Expert Opinion

Novel $GABA_B$ receptor positive modulators: a patent survey

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Importance of the field: Positive allosteric modulators of $GABA_B$ receptors may have a similar potential as positive modulators of $GABA_A$ receptors, the benzodiazepines discovered in 1957. The discovery of positive allosteric modulators of $GABA_B$ receptors at Novartis in Basel in 2000 opened the way to search for compounds, which activate $GABA_B$ receptors without the drawbacks of full agonists, such as desensitization, tolerance, muscle-relaxant effects, hypothermia, and central and gastrointestinal side effects.

Areas covered in this review: Numerous animal experiments point out that several indications can be addressed with positive modulators of $GABA_B$ receptors, such as depression, anxiety, schizophrenia, neuropathic and chronic pain and treatment of craving for drugs of abuse, such as alcohol, cocaine and nicotine. Peripherally acting compounds may be valuable drugs for the treatment of gastroesophageal reflux disease and irritable bowel syndrome.

What the reader will gain: An overview on 19 patents in this field, of the different scaffolds for positive modulators of $GABA_B$ receptors and of the major players in the field.

Take home message: The search for subtype selective benzodiazepine receptor ligands has proved to be extremely difficult. Positive modulators of $GABA_B$ receptors may provide novel anxiolytic drugs faster.

Keywords: agonists, allosteric binding site, desensitization, $GABA_B$ receptors, orthosteric binding site, positive allosteric modulators

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1. Introduction

Allosteric modulators are molecules that bind to a site on a neurotransmitter or hormone receptor, which is topographically distinct from the orthosteric binding pocket for agonists and competitive antagonists [1]. GABA, baclofen and all $GABA_B$ receptor agonists or antagonists bind to the Venus flytrap module on the N-terminal extracellular domain of the $GABA_{B1}$ receptor, whereas the positive allosteric modulators (PAMs) bind at the heptahelical domain of the $GABA_{B2}$ receptor, more precisely GS39783 binds at the 6th transmembrane domain. For a beautiful color drawing of the orthosteric and allosteric $GABA_B$ receptor binding sites see **Figure 3** in Ref. [2]. Allosteric modulators have little or no intrinsic agonistic activity of their own, but induce conformational changes in the receptor protein, which alters its interaction with the endogenous neurotransmitter.

No negative allosteric modulators (NAMs) of $GABA_B$ receptors have been found so far.

The first PAMs of $GABA_B$ receptors were discovered by Stephan Urwyler and colleagues at the Novartis Institute of Biomedical Sciences in Basel in 2000. In a high throughput screen using a GTPγ[^{35}S] assay in membranes from CHO-K1 cells stably transfected with human $GABA_{B1b}$ and rat $GABA_{B2}$ cDNAs CGP7930 (**Figure 1**) and the corresponding aldehyde CGP13501 were identified as positive modulator of $GABA_B$ receptor function [3,4].

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Article highlights.

- Positive allosteric modulators (PAMs) of $GABA_B$ receptors bind at the 6th transmembrane domain of the $GABA_{B2}$ receptor, whereas endogenous GABA and all $GABA_B$ receptor agonists and antagonists bind at the Venus flytrap N-terminal extracellular domain of the $GABA_{B1}$ receptor.
- PAMs do not elicit desensitization, tolerance, muscle-relaxant effects, hypothermia, and central and gastrointestinal side effects contrary to $GABA_B$ receptor agonists.
- PAMs show anxiolytic and antidepressant-like effects with excellent bioavailabilities.
- PAMs significantly reduce the craving for drugs of abuse, such as alcohol, cocaine and nicotine.
- PAMs alleviate neuropathic and chronic nociceptive pain.
- Ten patents of AstraZeneca describe peripherally acting PAMs as novel drugs for the treatment of gastroesophageal reflux disease (GERD).

This box summarizes key points contained in this article.

CGP7930 potentiated GABA-stimulated GTPγ[^{35}S] binding at low micromolar concentrations and was inactive in the absence of GABA. It increased both agonist potency and maximal effects: the EC_{50} for CGP7930 in the presence of 1 μM GABA in recombinant $GABA_B$ receptor heterodimer expressed in CHO cells was 4.6 μM, which decreased to 1.87 μM in the presence of 20 μM GABA. The maximal effect was dose dependent: GABA at 20 μM stimulated basal activity to 301%, in the presence of 1 μM CGP7930 to 328%, in the presence of 3 μM CGP7930 to 377%, in the presence of 10 μM CGP7930 to 394% and in the presence of 30 μM CGP7930 to 427% (see **Figure 2** [3] with permission from ASPET).

A more sensitive assay was employed by Pin and colleagues [5]. The $GABA_B$ receptor efficiently activated phospholipase C when co-expressed with the chimeric G-protein Gqi9. CGP7930 stimulated inositol *tris*-phosphate production even in the absence of added GABA suggesting that the compound acted as a partial $GABA_B$ receptor agonist. For compounds with such a pharmacological profile Schwartz and Holst have coined the term "ago-allosteric modulators" ([6,7]; see also [8]). The Pin group also found the precise site of interaction of CGP7930 on the heptahelical domain of the $GABA_{B2}$ receptor [5,9].

