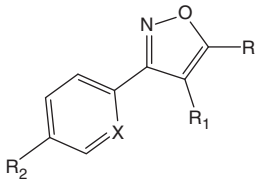
Table 2. A list of the patents and representative compounds (Hoffmann-La Roche).

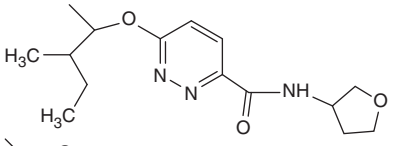
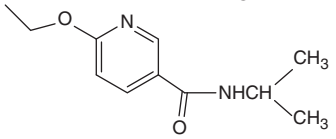
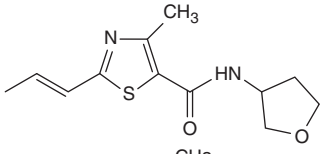
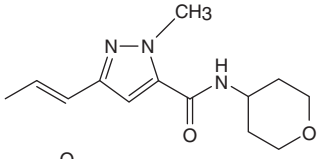
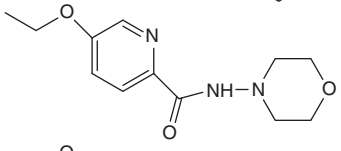
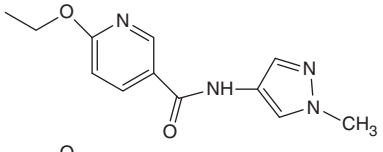
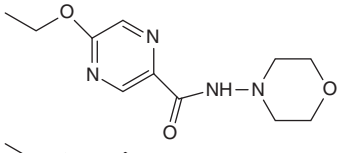
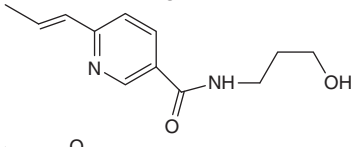
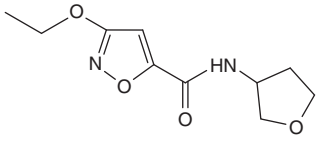


Patent	Compounds*	X	R	R_1	R_2	α5 K_i (nM)
WO2007074078 [34]	**135**	CH			H	1.64
WO2007082806 [35]	**68**	CH			H	5.5
WO2007074089 [36]	**1**	CH	CH_3		H	77.9
WO2007071598 [37]	**127**	CH	CH_3		H	1.7
US2007105922 [38]	**21**	CH	CH_3		H	1.4
US2007066668 [39]	**162**	CH	CH_3		Cl	1.4
WO2007137954 [40]	**50**	CH	CH_3		H	7.3
WO2009000662 [41]	**40**	CH	CF_3		F	4.1
WO2009071476 [42]	**311**	N	CH_3		Cl	0.1
US2009143385 [43]	**102**	N	CH_3		H	0.9
US2009143407 [44]	**3**	CH	CH_3		H	1.2

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡n-butyl is the substituent in 3-position of isoxazole.

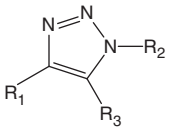
Table 2. A list of the patents and representative compounds (Hoffmann-La Roche) (continued).

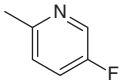
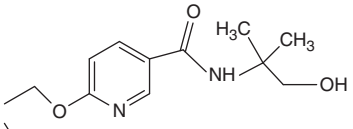
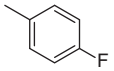
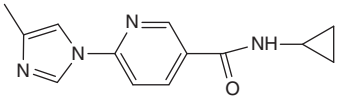


Patent	Compounds*	X	R	R_1	R_2	α5 K_i (nM)
WO2010127968 [45]	**8**	CH	CH_3		H	6.9
WO2010112475 [46]	**19**	CH	CH_2OH		Cl	0.4
WO2010127974 [47]	**69**	CH	CH_3		H	0.3
WO2010127975 [48]	**125**	CH	CH_3		F	0.4
WO2010127976 [49]	**39**	CH	CH_2OH		F	0.8
WO2010127978 [50]	**23**	‡	CH_3		^	2.2
US2010256154 [51]	**12**	N	CH_3		F	0.3
US2010216845 [52]	**4**	CH	CH_3		H	2.0
US2010210651 [53]	**16**	N	CH_3		H	1.4

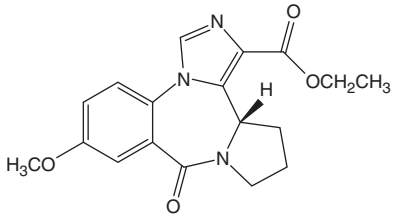
*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡n-butyl is the substituent in 3-position of isoxazole.

Table 3. A list of the patents and representative compounds (Hoffmann-La Roche).



Patent	Compounds*	R_1	R_2	R_3	α5 K_i (nM)
WO2012062687 [54]	**37**		CH_3		1.7
WO2012076590 [55]	**42**	CH_3			3.2

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.



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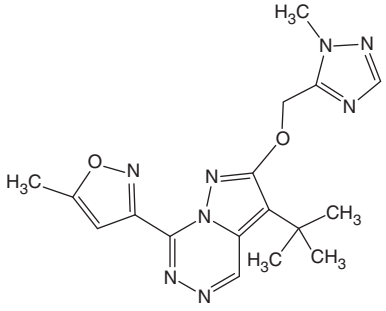
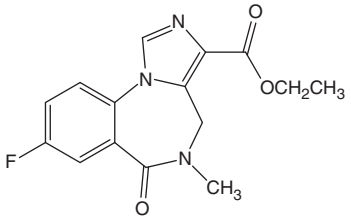
WO2012051707 (The Governing Council of The University of Toronto) [56]
WO2011024115 (Centre National de la Recherche Scientifique-CNRS) [62]

