Chapter 3
The Allosteric Modulation of the $GABA_B$ Receptor: A Medicinal Chemistry Perspective

Claudia Mugnaini and Federico Corelli

Abstract Since its cloning, the $GABA_B$ receptor has progressively become a target for potential drugs to be used in the treatment of a wide range of pathological conditions such as spasticity, pain, drug addiction, epilepsy, anxiety, mood disorders. Baclofen, the only $GABA_B$ receptor agonist currently approved for the treatment of muscle rigidity and spasm associated with multiple sclerosis or spinal cord injury, suffers from a number of side effects which hamper its clinical use. As a result, there has been a strong impetus for the development of positive allosteric modulators that modulate the physiological mechanisms of GABAergic regulation and are expected to have a much lower side effect potential than orthosteric ligands. Herein, the major structural classes of $GABA_B$ allosteric modulators are described with an emphasis on structure–activity relationships (SAR) and synthesis of the main representatives of each class. Medicinal chemistry strategies to overcome issues related to allosteric modulators development are also discussed.

Keywords $GABA_B$ positive allosteric modulators • $GABA_B$ NAMs • SAR • Baclofen • Spasticity • Drug addiction

3.1 Introduction

γ-Aminobutyric acid (GABA) (Fig. 3.1) is the most important and abundant inhibitory neurotransmitter in the mammalian brain (Krnjević and Schwartz 1967). It is a small, neutral amino acid characterized by high hydrophilicity and water solubility which exerts its function via ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors.

The $GABA_B$ receptor was cloned in 1997 (Kaupmann et al. 1997), 17 years after its identification (Bowery et al. 1980) and 35 years after the first synthesis of baclofen (Keberle et al. 1969) (Fig. 3.1), a lipophilic GABA derivative endowed

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G. Colombo (ed.), *$GABA_B$ Receptor*, The Receptors 29,
DOI 10.1007/978-3-319-46044-4_3

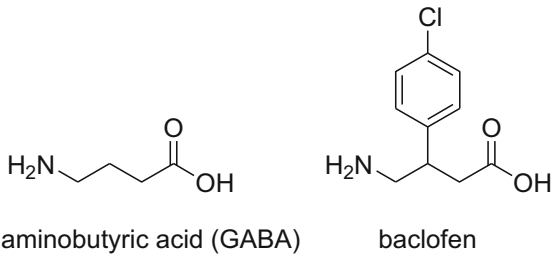


Fig. 3.1 Chemical structure of γ-aminobutyric acid (GABA) and baclofen

with high affinity and strong intrinsic activity for this receptor (see Chaps. 1 and 17 of this book). Since then, $GABA_B$ receptor has become an intriguing target for developing orthosteric as well as allosteric ligands as potential drugs for the treatment of a wide range of pathological conditions.

The structure and function of $GABA_B$ receptors are covered extensively in Chaps. 4 and 6 of this book. $GABA_B$ receptors have been identified not only in the central nervous system but also in the peripheral nervous system and organs (see Chap. 5 of this book). They are involved in several physiological and pathophysiological events, such as spasticity, pain, cognitive function, anxiety, mood disorders, epilepsy, and drug addiction (Benarroch 2012; Froestl 2010a; Balerio and Rubio 2002; Kumar et al. 2013; Philips and Reed 2014). A growing body of evidence showed that they are also involved in tumor development and tumor cell proliferation and migration, in accordance to the observation that neurotransmitters have modulatory roles in tumor cells (Jiang et al. 2012).

Baclofen (Fig. 3.1) is the only $GABA_B$ receptor agonist currently approved for clinical use in the treatment of muscle rigidity and spasm associated with multiple sclerosis or spinal cord injury (Sachais et al. 1977; for details about the clinical use of baclofen, see Froestl 2010) and it has also shown efficacy toward overactive bladder (Abraham and Goldman 2015), gastroesophageal reflux disease (Lehmann et al. 2010), addiction disorders (Agabio and Colombo 2014; Haney et al. 2006), and anxiety (Cryan and Kaupmann 2005) (see also Chap. 2 of this book). Some data are also available describing baclofen able to reduce the incidence of some carcinogen-induced gastrointestinal cancers in rats (Tatsuta et al. 1990) as well as human hepatocarcinoma cell growth (Wang et al. 2008) (see also Chaps. 10–17 of this book). However, the clinical use of baclofen is hampered by a number of side effects including sedation, dizziness, nausea, muscle weakness, and mental confusion which appear when the drug, owing to its poor brain penetration, is administered in high doses. This has been largely overcome, in the treatment of spasticity, by the introduction of intrathecal administration. Nevertheless, the focal injection of the agonist cannot be used in the treatment of pathological states such as drug addiction, pain, depression, and anxiety.