CGP7930 showed antidepressant-like effects in the modified forced swimming test significantly decreasing immobility time and showed also anxiolytic properties in the elevated zero maze test significantly increasing time spent in the open area of the maze [10] and in the stress-induced hypothermia test [11]. For an excellent review on the multiple properties of CGP7930 see [12].

In 2003 the more potent PAM GS39783 (**Figure 1**) was described, which increased the potency of GABA about eightfold: EC_{50} of GABA in the absence of GS39783 was 3.59 μM and in the presence of 30 μM GS39783 was 0.45 μM. The maximal effects increased from 100 to 217% [13]. GS39783 showed anxiolytic-like effects in the elevated plus maze in rats and the elevated zero maze in mice and rats [14] and decreased anxiety in the light-dark box, but did not show any effects in the forced swim test [15]. GS39783 blocked the MK-801- and amphetamine-induced hyperactivity in a dose dependent manner [16].

Through point mutations G706T and A708P Novartis molecular biologists were able to locate precisely the binding site of GS39783 in the 6th transmembrane domain of the $GABA_{B2}$ receptor [17].

Both drugs efficiently reduced the craving for drugs of abuse: CGP7930 reduced alcohol intake in alcohol-preferring rats [18,19] and counteracted the cocaine-discontinuation-induced enhancement of immobility time in the forced swimming test [20,21]. GS39783 reduced alcohol self-administration in alcohol-preferring rats [18,22,23]. GS39783 also attenuated the reward-facilitating effects of cocaine in rats [24-26] and of nicotine in rats [27,28].

CGP7930, CGP13501 and GS39783 are commercially available from Sigma–Aldrich and from Tocris Bioscience.

2. Novartis patents

Novartis patented the use of PAMs for the treatment of gastrointestinal esophageal reflux disease [29].

Novartis chemists optimized the potentially genotoxic lead structure of the pyrimidine derivative GS39783, in collaboration with NIH, in particular NIDA and NIMH (Grant U01 MH069062) to obtain non-toxic positive modulators of $GABA_B$ receptors, such as NVP-BHF177 (**Figure 1**; [30,31], see also [8]). The primary goal of the NIH collaboration was to find novel drugs for the treatment of craving after smoking cessation [28], but NVP-BHF177 also reduced alcohol intake [32]. NVP-BHF177 crosses the blood–brain barrier and is devoid of *in vitro* genotoxicity. It did not cause motor-incoordinating side effects in mice up to doses of 100 mg/kg unlike the $GABA_B$ receptor agonist baclofen. BHF177 showed anxiolytic properties in the mouse stress-induced hypothermia test [33]. Variation of the pyrimidine-2 substituent led to additional interesting potent compounds such as NVP-BIK998 (R = CN), NVP-BIK834 (R = OMe) and NVP-BIK877 ($R = NMe_2$). For a comparison of the positive allosteric modulatory effects of five Novartis compounds see **Table 1**, which shows the potentiation of GABA-stimulated GTPγ[^{35}S] binding with 2.5 and 25 μM of PAM, the EC_{50} values and the maximal effects. The patent describes 64 examples with full experimental details.

3. Addex Pharma patent

Addex is specialized in the discovery of allosteric modulators (the allosteric modulation company). It discovered a positive