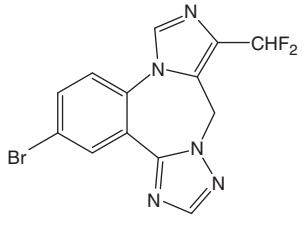


MRK-016

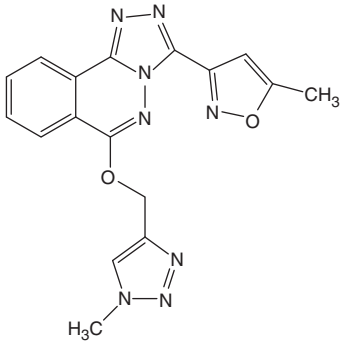


Flumazenil

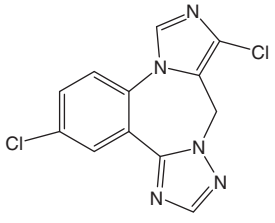
WO2009016329 (Cambridge Enterprise Ltd.) [59]



RO4938581



α5IA

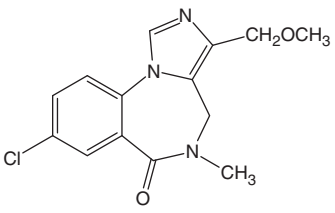


RO4882224

WO2011024115 (Centre National de la Recherche Scientifique-CNRS) [62]
US20121574445 (The Board of Trustees of the Leland Stanford University) [63]

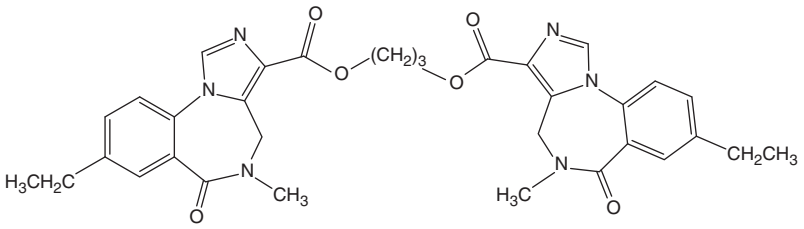
Figure 1. Structures of known α5 inverse agonists.

treatment of memory deficit. Among the drugs/compounds used in this patent were the α-5 inverse agonists PWZ029 [65] and XLi356 (**Figure 2**) [66]. From a full panel of receptor binding was observed that XLi356 does not bind to other types of receptors and, with PWZ029, were found to reverse scopolamine-induced memory deficit (1 mg/kg) in mice at the dose of 10 mg/kg. XLi356 and PWZ029 were not able to reverse audio cued memory (amygdale-driven) suggesting that the



PWZ-029 K_i (nM) $\alpha5$ = 38.8; $\alpha1/\alpha2/\alpha3$ = 300

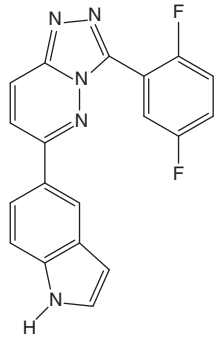
US7595395 (Wisys Technology Foundation) [64]
US20100130479 (Cook) [67]



XLi356 K_i (nM) $\alpha5$ = 107; $\alpha1/\alpha2$ > 2000; $\alpha3$ = NT

US7595395 (Wisys Technology Foundation) [64]

Figure 2. Examples of known α5 inverse agonists claimed by Wisys Technology Foundation and James Cook.



Example 1 NAM α5 $GABA_A$-R; PAM α7 AchR

WO20100104843 (The Regents of the University of California) [68]

Figure 3. Example of compound claimed by The Regents of the University of California.

effects of these compounds may be mediate through α5 receptors located in the hippocampus (highly associated with contextual memory).

This patent has been continuing-in-part of patent application US20100130479 by James Cook [67] in which the compound PWZ029, already studied for its cognitive enhancing effect in rodents, was evaluated as cognitive enhancer in a test of "executive function" in monkeys (Object Retrieval with Detours [ORD]).

2.2 Selective α5 $GABA_A$ inverse agonists and α7 nACh agonists

The Regents of the University of California, taking in account the validity of the multi-targeted therapy, presented an invention [68] of dual compounds as α5 $GABA_A$-Rs NAM and α7 nAChRs PAM (**Figure 3**). Electrophysiological methods were reported: several compounds were found to have maximum positive modulation at α7nAChRs greater than 500% (till 1000%) at 10 μM. Other compounds showed maximum negative modulation of α5 $GABA_A$-Rs from 5 to 50% at 10 μM. Behavioral tests were performed to evaluate the effect on memory: the compounds of the invention exhibited activity in the radial arm maze paradigm in a range between 0.1 and 10 mg/kg i.p. The tested compounds did not disrupt rotarod performance, thus the possible CNS-depressant effect being excluded.

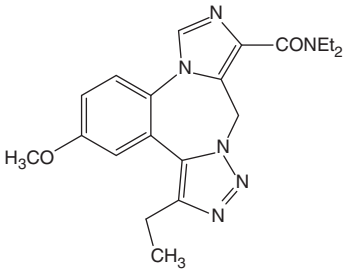
2.3 Selective α5 $GABA_A$ agonists

Based on studies [69] that linked the age-related cognitive decline and schizophrenia to a reduction of hippocampal expression of α5 subunit of the $GABA_A$-R, it has been suggested the usefulness of α5-agonists in the treatment of CNS disorders. Agenebio, Inc. filed two applications in this field [70,71]. In these patents, the preferred compounds with benzodiazepine or pyridazine scaffold (**Figure 4**) showed an EC_{50} that induced greater than 5% potentiation of the GABA-evoked chlorine current, showing a strong positive allosteric modulatory effect on the α5 $GABA_A$-Rs.

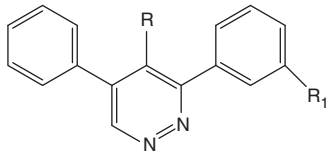
2.4 Selective α2/α3 $GABA_A$ agonists

A series of cinnoline and fused quinoline derivatives have been claimed by Astrazeneca AB as $GABA_A$-R modulators [72-75]. The inventions disclosed derivatives able to module the function and activity of GABA and $GABA_A$-Rs in mammalian subjects, to treat anxiety disorders, cognitive disorders associated with mood and schizophrenia. The therapeutic effect of certain compounds as α2/α3 $GABA_A$ agonists, in the cognitive impairment associated with schizophrenia, may be demonstrated by using the EEG protocol. These compounds attenuated the high-frequency EEG deficits present in schizophrenic patients. In these international applications, [72-75], $GABA_A$-R binding assay was performed as reported in **Table 4**.