This consciousness has provided a strong impetus for the development of positive allosteric modulators that potentiate $GABA_B$ receptor-mediated actions. In contrast to baclofen and other $GABA_B$ agonists, that activate constantly and everywhere the receptor, positive allosteric modulators are indeed expected to modulate the

physiological mechanisms of GABAergic regulation, enhancing receptor activity only when and where needed, that is when and where GABA is released to act on the $GABA_B$ receptor. On the basis of their mechanism of action, positive allosteric modulators are expected to have a much lower side effect potential than orthosteric ligands (see Chap. 18 of this book).

This chapter will focus exclusively on allosteric modulators of $GABA_B$ receptor with an effort to group known ligands according to their chemical structure. This approach is expected to help medicinal chemists working in the field to explore the chemical space around *old* scaffolds and eventually to design *new* ones on the basis of established structure–activity relationships (SAR).

3.2 Positive Allosteric Modulators of $GABA_B$ Receptors

3.2.1 CGP7930 and CGP13501

The first $GABA_B$ receptor positive allosteric modulators were discovered by Urwyler and colleagues at the Novartis Institute for BioMedical Research in Basel in 2001, by means of a high-throughput screening (Urwyler et al. 2001). Compound CGP7930, structurally close to the general anesthetic agent propofol, and its aldehyde analog CGP13501 (Fig. 3.2) potentiated GABA-induced signals at low micromolar concentrations without stimulating [^{35}S]GTPγS binding in the absence of GABA (Urwyler et al. 2001; Adams and Lawrence 2007).

CGP7930 increased both agonist potency and maximal effect, which was shown to be dose dependent. Only in 2004, Pin and colleagues, using a more sensitive assay, demonstrated that CGP7930 acts as a partial $GABA_B$ receptor agonist, and thus can be classified as an ago-allosteric modulator. Moreover, they were able to localize its binding site on the heptahelical domain of the $GABA_{B2}$ subunit (Binet et al. 2004; Pin and Prézeau 2007). Besides the ability to lower the drug-seeking behavior with substances such as alcohol, nicotine, and cocaine, CGP7930 showed in vivo antidepressant and anxiolytic properties.

The synthesis of CGP13501 and CGP7930 was accomplished starting from propofol (Scheme 3.1), which on treatment with formaldehyde in the presence of base

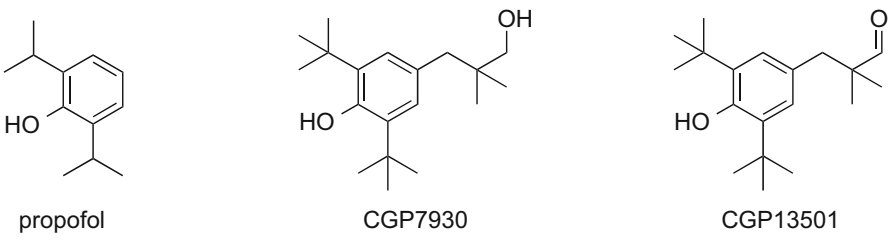
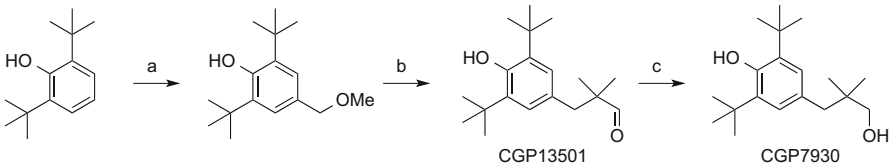


Fig. 3.2 Chemical structure of CGP7930 and CGP13501



Scheme 3.1 Reagents and conditions: (*a*) CH_2O, KOH, MeOH, reflux; (*b*) 2-methylpropanal, KOH, MeOH, 65 °C; (*c*) $NaBH_4$, EtOH, reflux