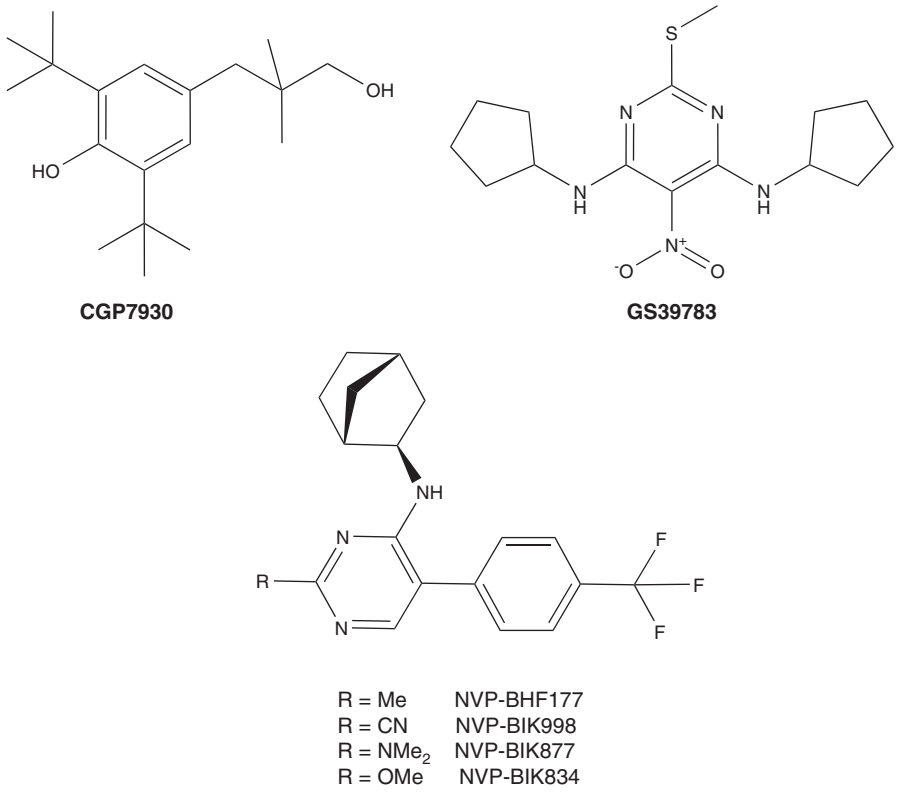


Figure 1. Novartis compounds [3,4,13,30,31].

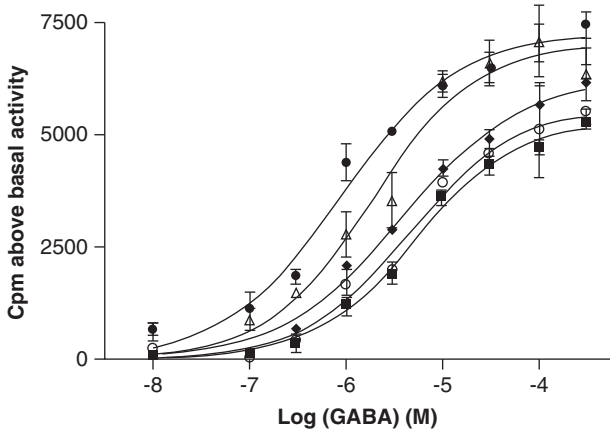


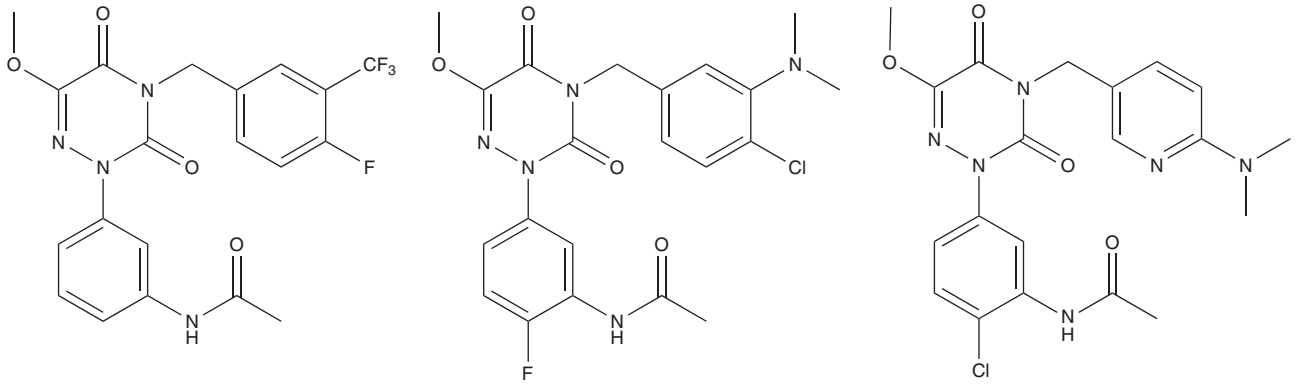
Figure 2. Concentration–response curves for GABA in the GTPγ[^{35}S] binding assay in the absence (■) and in the presence of CGP7930 (○, 1 μM; ◆, 3 μM; △, 10 μM; ●, 30 μM).
Reproduced with permission from [3].

modulator of mGlu2 receptors ADX-71149 (structure not communicated) for the potential treatment of schizophrenia, NAMs for mGlu2/3 receptors for the potential treatment of depression, a PAM of mGlu5 receptors ADX-63365 (a 1,2,4-oxadiazole derivative) for the potential treatment of schizophrenia, and ADX-48621 (an imidazo [1,2-a]pyridine derivative with structural similarity to MPEP, which is currently in Phase I clinical trials), a NAM of mGlu5 receptors for the potential treatment of levadopa-induced dyskinesia. With ADX-71943 (structure not communicated) Addex has discovered an orally available PAM for $GABA_B$ receptors for the treatment of osteoarthritis pain and for chronic nociceptive pain. "It is expected that ADX-71943 will enter clinical testing in the fourth quarter of 2010" (Company press release of February 8, 2010). In the patent of Addex 335 novel triazinedione derivatives are described in five tables (containing 7, 20, 20, 269 and 19 compounds, respectively) characterized with melting points, NMR and mass spectra [34].