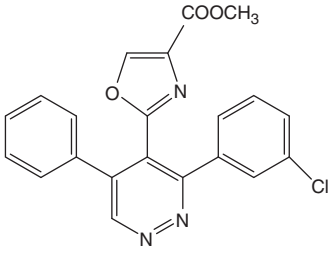
A patent application from the Zurich University [76] reported to the use of α2/α3 $GABA_A$ agonists or partial agonists for the treatment of neuropathic pain. *In vivo* tests to assess the sensitivity to tonic nociceptive stimulation (formalin test) and the pro- or antinociceptive effect in the chronic constriction injury (CCI) model were reported for selected known



Compound 7
WO2012068149 [70]
GABA $\alpha5$ EC_{50} % potentiation = 11.1



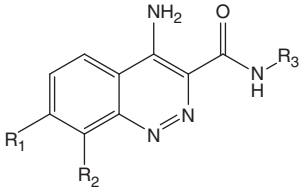
Compound 4
WO2012068161 [71]
GABA $\alpha5$ EC_{50} % potentiation = 42

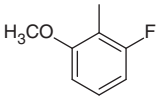
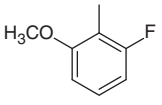
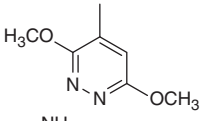
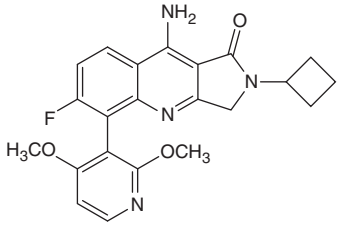


Compound 27
WO2012068161 [71]
GABA $\alpha5$ EC_{50} % potentiation = 30.5

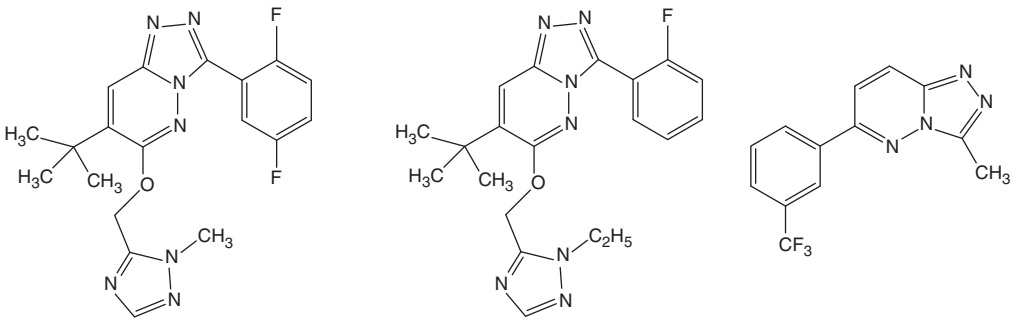
Figure 4. Examples of compounds claimed by Agenebio, Inc.

Table 4. A list of the patents and representative compounds (Astrazeneca AB).

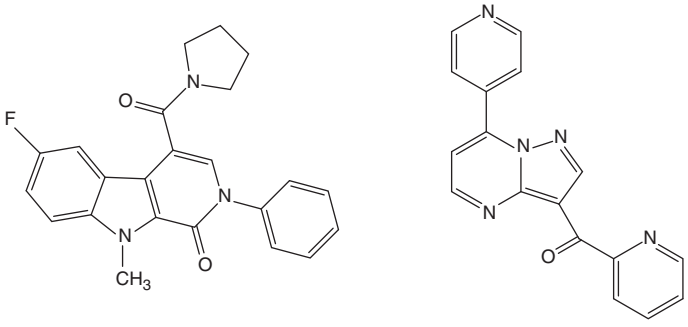


Patent	Compounds*	R_1	R_2	R_3	K_i (nM)
WO2007073283 [72]	**64**	H		$(CH_2)_2CH_3$	$\alpha2$ = 0.144
WO2008155573 [73]	-‡	H		CH_2CH_3	$\alpha2$ = 0.301
WO2011021979 [74]	**I**	F			$\alpha1$ = 0.11 ± 0.03 $\alpha2$ = 0.65 ± 0.45 $\alpha3$ = 0.99 ± 0.16 $\alpha5$ = 35.3 ± 8.5
WO2008155572 [75]	-‡				$\alpha2$ = 0.774

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡Number not reported.



L838417 **TPA023** **CL218872**

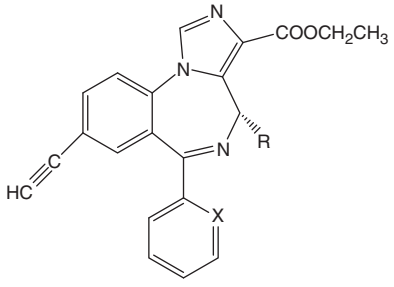


SL651498 **Ocinaplon**

WO2006061428 (Universitat Zurich) [76]

Figure 5. Examples of compounds claimed by Universitat Zurich.

Table 5. A list of the patents and representative compounds (Cook and Wisys).

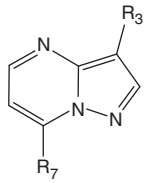


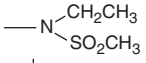
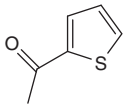
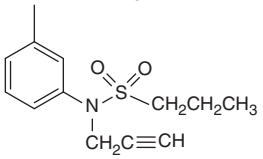
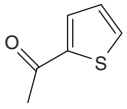
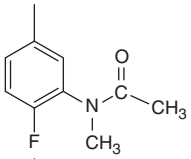
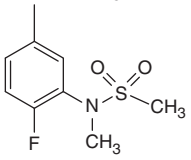
Patent	Compounds	R	X	K_i (nM)			
				α1	α2	α3	α5
US2010317619 [77]	**XHe-II-53**	H	CH	287	45	96	138
	JY-XHe-053	H	CF	21.99	12.34	34.9	0.671
	HZ166	H	N	118	148	365	76.88
US7618958 [78]	**SH-053-*s*-CH_3**	CH_3	CH	Anxiolytic activity without suppressing locomotor activity 56 – 100 mg/kg			

compounds, L838417, TPA023, CL218872, SL651498, Ocinaplon (10 mg/kg p.o.) versus diazepam (0.09 mg/kg i.t. or 10 mg/kg p.o.) (**Figure 5**). For some of these compounds, detailed information is available in the references cited in the same patent and in the recent literature [21].