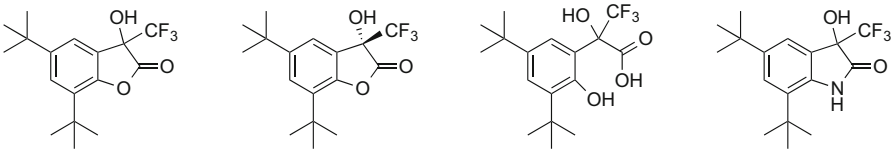


Fig. 3.3 Chemical structure of *rac*-BHFF and (+)-BHFF

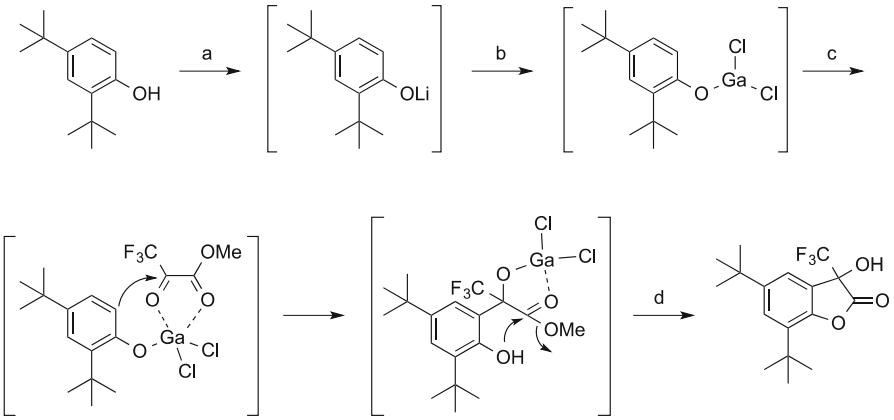
was converted into the corresponding 4-methoxymethyl derivative (Kharasch and Joshi 1957). Subsequent reaction with 2-methylpropanal gave CGP13501, which was then reduced in almost quantitative yield to CGP7930 (Kerr et al. 2006).

3.2.2 rac-*BHFF*

Scientists at Hoffmann-La Roche took up the structure of CGP7930 and elaborated it further, synthesizing novel fluorinated 2-hydroxypropionic acid derivatives and their lactone analogs 3-hydroxybenzofuran-2-ones *rac*-BHFF and (+)-BHFF (Fig. 3.3) (Malherbe et al. 2007; Alker et al. 2008).

rac-BHFF increased the potency and the efficacy with which GABA stimulated [^{35}S]GTPγS binding to membrane preparations from cells enhancing, at 0.3 μM, the EC_{50} of GABA in recombinant cells more than 15-fold. The most potent compound (+)-BHFF, which represents the first example of stereoselectivity at the $GABA_B$ allosteric binding site, increased this value by a factor of 87-fold. During pharmacokinetic studies in mouse plasma after p.o. administration of *rac*-BHFF, only the hydrolyzed form of BHFHP could be measured because quantitative hydrolysis of *rac*-BHFF under the analytical conditions occurred. As *rac*-BHFF and its hydroxy-acid BHFHP proved to be equipotent as enhancers at $GABA_B$ receptor, it was assumed that both compounds may be present in vivo because of rapid interconversion and may contribute to the observed effects in vivo. Isosteric replacement of oxygen atom by a NH group led to a hydrolytically stable lactam analog of BHFF, encoded BHFI (Malherbe et al. 2008).

In spite of the challenging structure of *rac*-BHFF, showing a benzofuran ring crowded with diverse functional groups, its synthesis could be efficiently performed



Scheme 3.2 Reagents and conditions: (*a*) *n*-BuLi, THF, −70 to 20 °C; (*b*) $GaCl_3$, DCE, −10 to 80 °C; (*c*) CF_3COCO_2Me, DCE, 0–20 °C; (*d*) DCE, 28–80 °C

through a gallium(III) chloride-mediated one-pot procedure (Scheme 3.2). Thus, the gallium phenolate obtained from 2,4-di-*tert*-butylphenol was reacted with the highly electrophilic methyl trifluoropyruvate to give regiospecifically an aldol-type intermediate, which subsequently underwent lactonization to *rac*-BHFF. The racemic compound was resolved into the two enantiomers by chiral-phase HPLC and the *R* and *S* configuration could be assigned to the dextrorotatory and levorotatory isomers, respectively, on the basis of X-ray crystallographic analysis of a suitable derivative (Alker et al. 2008).

3.2.3 Arylalkylamines

A variety of phenylalkylamines, including fendiline, a coronary vasodilator, are potent allosteric modulators at extracellular Ca^{2+}-sensing receptors. In 2002 three arylalkylamines (fendiline, prenylamine, and F551) were reported by Kerr and Ong as new $GABA_B$ receptor positive allosteric modulators (Kerr et al. 2002). These compounds showed little or no hyperpolarizing response in the absence of baclofen, but potentiated responses to baclofen and produced a leftward shift of the baclofen concentration–response curve, with a marked increase in the maximal hyperpolarization obtained with baclofen alone, indicative of positive allosteric modulation at $GABA_B$ receptors. Nevertheless, in 2004 Urwyler et al. demonstrated that these compounds are not allosteric modulators of $GABA_B$ receptors. They proposed, instead, that these phenylalkylamines could act at distinct sites in the complex circuitry in brain tissue slice preparations, possibly even at different cells ("downstream effects") or, alternatively, at the effector level. In fact, many examples of "receptor crosstalk" have been documented (Urwyler et al. 2004).