The compounds were evaluated in a [^{35}S]GTPγS binding assay in rat cortical membranes and 23 compounds were found with EC_{50} < 100 nM (all compounds were from Table 4 in the patent). Additional tests employed were the marble burying test as a model of anxiety in mice, the Vogel conflict drinking test as a model of anxiety in rats and the elevated plus maze test as a model of anxiety in mice and rats. Inflammatory, neuropathic and post-operative pain is also claimed, but no experimental results are presented. As examples of potent structures, compounds with EC_{50} < 100 nM, compounds 4.118, 4.231 and 4.234 are shown in **Figure 3**. None of the structures of the front runner compounds, such as ADX-71943, ADX-1a, ADX-1b or ADX-71441 was communicated so far.

Table 1. Biological data to Novartis compounds (see Figure 1).

Compound	2.5 μM compound 1 μM GABA Effect in %	25 μM compound 1 μM GABA Effect in %	pEC_{50}	E_{max} in %
GS39783	132	153	6.13	146
NVP-BHF177	122	110	5.78	183
NVP-BIK998	117	127	5.92	198
NVP-BIK877	115	131	5.69	191
NVP-BIK834	94	126	5.78	167



ADDEX WO 2008/056257
Compound 4.118

ADDEX WO 2008/056257
Compound 4.231

ADDEX WO 2008/056257
Compound 4.234

Figure 3. Addex compounds [34].

4. F. Hoffmann-La Roche patents

Roche scientists contributed significantly to the research of PAMs of $GABA_B$ receptors with five patents and two publications. They took up both leads of Novartis and developed them further.

The pyrimidine lead GS39783 was developed into 4-(sulfanyl-pyrimidine-4-ylmethyl)-morpholine derivatives [35]. Forty-three examples of pyrimidine derivatives are described in the patent, which were tested in an intracellular Ca^{2+} mobilization assay in CHO cells stable expressing human $GABA_BR1aR2a$ and Gq16. The E_{max} values (%) in the range of 0% for 10 nM GABA alone and 100% for 10 μM GABA alone were from 46 to 74%, the EC_{50} values in the presence of 10 nM GABA varied from 1.7 to 8.2 μM, the GABA shift (= log[EC_{50}(GABA + 10 μM compound)/EC_{50} (GABA alone)]) ranged from -0.48 to -0.92). See the first structure in **Figure 4**. The corresponding biological data for examples 1, 2 and 35 are shown in **Table 2** providing data from the intracellular Ca^{2+} mobilization assay (maximum enhancing effects and potency, i.e., EC_{50} values) and in the GABA shift assay, which determines concentration–response curves of GABA (0.0003 – 30 μM) in the absence and presence of 10 μM PAM.

Entirely new structural classes were presented in the 2006 patents of Roche, quinolines and thieno-pyridine derivatives. Fifty-eight examples of quinoline derivatives are described in [36], which were tested in the same intracellular Ca^{2+} mobilization assay in CHO cells stable expressing human $GABA_BR1aR2a$ and Gq16. The E_{max} values (%) in the range of 0% for 10 nM GABA alone and 100% for 10 μM GABA alone were from 58 to 124%, the EC_{50} values in the presence of 10 nM GABA varied from 0.33 to 2.2 μM, the GABA shift (= log[EC_{50}(GABA + 10 μM compound)/EC_{50} (GABA alone)]) ranged from -0.7 to -1.2). See the second structure in **Figure 4**. The corresponding biological data for examples 3, 11 and 33 are shown in **Table 2**.

Twenty-one examples of 3-methanesulfonyl-quinolines are presented in [37], which were tested in the same intracellular Ca^{2+} mobilization assay in CHO cells stable expressing human $GABA_BR1aR2a$ and Gq16. The E_{max} values (%) in the range of 0% for 10 nM GABA alone and 100% for 10 μM GABA alone were from 26 to 56%, the EC_{50} values in the presence of 10 nM GABA varied from 0.6 to 2.1 μM, the GABA shift