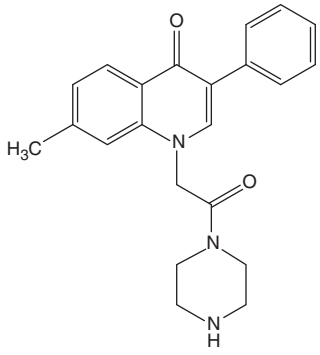
James Cook and Wisys Technology Foundation have patented [77,78] a series of compounds with benzodiazepine scaffold fused with a heterocyclic five-membered ring (**Table 5**), with selective agonist efficacy at $GABA_A$ α2/α3 in comparison with α1, despite the high affinity to all subtype receptors.

Table 6. A list of the patents and representative compounds (Ferrer Internacional SA).



Patent	Compounds*	R_3	R_7	K_i (nM)	
				α1	α2
EP1648896 [79]	**15**	NO_2		11.1	295.8
EP1648897 [80]	**19**			0.5	4520
EP1899343 [81]	**2**			2.1	20
WO2008015253 [82]	**5**	NO_2		-‡	-

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡Only specific binding, expressed as % inhibition, is reported (98.2% at 10^{-5} M). No IC_{50} or K_i values are reported.



Compound 15 α1 affinity I% = 87.3
US20100168099 (Ferrer Internacional SA) [83]

Figure 6. Examples of known compounds claimed by Ferrer Internacional SA.

Cook and co-workers reported in a US application [77] methods for suppression, alleviation and prevention of the neuropathic and inflammatory pain. Preferred compounds (XHe-II-53, JY-XHe-053, and HZ166) were tested (8 – 150 mg/kg) for the ability to suppress the pain (thermal hyperalgesia and mechanical sensitivity) in the chronic constriction injury (CCI) and the inflammatory pain with the formalin test models. The ability of these compounds to suppress pain with minimal ataxic and sedative effects is reflecting their selectivity for α2 or α3 compared to α1.

For the treatment of anxiety, Wisys Technology Foundation has patented [78] a series of compounds tested in a rodent model of anxiety and locomotor activity. The compound SH-053-*s*-CH_3 showed anxiolytic activity without suppressing locomotors activity from 56 until to 100 mg/kg.

2.5 Selective α1 $GABA_A$ agonists

A series of patents claiming several bicyclic heteroaromatic compounds with pyrazolopyrimidine, imidazopyrimidine, imidazopyridine, imidazopyridazine and quinolone scaffolds were registered by Ferrer Internacional S.A. [79-87]. The inventions describe novel classes of subtype-selective α1 relative to α2 $GABA_A$-R ligands, useful in the treatment and prevention of sleep disorders, insomnia and epilepsy. Many of the novel compounds with pyrazolopyrimidine scaffold (**Table 6**) [79-82] and the quinolone derivatives (**Figure 6**) [83] were evaluated *in vitro* and *in vivo*. Good α1 affinity values were evidenced and the *in vivo* effects were assessed by predictive sedation-hypnosis tests in

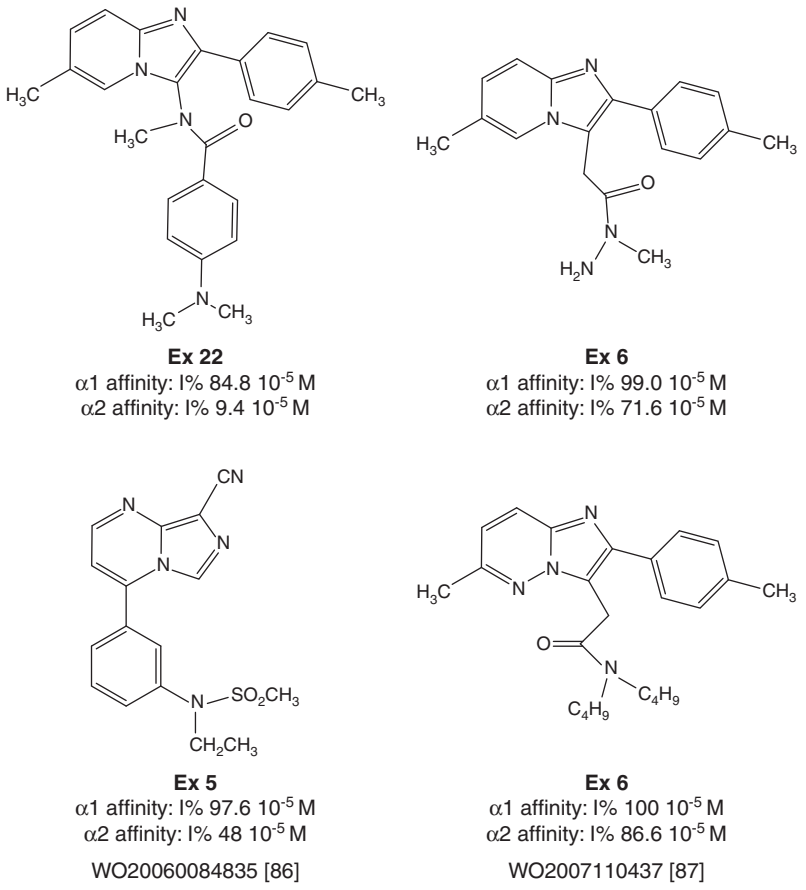


Figure 7. Examples of compounds claimed by Ferrer Internacional SA.

mice (expressed as percentage of crossings of treated animals versus controls, 89 – 95%) with respect to the reference compound zaleplon (84.9%).