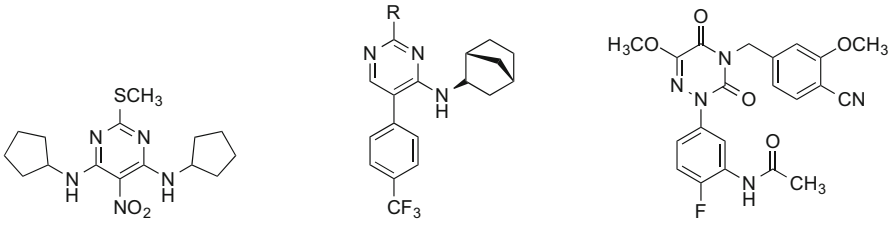
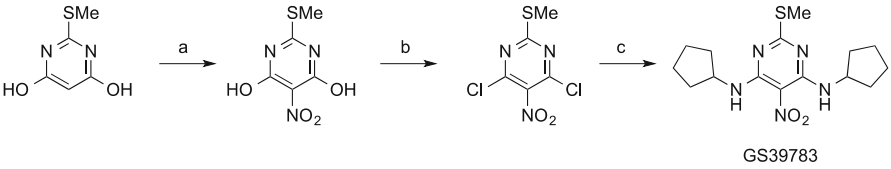


Fig. 3.4 Chemical structure of pyrimidines and related six-membered heterocyclic analogs



Scheme 3.3 Reagents and conditions: (*a*) HNO_3, H_2SO_4, 0–5 °C; (*b*) $POCl_3$, *N*,*N*-diethylaniline, reflux; (*c*) cyclopentylamine, EtOH, reflux

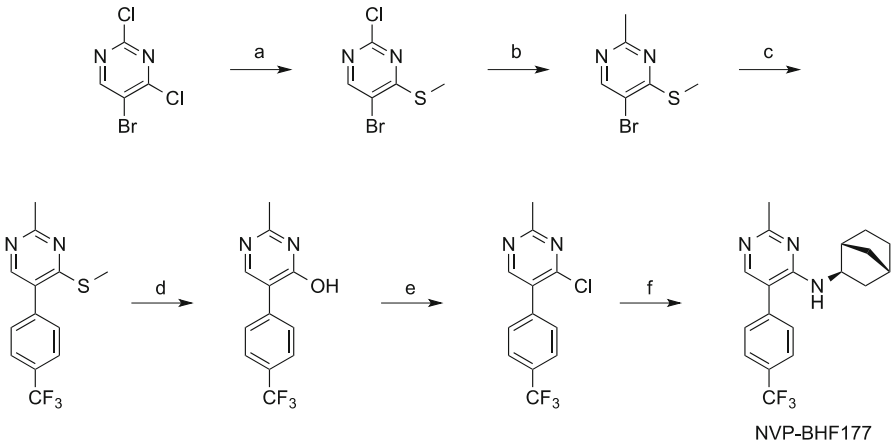
3.2.4 Pyrimidines and Related Six-Membered Heterocyclic Analogs

In 2003, GS39783 (Fig. 3.4) and structurally related compounds were described as novel and more potent positive allosteric modulators of the $GABA_B$ receptor (Urwyler et al. 2003).

GS39783 was synthesized in a straightforward manner (Scheme 3.3) by nitration of 2-methylthio-4,6-pyrimidinedione, followed by chlorination and displacement of both chlorine atoms with an excess of cyclopentylamine (Fisher 1972).

Like CGP7930, GS39783 acted through a dual mechanism, by enhancing at the same time the affinity and the maximal efficacy of GABA by approximately eight-fold and twofold, respectively. It showed anxiolytic-like effects in the elevated plus maze in rats and the elevated zero maze in mice and rats (Cryan et al. 2004) and decreased anxiety in the light–dark box, but it did not show any effects in the forced swim test (Mombereau et al. 2004) (see also Chaps. 12 and 18 of this book). Moreover, it reduced alcohol self-administration in alcohol-preferring rats (Orrù et al. 2005; Maccioni et al. 2012) and attenuated the reward facilitating effects of cocaine (Slattery et al. 2005) and nicotine (Paterson et al. 2008) in rats (see also Chaps. 14 and 15 of this book). Through point mutations, Novartis scientists were able to locate precisely its binding site in the sixth transmembrane domain of the $GABA_{B2}$ receptor (Dupuis et al. 2006).

In order to obtain molecules devoid of genotoxicity, the chemical structure of GS39783 was further elaborated by synthesizing a number of trisubstituted



Scheme 3.4 Reagents and conditions: (*a*) MeSNa, THF, rt; (*b*) i. HI 57 %, rt; ii. MeZnCl, $Pd(PPh_3)_4$, THF, rt to 60 °C; (*c*) 4-(trifluoromethyl)benzeneboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, EtOH, toluene, water, 110 °C; (*d*) HCl 37 %, MeOH, reflux; (*e*) $POCl_3$, cat. DMF, 80 °C; (*f*) *exo*-2-aminonorbornane, THF, 80 °C

pyrimidine derivatives in which the nitro group was replaced by a 4-trifluoromethylphenyl group and one of the two cyclopentylamino moieties was removed (Fig. 3.3) (Floersheim et al. 2006). As a means to introduce molecular diversity at position 4 of the pyrimidine ring at a late stage of the synthesis, a seven-step procedure was developed, starting from the commercially available 5-bromo-2,4-dichloropyrimidine (Scheme 3.4).