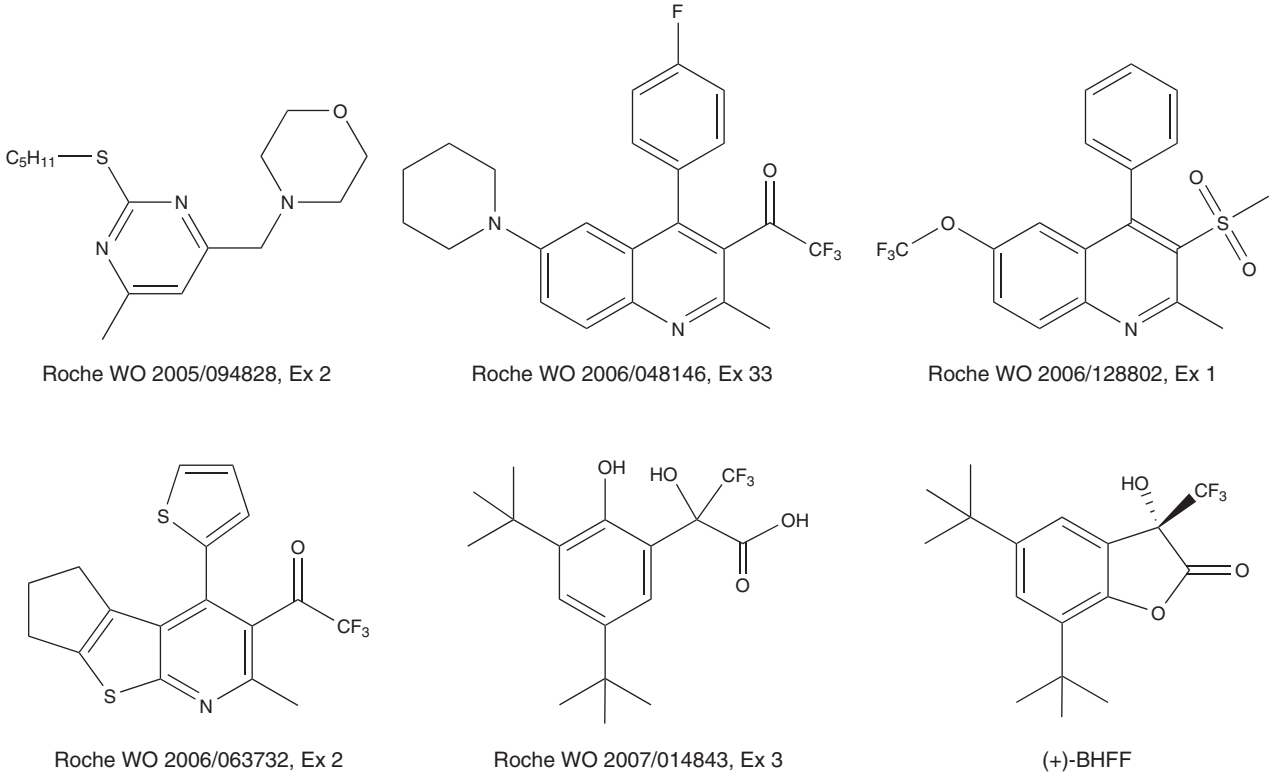


Figure 4. Roche compounds [35-42].

Table 2. Biological data to Roche compounds (see Figure 4).

Example	Intracellular Ca^{2+} mobilization assay in CHO-$GABA_B$R1aR2a-Gα16 cell		GABA shift
	E_{max} (%) at 10 nM GABA alone = 0% 10 μM GABA alone = 100%	EC_{50} (μM) at 10 nM GABA	Log [EC_{50} (GABA + 10 μM cp)/ EC_{50} (GABA alone)]
WO2005/094828			
1	46	2.2	-0.75
2	52	1.7	-0.55
35	62	1.8	-0.92
WO2006/048146			
3	124	0.8	-1.20
11	65	0.8	-1.00
33	62	0.33	-0.90
WO2006/128802			
1	56	0.85	-0.7
9	48	0.6	-1.2
11	26	0.8	-0.8
WO2006/063732			
2	59	1.3	-0.9
16	69	2.4	-0.9
21	66	1.9	-1.1
WO2007/014843			
3	70	0.32	-1
9	62	0.36	-1.3

(= log[EC_{50}(GABA + 10 μM compound)/EC_{50} (GABA alone)]) ranged from -0.45 to -1.2). See the third structure in **Figure 4**. The corresponding biological data for examples 1, 9 and 11 are shown in **Table 2**.

Thieno-pyridine derivatives were also found to have properties of PAMs at $GABA_B$ receptors. Roche scientists described 42 thieno-pyridine compounds in patent [38], which were tested in the same intracellular Ca^{2+} mobilization assay in CHO cells stable expressing human $GABA_B$R1aR2a and Gq16. The E_{max} values (%) in the range of 0% for 10 nM GABA alone and 100% for 10 μM GABA alone were from 26 to 56%, the EC_{50} values in the presence of 10 nM GABA varied from 0.6 to 2.1 μM, the GABA shift (= log [EC_{50}(GABA + 10 μM compound)/EC_{50} (GABA alone)]) ranged from -0.45 to -1.2). See the fourth structure in **Figure 4**. The corresponding biological data for examples 2, 16 and 21 are shown in **Table 2**.

In the fifth Roche patent 13 examples of novel 2-hydroxy-propionic acid and 3-hydroxy-benzofuran-2-one derivatives are described [39], which are optimized compounds of the lead structure CGP7930. See the fifth structure in **Figure 4**. The corresponding biological data for examples 3 and 9 are shown in **Table 2**.