As concerning the imidazole derivatives (**Figure 7**) in which the heterocyclic ring is variously fused with pyridine, pyrimidine and pyridazine moieties, the *in vitro* and *in vivo* tests were reported in four patents [84-87]. Certain compounds exhibited high selectivity for the α1 $GABA_A$ subtype receptors with respect to the α2 ones, suggesting their possible use in diseases or conditions in which preferential activity on the α1 receptors is desirable, such as insomnia, anesthesia, induction of sleep and sedation. Affinity data were expressed as percentage inhibition (I%) at 10^{-5} for α1 and for α2 subunits and the percentage inhibition of motor activity was in the 85 – 92% range.

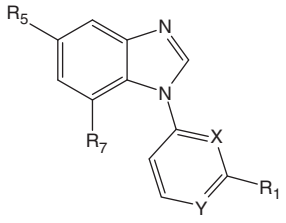
3. $GABA_A$ receptor modulators

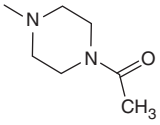
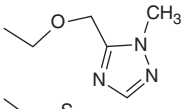
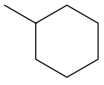
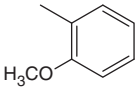
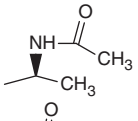
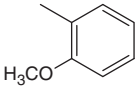
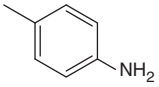
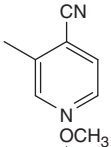
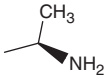
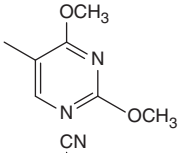
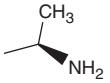
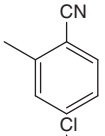
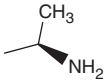
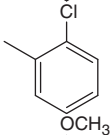
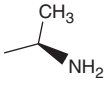
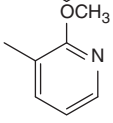
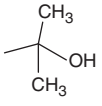
Many companies and institutions reported in several patents on ligands binding to the $GABA_A$/Bz site that could be used in the treatment and/or prevention of a variety of disorders of the central nervous system in particular in anxiety and related diseases. *In vitro* inhibition of ^{3}H-flunitrazepam (^{3}H-FNM) binding was performed and the affinity value of the tested substance, which inhibits the specific binding of ^{3}H-FNM by 50%, was given as IC_{50} or K_i values. All reported compounds may be also useful in their labeled form as radiotracers for positron emission tomography (PET) imaging or for single photon emission computerized tomography (SPECT).

Neurosearch has filed a series of 17 [88-104] patents concerning novel benzimidazole derivatives (**Table 7**) and 4 patents [105-108] describing imidazole derivatives (**Table 8**), active from the submicromolar to the picomolar range (IC_{50} 0.10 – 0.00029 μM).

Neurogen published 11 patents and/or applications [109-119] in which are reported numerous heterocyclic compounds (around 2400 derivatives) exhibiting K_i values in the range of 1 μM – 10 nM. To rationalize all these compounds we have summarized the most representative chemical scaffolds and structures in **Figures 8**, **9** [109,116-119], in **Table 9** [110,113-115,120], **Table 10** [111] and **Table 11** [112]. In **Table 9**, for the sake of simplicity, we also report the structures described by Yuelian, in the patent

Table 7. A list of the patents and representative compounds (Neurosearch).



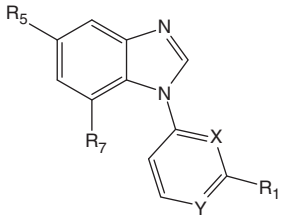
Patent	Compounds*	X	Y	R_1	R_5	R_7	IC_{50} (μM) I%‡
WO2006111517 [88]	**23**	CH	CH		CH_3	H	0.0026
WO2006111516 [89]	**22a**	CH	CH			H	0.0021
WO2006108800 [90]	**-**§	CH	CH			H	0.0048
US2006148856 [91]	**-**§	CH	CH			H	75%
WO2007065864 [92]	**12g**	CH	CH			H	0.0026
WO2007110374 [93]	**9aac**	CH	CH			H	0.00065
US2007021482 [94]	**6**	CH	CH	H			0.0042
WO2010055124 [95]	**8a**	CH	CH			H	0.0085
WO2010055125 [96]	**8f**	CH	C-F			H	0.0026
WO2010055126 [97]	**8a**	N	CH			H	0.0049
WO2010055127 [98]	**8a1**	CH	N			H	0.019
WO2010055128 [99]	**7d**	CH	C-F			H	0.00029

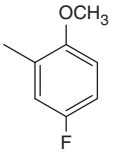
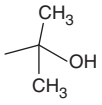
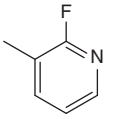
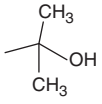
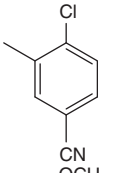
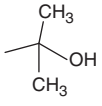
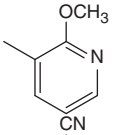
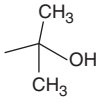
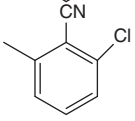
*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.

‡Specific binding (I%) must be obtained, before calculation of IC_{50}.

§Number not reported.

Table 7. A list of the patents and representative compounds (Neurosearch) (continued).



Patent	Compounds*	X	Y	R_1	R_5	R_7	IC_{50} (μM) I%‡
WO2010055129 [100]	**7a**	CH	C-F			H	0.00049
WO2010055130 [101]	**7a**	N	CH			H	0.0023
WO2010055131 [102]	**7g**	N	CH			H	0.0013
WO2010055132 [103]	**7a**	CH	N			H	0.0012
WO2010055133 [104]	**7b**	CH	N		H	H	0.016

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡Specific binding (I%) must be obtained, before calculation of IC_{50}.
§Number not reported.

application [120] because of the similarity of the nucleus. Preferred compounds of this inventions exhibited K_i < 100 nM and the most preferred ones had K_i values < 10 nM. In **Table 11** we report three compounds and the most representative general structures chosen among the numerous derivatives reported in the considered patent [112].