Treatment of this trihalogenated pyrimidine with sodium methanethiolate in THF at room temperature resulted in the regioselective displacement of the chlorine atom in position 4, leading to 5-bromo-2-chloro-4-methylthiopyrimidine. This was subjected to halogen exchange to give the corresponding 2-iodo derivative that in turn underwent a Negishi cross coupling with methylzinc chloride to afford the 2-methylpyrimidine intermediate, followed by reaction with 4-trifluoromethylbenzeneboronic acid under the classical conditions of the Suzuki–Miyaura reaction. In order to enhance its reactivity toward nucleophiles, this intermediate was first oxidized to the methanesulfonyl analog, which, however, reacted cleanly with only few amines. Therefore, the methylthio derivative was hydrolyzed to the 4-hydroxypyrimidine (4-pyrimidinone) derivative, which was then transformed into the corresponding 4-chloro compound. This key intermediate was reacted with a variety of amines to yield a small library of 4-aminosubstituted compounds (Guery et al. 2007). Among them, NVP-BHF177, bearing a methyl group at the 2-position and the *exo*-2-norbornanylamino group at the 4-position of the pyrimidine ring, proved to be devoid of in vitro genotoxicity and showed antinicotine and antialcohol effects as well as anxiolytic properties in the mouse stress-induced hyperthermia test (Paterson et al. 2008; Maccioni et al. 2009; Vlachou et al. 2011; Li et al. 2015).

In 2008, Addex Therapeutics patented more than 300 novel triazinedione derivatives identified from a high-throughput screening campaign of their corporate chemical library followed by lead optimization process (Riguet et al. 2008).

Among all the compounds, which were evaluated in a [^{35}S]GTPγS binding assay in rat cortical membranes, 23 compounds displaying $EC_{50} < 100$ nM were selected for additional evaluation in different animal models of anxiety and pain. ADX71943 (Fig. 3.4), a potent and selective $GABA_B$ receptor positive allosteric modulator endowed with a peripheral mode of action, was initially chosen for preclinical development, but due to its inadequate safety profile, it was further characterized as a pharmacological tool compound. ADX71943 showed consistent and target-related efficacy in tests of disorders that have a significant peripheral component (acute and chronic pain), while having no effect in those associated with centrally mediated anxiety-like reactivity and side effects (Kalinichev et al. 2014a). ADX71441, a triazinedione whose exact structure has not been disclosed so far, demonstrated excellent preclinical efficacy and tolerability in several rodent models of pain, anxiety, addiction (Hwa et al. 2014), and overactive bladder (Kalinichev et al. 2014b) and has also proven efficacy in a genetic model of Charcot–Marie–Tooth Type 1A disease (see also Chap. 18 of this book). It is therefore in the Addex pipeline as a phase 1 clinical candidate.

3.2.5 Quinolines and Bicyclic Congeners

Quinolines **1** (Fig. 3.5) (Malherbe et al. 2006a), 3-methanesulfonylquinolines **2** (Malherbe et al. 2006b), and thienopyridine **3** (Malherbe et al. 2006c) are representative molecules of new structural classes presented by Hoffmann-La Roche in 2006 as $GABA_B$ receptor positive allosteric modulators potentially useful in the treatment of CNS disorders.

Three years later, AstraZeneca presented a number of substituted quinolines, structurally correlated to those described by Hoffmann-La Roche (i.e., compound **4**, Fig. 3.5) (Cheng and Karle 2009), more than 100 substituted xanthines (i.e., compound **5**) (Cheng et al. 2008), and some examples of substituted pteridines (i.e., compound **6**) (Cheng et al. 2009). All of these compounds showed EC_{50}values in the submicromolar range in the [^{35}S]GTPγS binding test and were potentially useful in the treatment of gastroesophageal disease (GERD), irritable bowel syndrome (IBS), and other gastrointestinal pathological states.

The synthesis of representative compounds **1**, **5**, and **6** is described in Schemes 3.5, 3.6, and 3.7, respectively. Starting from 4-bromoaniline, the suitably substituted benzophenone derivative was prepared and submitted to a Friedländer reaction for the construction of the quinoline ring. Substitution of the bromine atom of the quinoline by piperidine in a Pd-catalyzed amination reaction gave compound **1** (Scheme 3.5).

For the synthesis of **5**, the key intermediate ethyl 4-amino-2-ethyl-1-methylimidazole-5-carboxylate was prepared through a five-step procedure from propionitrile and then converted into a ureido derivative by acylation of its amino group with 3,4-dichlorobenzylisocyanate. Sodium methoxide-catalyzed intramolecular amidation allowed to install the xanthine ring, which was in turn alkylated with 1-bromopinacolone to yield **5** (Scheme 3.6).