The most potent compound is (+)-BHFF [40] (last structure in **Figure 4**). In the [^{35}S]GTPγS binding assay in CHO cells stably expressing $G_{\alpha16}$-$GABA_{B(1a,2a)}$ receptors the EC_{50} for GABA increased by a factor of 15.3-fold in the presence of 0.3 μM of racemic BHFF reaching an E_{max} of 149%, for (+)-BHFF the EC_{50} for GABA increased by a factor of 87.3-fold in the presence of 0.3 μM of (+)-BHFF reaching an E_{max} of 181%. Racemic BHFF reversed stress-induced hypothermia at doses of 3, 10, 30 and 100 mg/kg in mice. The mean absolute p.o. bioavailability was 100% [40]. The synthesis of racemic BHFF was described in all experimental details in [41]. This compound is also commercially available at Tocris Bioscience.

A very recent report [42] describes the actions of BHFF in the [^{35}S]GTPγS binding to be as strong as the one's of baclofen in different mouse brain regions.

5. AstraZeneca patents

The most extensive effort to discover novel structures of positive modulators of $GABA_B$ receptors has been contributed by scientists from AstraZeneca in ten patents between 2006 and 2009 to find novel drugs for the treatment of gastrointestinal diseases, such as gastroesophageal reflux disease (GERD) and irritable bowel syndrome (IBS).

The potencies of the PAMs were measured in the [^{35}S]GTPγS binding test. Patent WO2006/001750 [43] describes 120 imidazole derivatives. Selected compounds showed EC_{50} values of 0.3 – 2.3 μM. See the first structure in **Figure 5** with an EC_{50} of 0.3 μM.

WO2007/073298 [44] describes six examples of substituted imidazoles, the most potent of which is shown as second structure in **Figure 5** with an EC_{50} of 2.27 μM in the [^{35}S]GTPγS binding test.

WO2007/073299 [45] describes 27 examples of substituted imidazoles, the most potent of which displayed EC_{50} values of 3.68 and 5.54 μM, respectively, in the [^{35}S]GTPγS binding test (structures not shown). WO2007/073300 [46] describes 98 examples of substituted imidazoles, the most potent of which displayed EC_{50} values of 2.47, 5.13 and 2.53 μM, respectively, in the [^{35}S]GTPγS binding test (structures not shown). WO2008/130313 [47] describes 20 examples of substituted imidazoles, the most potent of which displayed an EC_{50} of 0.81 μM in the [^{35}S]GTPγS binding test (third structure in **Figure 5**).

WO2007/073296 [48] describes 37 examples of substituted pyrazoles, oxazoles, thiazoles, the most potent of which are shown as fourth and fifth structure in **Figure 5** with EC_{50} values of 1.96 and 7.23 μM, respectively, in the [^{35}S]GTPγS binding test. WO2007/073297 [49] describes three examples of substituted pyrazoles, of which Example 3 is shown as last structure in **Figure 5**. No EC_{50} value in the [^{35}S]GTPγS binding test was communicated in this patent.

WO2009/041904 [50] describes 25 examples of substituted quinolines, the most potent of which is shown in **Figure 6** with an EC_{50} value of 0.3 μM (mean agonist potencies) in the [^{35}S]GTPγS binding test. These compounds are close follow-up structures of the Roche patents [36-38].

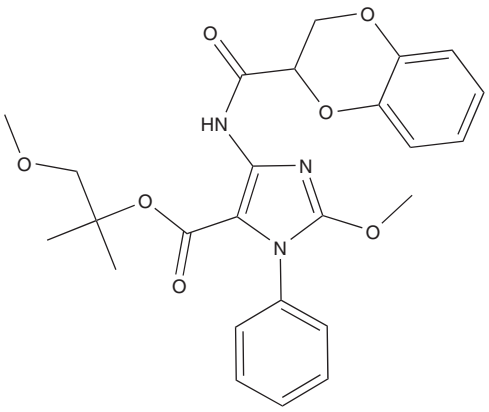
WO2008/130314 [51] describes 103 examples of substituted xanthines, the most potent of which is shown in **Figure 6** with an EC_{50} of 0.6 μM in the [^{35}S]GTPγS binding test. WO2009/041905 [52] describes 20 examples of substituted pteridines, the most potent of which is shown in **Figure 6** with EC_{50} of 0.6 μM in the [^{35}S]GTPγS binding test.

6. Johann Wolfgang Goethe University Frankfurt/main patent

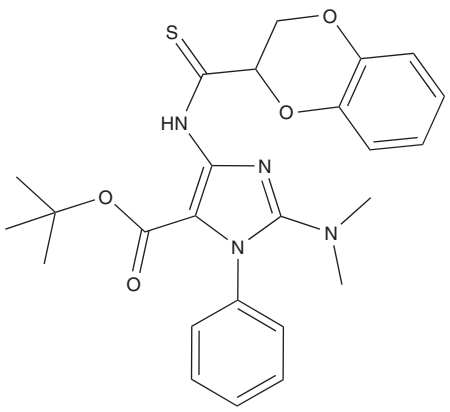
The patent [53] claims that $GABA_B$ receptor agonists and PAMs are useful for the treatment of inflammatory skin diseases. Particularly claimed are baclofen, CGP44532 (both full $GABA_B$ receptor agonists) and CGP7930, CGP13501 and GS39783.