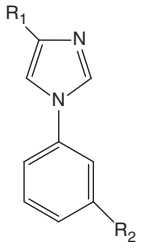
Helicon filed patent applications on pyrazoloquinolines [121,122], pyrazolonaphthyridines [123], and dipyrazolopyridines [124]. General binding data are reported and expressed as percentage of inhibition (I%) (**Table 12**).

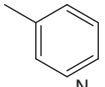
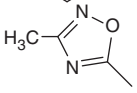
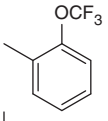
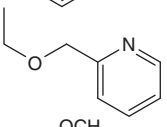
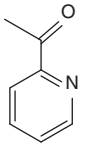
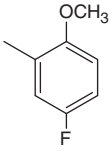
A series of quinolone derivatives fused with isothiazole and/or isoxazole rings were registered by Forskarpatent I Syd AB [125] together with their binding mode in a pharmacophoric model. The affinity for $GABA_A$/Bz (rat cortical membranes) was also measured. The K_i range of these novel compounds was of 1.9 – 18 nM and the company claimed a method for treating anxiolytic, anticonvulsant, sedative-hypnotic, and myorelaxant conditions in mammalians, including humans (**Figure 10**).

4. Labeled compounds

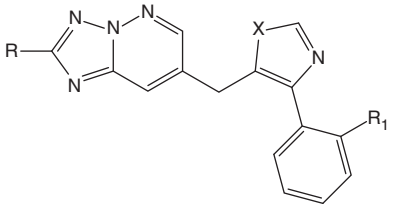
Concert Pharmaceutical, Inc. patented the deuterated form of several known compounds, since they show a higher metabolic stability but a comparable pharmacological activity with respect to non-deuterated analogs. The determination of metabolic stability was performed *in vitro*, evaluating the "percentage parent remaining" (% PR) that refers to the percentage of starting material remaining at a specified time point (i.e., 30 min). Concert Pharm., in the field of $GABA_A$ ligands seems to be a newcomer and filed five patents [126-130] in which reported several deuterated analogs in the "various possible position" of the considered structures. The deuterated analogs of TPA023 (α1 antagonist; α2/α3 partial

Table 8. A list of the patents and representative compounds (Neurosearch).



Patent	Compounds*	R_1	R_2	IC_{50} (μM) ‡
WO2007042543 [105]	-§		CN	0.10
WO2007042544 [106]	-§			0.017
WO2007042545 [107]	**11a**	$-COOC_2H_5$		0.024
WO20070472546 [108]	**14**			0.0027

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡Specific binding (I%) must be obtained, before calculation of IC_{50}.
§Number not reported.



WO2006093911 [109] (Neurogen)
R = alkyl; R_1 = CN, F; X = S, O
K_i < 1 μM

Figure 8. Example of compound claimed by Neurogen.

agonist), NS11394 (α3 agonist), α5IA (α5 inverse agonist) L838417 (α1 antagonist; α2/α3/α5 agonist), and pagoclone (α3 full agonist; α1/α2/and α5 partial agonist) are shown in **Figure 11**.

An important aspect developed by GE Healthcare Ltd is related to compounds useful for *in vitro* diagnostics and *in vivo* imaging of the $GABA_A$-Rs. In particular, these inventions [131,132] deal with quinoline and carboline derivatives showing K_i value ≤ 20 nM at the $GABA_A$/Bz site from rat cerebellar membranes. It was claimed that these novel compounds may be prepared with a detectable label, for example, ^{18}F for PET or SPECT imaging methods. In **Figure 12** are reported the only two labeled synthesized compounds claimed in this patent.

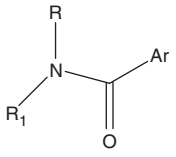
5. Patents of natural products and derivatives

Wien University has filed a patent application [133] for piperine derivatives as new anxiolytics with significantly reduced sedative properties without affect transient receptor potential vanilloid 1 (TRPV1). SCT-64 (ex 35) and SCT-66 (ex 36) (**Figure 13**) show dose-dependent anxiolytic activity at 0.3 – 10 mg/kg in elevated plus maze (EPM) test.

6. Expert opinion

This review presents an overview of the most interesting $GABA_A$-R modulators patented starting with 2006 till the present period, and can be considered an update of the previously published reviews [134,135].

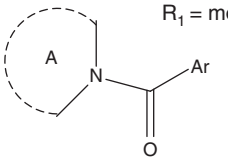
The Bz-binding site on $GABA_A$-R has historically received much attention and since 1980 century many efforts were made to develop a pharmacophore/receptor model for agonists, antagonists and inverse agonists, by using a variety of



US20061353367 [116]
Ar = Ph, Py
R = alkyl
R_1 = heteroaryl methyl

US2006160842 [117]
Ar = Ph, Tiofene, Furane
R = alkyl
R_1 = heteroaryl methyl

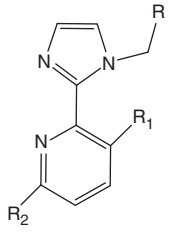
US2008032975 [119]
Ar = tetrahydroindazole, indole
R = H
R_1 = monociclyc aryl/heteroaryl system

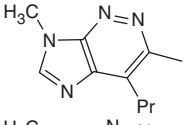
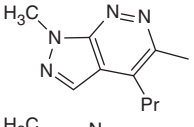
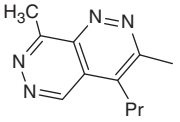
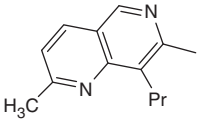
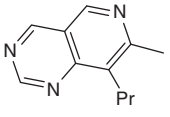


US2008146546 [118]
Ar = heteroaromatic bicyclic system
A = piperidine, pirrolidine, morfoline

Figure 9. Other compounds claimed by Neurogen.