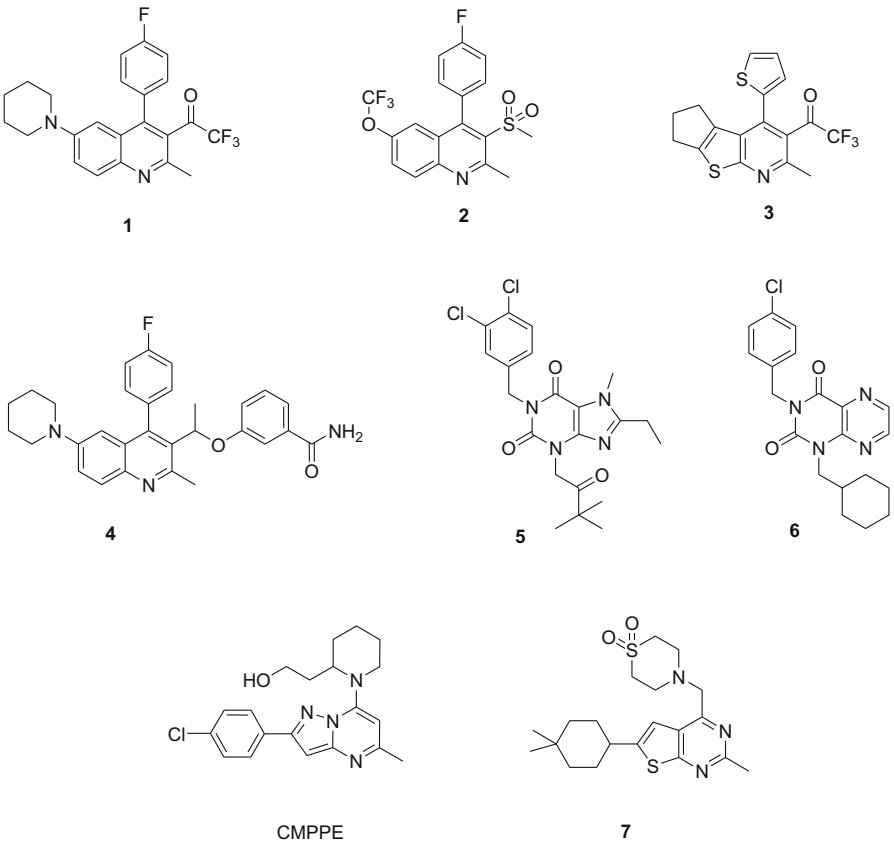
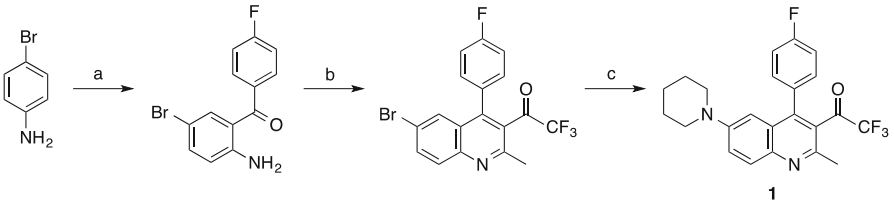


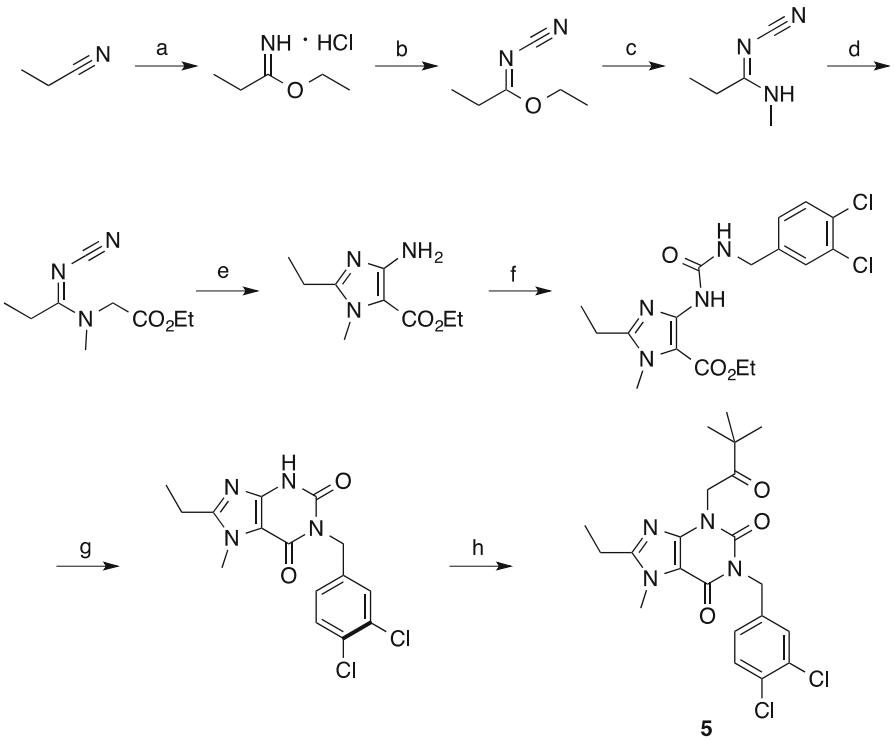
Fig. 3.5 Chemical structures of quinolines and bicylic congeners