7. Conclusion

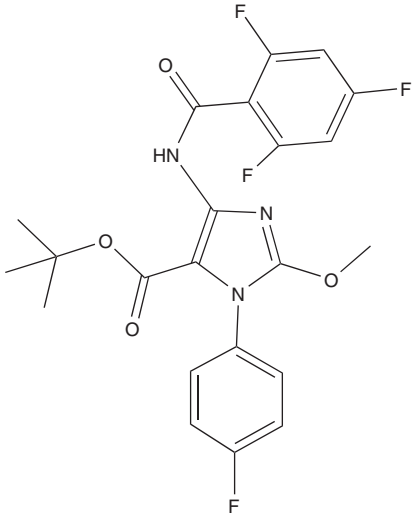
Since the humble beginnings in 2001 and 2003 with the publications of the properties of CGP7930 and GS39783 found by high throughput screening at Novartis [3,13] significant progress has been achieved by medicinal chemists of Roche, AstraZeneca and Addex (in chronological order of the publishing dates of their patents) discovering additional novel scaffolds of PAMs of $GABA_B$ receptors with respectable EC_{50} values. However, it is still early days in order to bring optimized compounds into development, whose requirements are getting more severe every year.



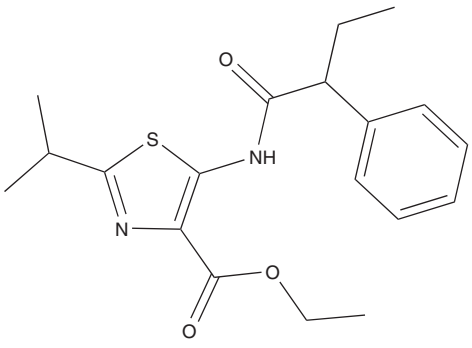
AstraZeneca WO 2006/001750
page 70, EC_{50} = 0.3 μM



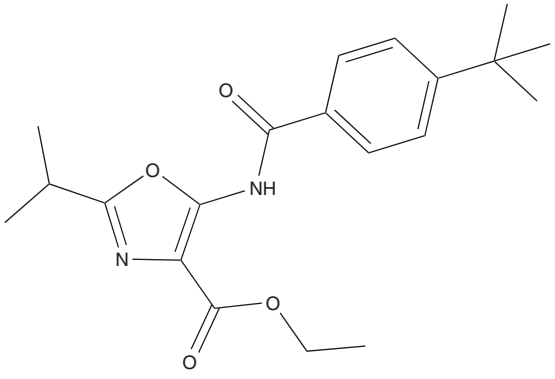
AstraZeneca WO 2007/073298
Ex 6, EC_{50} = 2.27 μM



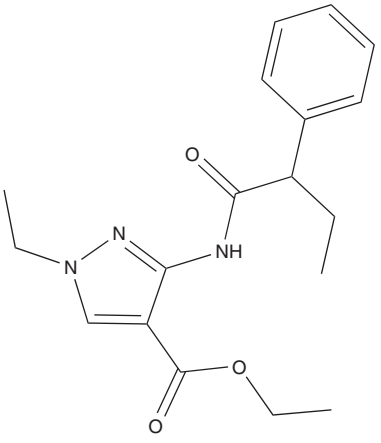
AstraZeneca WO 2008/130313
Ex 8, EC_{50} = 0.81 μM



AstraZeneca WO 2007/073296
Ex 16, EC_{50} = 1.96 μM



AstraZeneca WO 2007/073296
Ex 21, EC_{50} = 7.23 μM



AstraZeneca WO 2007/073297
Ex 3, no EC_{50}

Figure 5. AstraZeneca compounds (1). Five membered rings [43-49].