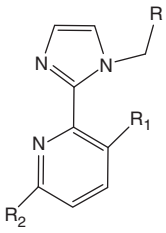
Table 9. A list of the patents and representative compounds (Neurogen and Xu Yuelian).

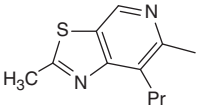
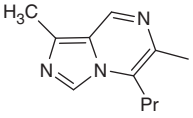
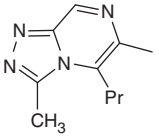
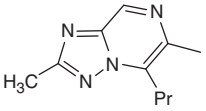
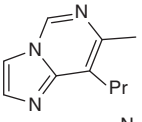
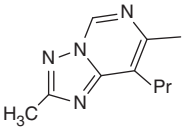
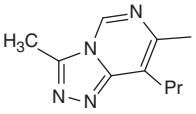
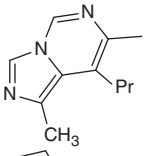
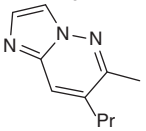
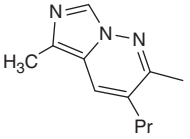
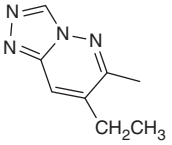


Patent	Compounds*	R	R_1	R_2
WO2006078891 Neurogen [110]	**63**		F	H
	73		F	H
	79		F	H
	90		F	H
	96		F	H

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.

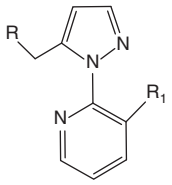
Table 9. A list of the patents and representative compounds (Neurogen and Xu Yuelian) (continued).

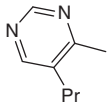
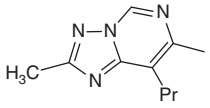
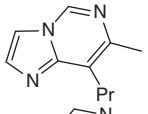
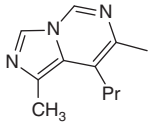
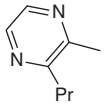


Patent	Compounds*	R	R_1	R_2
	102		F	H
US2006247245 Xu Yuelian [120]	**Ex 5**		F	H
	Ex 7		H	H
	Ex 9		F	H
EP1880998 Neurogen [113]	**106**		H	F
	130		F	H
	186		F	H
US7271170 Neurogen [114]	**213**		H	F
US20090023737 Neurogen [115]	**111**		H	F
	126		H	F
	128		H	F

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.

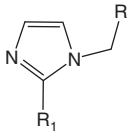
Table 10. A list of the patents and representative compounds (Neurogen).

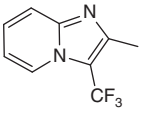
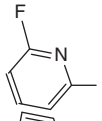
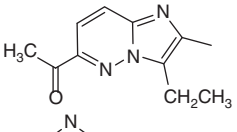
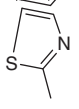
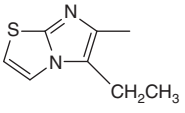
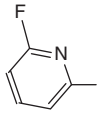
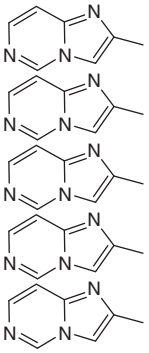


Patent	Compounds*	R	R_1
WO2006052546 [111]	**106**		F
	125		F
	215		F
	217		F
	307		F

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.

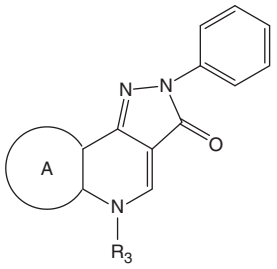
Table 11. A list of the patents and representative compounds (Neurogen).

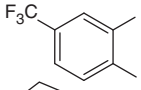
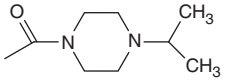
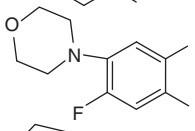
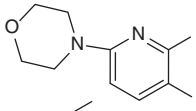
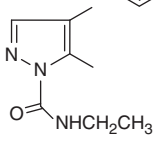


Patent	Compounds*	R	R_1
US20060014746 [112]	**Ex 9**		
	Ex 11		
	Ex 13		
General structures in US20060014746 [112]			Aryl/Heteroaryl (further substituted)

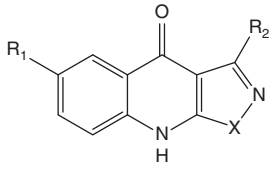
*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.

Table 12. A list of the patents and representative compounds (Helicon).



Patent	Compounds*	A	R_3	IC_{50} (nM) or I%‡
WO2008154442 [121]	**45**			< 1.0
WO2008154438 [122]	**19a**		H	< 1.0
WO2008154447 [123]	**6c**		H	< 1.0
WO2012051213 [124]	**6b**		H	> 80%

*Original number compound used in the cited patents: selection, made by the authors, of the most representative structures.
‡Specific binding must be obtained, before calculation of IC_{50}.
I%: Percentage of inhibition at 0.1 μM.



WO2009123536 [125] (Forskapatent I Syd AB)

X = S, O
R_1 = H, CH_3, OCH_3, NO_2, Br, C_2H_5, C_3H_7,
R_2 = $PhCH_3$, PhBr, $NHCOCH_3$

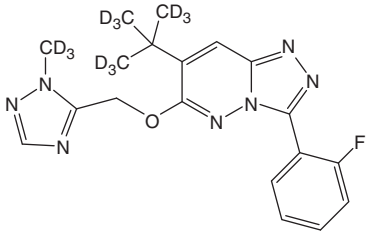
K_i range 1.9 – 18 nM

Figure 10. Examples of compounds claimed by Forskapatent I Syd AB.