Scheme 3.5 Reagents and conditions: (*a*) 4-fluorobenzonitrile, $AlCl_3$, BCl_3, DCE, reflux; (*b*) 1,1,1-trifluoro-2,4-pentanedione, $NaAuCl_4$, 2-propanol, reflux; (*c*) $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, *t*-BuOH, 120 °C

The pteridine derivative **6** was synthesized from methyl 2-aminopyrazine-3-carboxylate (Scheme 3.7) according to a procedure set up to enhance molecular diversity in position 1.

To this end, protection/deprotection steps were necessary in order to control the regiochemistry of the *N*-alkylation reactions. Thus, the starting compound was

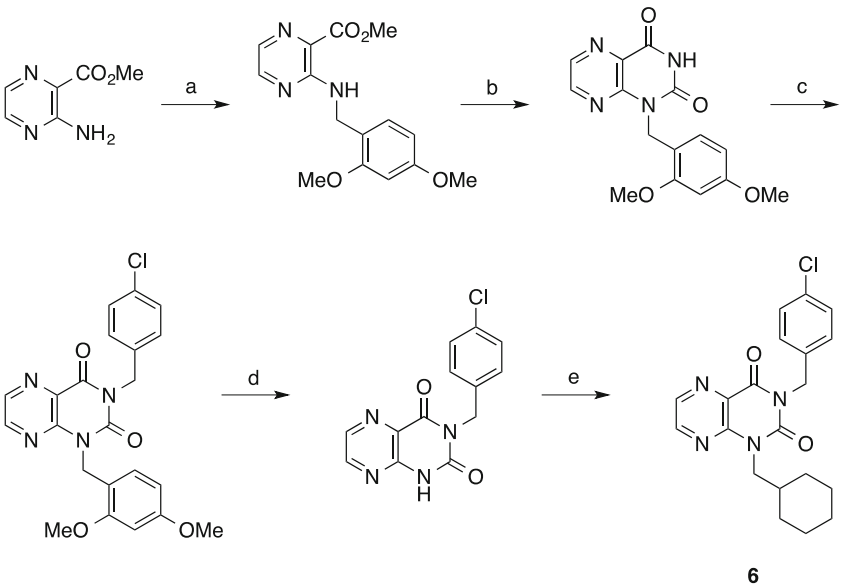


Scheme 3.6 Reagents and conditions: (*a*) HCl(g), EtOH; (*b*) cyanamide, K_2HPO_4, H_2O, 0 °C; (*c*) methylamine, EtOH, reflux; (*d*) ethyl bromoacetate, TBAI, DMF, rt; (*e*) MeONa, EtOH, reflux; (*f*) 3,4-dichlorobenzyl isocyanate, toluene, MW, 120 °C; (*g*) MeONa, MeOH, 100 °C; (*h*) 1-bromopinacolone, K_2CO_3, DMF, rt

subjected to reductive alkylation with 2,4-dimethoxybenzaldehyde and the product obtained was cyclized by treatment with trichloroacetylisocyanate, followed by cleavage of the trichloroacetyl group by sodium methoxide. Removal of the dimethoxybenzyl protecting group with trifluoroacetic acid led to a pteridine intermediate with a free NH group at 1-position, which could be alkylated with a variety of electrophiles to generate a library of pteridine derivatives. In particular, reaction with (bromomethyl)cyclohexane provided compound **6**.

In 2011, GlaxoSmithKline reported on the discovery of the new $GABA_B$ receptor positive allosteric modulator, CMPPE (Fig. 3.5), a pyrazolo[1,5-*a*]pyrimidine derivative identified by screening the GSK compound collection using the [^{35}S] GTPγS-binding assay (Perdonà et al. 2011). CMPPE was fully profiled in vitro and in rat models of food intake and locomotor activity, demonstrating its involvement in the regulation of food consumption without impairment on the animal locomotor activity.

In 2015 Astellas Pharma patented novel thieno[2,3-*d*]pyridine derivatives (i.e. compound **7**) for the prevention/treatment of diseases such as schizophrenia, cognitive disorder, pain (Shiraishi et al. 2015).