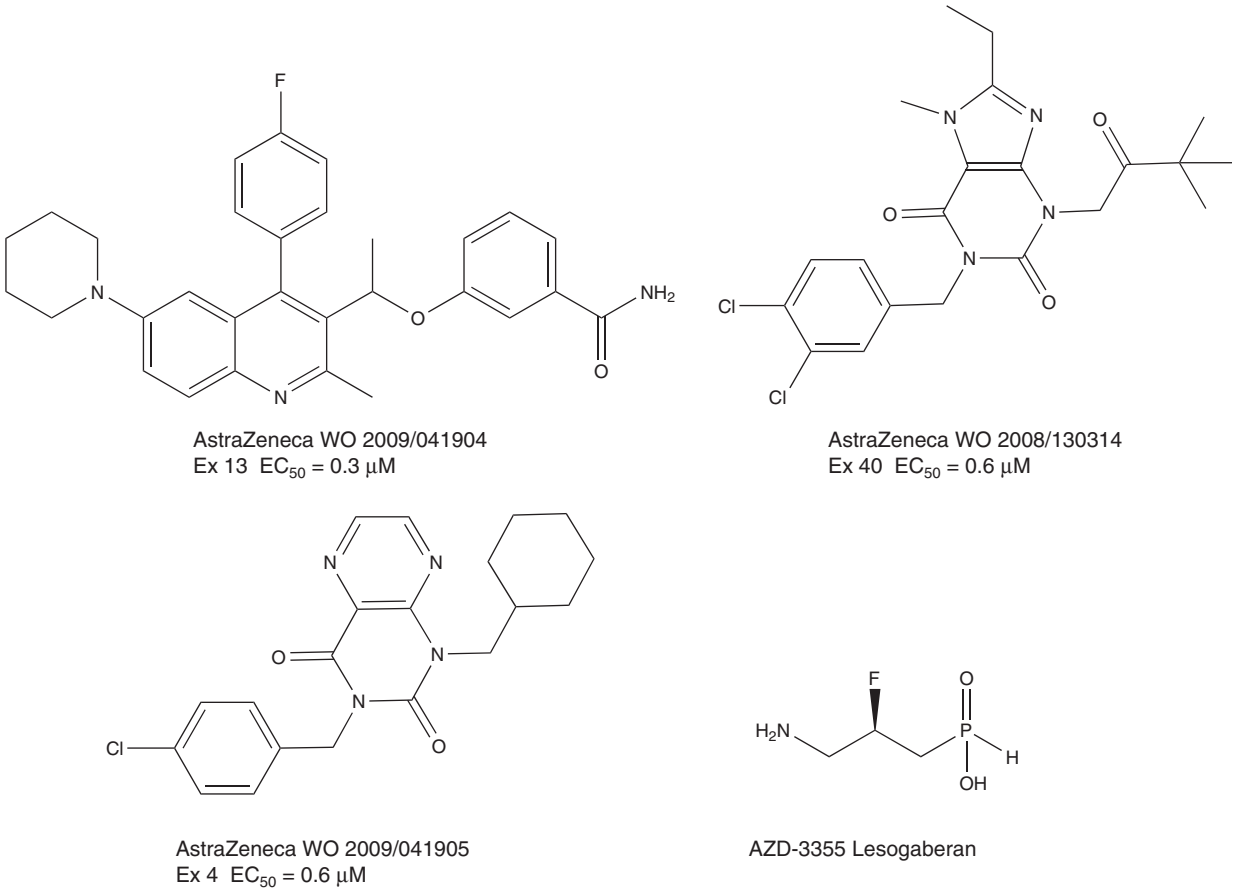


Figure 6. AstraZeneca compounds (2). Six membered rings [50-52] and Lesogaberan [57-59].

8. Expert opinion

Allosteric modulators act more physiologically than orthosteric ligands, targeting only endogenously activated receptors and not their whole population, which is why they are expected to produce less side effects and tolerance [9,54]. Experiments on desensitization were carried out in a recombinant GABA$_B$ receptor cell line. The potency of GABA to inhibit 7β-forskolin induced cAMP formation decreased after exposure to a saturating GABA concentration, but not after a combination of GABA and GS39783 that activated the receptor to the same extent. A significant decrease of cell surface receptors was found after GABA-induced desensitization unlike after the combined treatment with GABA and GS39783 ([54], see also [55,56]).

Four companies have invested substantial resources into the search of PAMs of GABA$_B$ receptors, Novartis, Roche, AstraZeneca and Addex (in chronological order of the publishing dates of their patents). However, the target indications of the four companies are significantly different. Novartis looked for novel anxiolytic drugs and, in collaboration with NIH, for drugs for the treatment of nicotine addiction, optionally also for alcohol and cocaine addiction. Addex found valuable drugs for the treatment of osteoarthritis pain and for chronic nociceptive pain. Roche characterized valuable novel anxiolytic drugs, whereas AstraZeneca concentrated on drugs for the treatment of GERD as follow-up compounds of their GABA$_B$ receptor full agonist AZD-3355 (Lesogaberan; see the last formula in **Figure 6**) currently the only drug interacting with GABA receptors in Phase II clinical trials [57-59].

These medicinal chemistry efforts have substantially enlarged the number of scaffolds, which lead to potent compounds and thus expanded our knowledge on structure–activity relationships significantly. It will be easier to find potent PAMs with this background. Nevertheless, it still will require substantial efforts to optimize drugs for a given indication to get all the ADMET parameters right as well, such as metabolic stability, brain penetration for the CNS indications (or no brain penetration for the peripheral indications), interactions with P-glycoprotein, cytochrome P450 enzymes, and hERG channels, sufficient water solubility, no alerts in Ames and genotoxicity tests, and several other parameters.

Addex plans to put their most advanced compound ADX-71943 into clinical trials in 2010 for the treatment of

pain, an indication with a very high medical need. At present there is no information available concerning potential development compounds of AstraZeneca, Novartis or Roche in this field.

Whether in the long term PAMs at $GABA_B$ receptors will be as successful as the PAMs at $GABA_A$ receptors, the benzodiazepines discovered two generations ago in 1957 [60], remains to be seen.

Declaration of interest

The author is an employee of AC Immune SA.

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