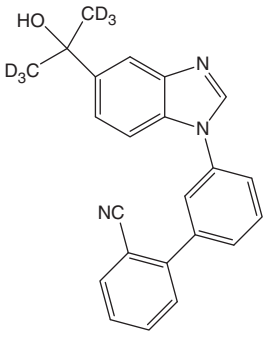
techniques (e.g., chemical synthesis, radioligand binding, and receptor mapping) affording thus Cook's model [136]. The determination of the crystal structure of water-soluble acetylcholine binding protein (AChBP) [137] has generated much interest also for the study of the $GABA_A$-R, and several comparative modeling studies have been proposed for the last one [138,139]. At the same time the discovery of molecular genetics and pharmacological approaches permitted to elucidate the relationship between the α-subunit and the pharmacological effect of classical benzodiazepines, and to develop selective anxiolytic, anticonvulsant, myorelaxant, sedative and promnemonic drugs. At the present it is particularly intriguing the use of the α-subtype selective ligands as synergic compounds in multi-targeted therapy for CNS diseases.

It is interesting to highlight that the majority of the patents, in the period 2006 – 2012, have been filed by companies (e.g., Hoffmann La-Roche, Neurogen Corp., Neurosearch A/S, and Wisys Technology Foundation, Inc.), extensively engaged in this research field. The α-subtype-selective ligands, developed by these companies, are claimed in the reviewed patent applications, and represent around 70% of the considered patents. Companies such as Ferrer Internacional S.A., Helicon Therapeutics, Inc., Agenebio, Inc., Astrazeneca AB, Concert Pharmaceutical, Inc., GE Healthcare Ltd, and some University, represent the remaining 30%, and are newcomers in this field.

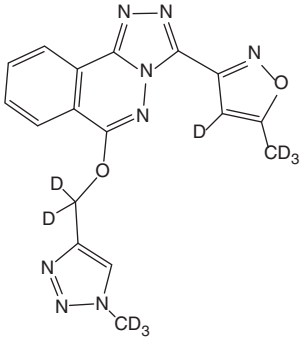
The structural heterogeneity of the compounds reported in the reviewed patents does not permit to individuate a pharmacophore moiety for subtype-selective ligands. The novel compounds claimed in these patents are mainly represented by heterocycles (mono- or bicyclic, eventually further substituted with other ring systems), while the previously described



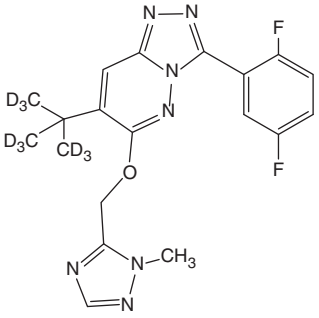
Compound 109 (analogue of TPA023)
WO2011005520 [126]



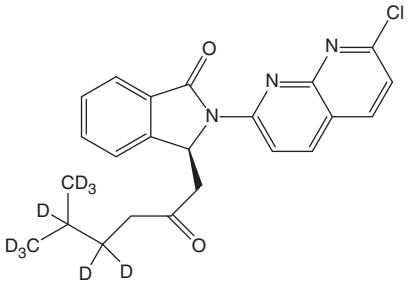
Compound 102 (analogue of NS11394)
WO2011047315 [127]



Compound 115 (analogue of α5IA)
WO2011020044 [128]

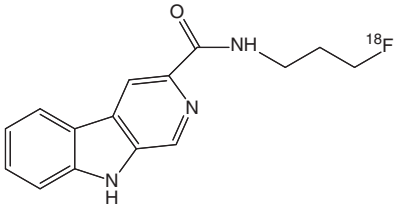


Compound 103 (analogue of L838417)
WO2010025407 [129]

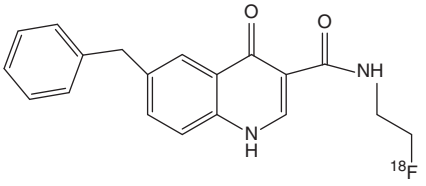


Compound 121 (analogue of pagoclone)
WO2012116288 [130]

Figure 11. Example of known compounds claimed from Concert Pharmaceutical.



4 [^{18}F]
WO2008132454 [131]

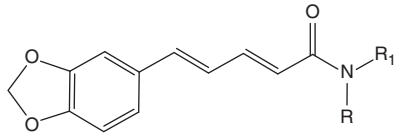


6b [^{18}F]
WO2009040377 [132]

Figure 12. Examples of compounds claimed from GE Healthcare Ltd.

benzodiazepine receptor ligands showed a planar nitrogen polycyclic scaffold. Thus, a major conformational flexibility in the structure seems to be tolerated for binding on $GABA_A$-R. For example Hoffmann La Roche "simplified" the rigid tetracyclic scaffold (imidazotriazolobenzodiazepine reported in the 2006 and 2007 patent applications), which led to isoxazole and triazole derivatives.

In addition to several patent applications claiming compounds acting as no-selective $GABA_A$-R modulators, most of the reviewed patents highlighted that different types of memory deficits related to AD, DS, mood disorders, schizophrenia and age-related cognitive decline, may be treated using α5-inverse agonists and/or α5-agonists. Moreover, taking into account that many neurotransmitters are involved in neurodegenerative



WO20110800313 [133] (Universitat Wien)

R = R_1 = *n*-Pr SCT-64 (**ex 35**)
R = R_1 = *i*-Bu SCT-66 (**ex 36**)

Figure 13. Example of compound claimed by Universitat Wien.

diseases, the use of a dual approach (allosteric modulators of α7-nAchR and/or α5-$GABA_A$-Rs) as claimed from The Regents of the University of California, might be a therapeutically relevant modality to ameliorate CNS disorders. The enhancer cognition topic covers a wide part of the research by the drug companies, as shown by the high number of filed patents.

Another attractive aspect that stands out in this review is the use of α2/α3-selective ligands for the prevention or suppression of neuropathic pain in addition to anxiolytic effects, which represents an intriguing approach, whose research is still in progress.

It is important to point out that the label strategy is another topic reported in several patents, either for improving pharmacokinetic properties without impairing pharmacodynamics properties or for generating diagnostic tools.

All these data show that since 2000 the study and development of subtype-selective receptor ligands is in progress with the aid of classic or more modern approaches. The promising aspect that arises is referred to the possibility to treat particular deficits that are not directly related to GABAergic neurotransmission.

Declaration of interest

This work was supported by a grant from the Italian Ministry for University and Research (MIUR). The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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