Scheme 3.7 Reagents and conditions: (*a*) 2,4-dimethoxybenzaldehyde, $NaBH(OAc)_3$, DCE, rt; (*b*) i. trichloroacetylisocyanate, DCM, rt; ii. MeONa, MeOH, 60 °C; (*c*) 4-chlorobenzyl chloride, K_2CO_3, DMF, rt; (*d*) trifluoroacetic acid, DCM, 100 °C; (*e*) (bromomethyl)cyclohexane, K_2CO_3, DMF, rt

3.2.6 Five-Membered Heterocyclic Amides

In seven patents between 2006 and 2008, AstraZeneca described approximately 200 molecules characterized by a five-membered heterocyclic scaffold functionalized with amide and ester groups. Examples of these compounds are reported in Fig. 3.6: imidazoles (i.e., compounds **8** and **9**) (Bauer et al. 2006, 2007a, b, c, 2008), pyrazoles (i.e., compound **10**) (Bauer 2007), oxazoles (i.e., compound **11**), thiazoles (i.e., compound **12**) (Bauer et al. 2007d).

The compounds were characterized by the [^{35}S]GTPγS-binding test and were proposed as new drug candidates for the treatment of different pathologies, such as GERD and IBS.

The 2-methoxyimidazole compound **9** (Scheme 3.8) was prepared by acylation with 2,4,6-trifluorobenzoyl chloride of the key intermediate *tert*-butyl 4-amino-2-methoxy-1-(4-fluorophenyl)-1*H*-imidazole-5-carboxylate. This was synthesized from dimethylcyanodithioimidocarbonate in three steps entailing: (i) the substitution of one methylthio group by 4-fluoroaniline, (ii) alkylation of the NH group by *tert*-butyl bromoacetate, (iii) substitution of the remaining methylthio group with a methoxy group and concurrent intramolecular *C*-acylation to generate the imidazole ring.

The synthesis of compound **10** was accomplished in an easy and fast way (Scheme 3.9) by alkylation with ethyl iodide of the commercially available ethyl

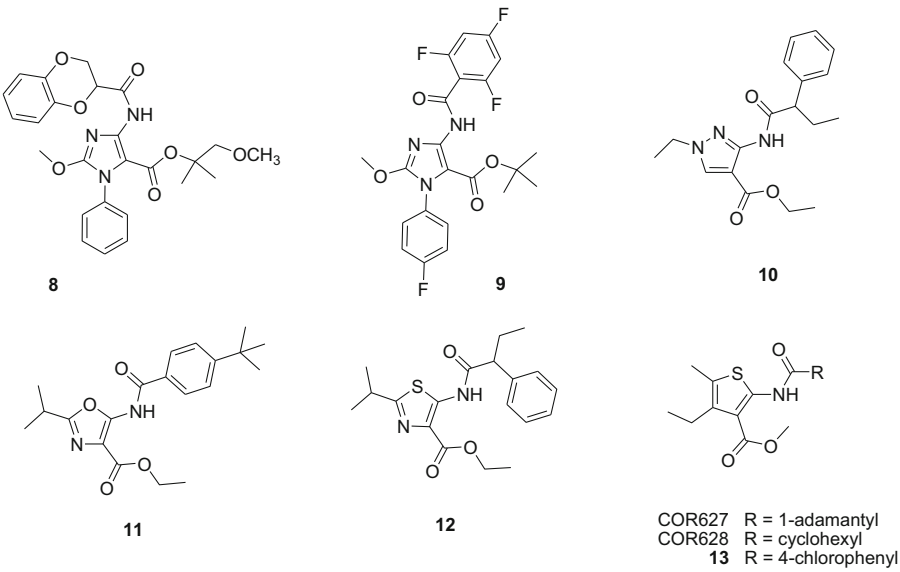
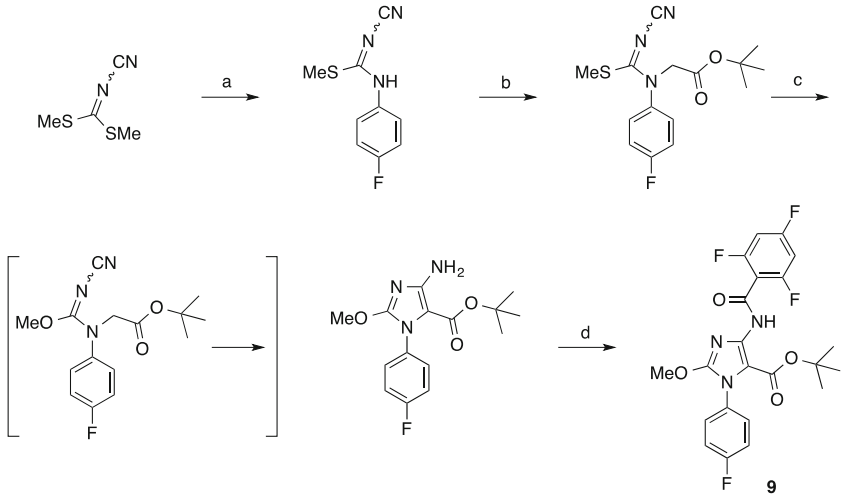


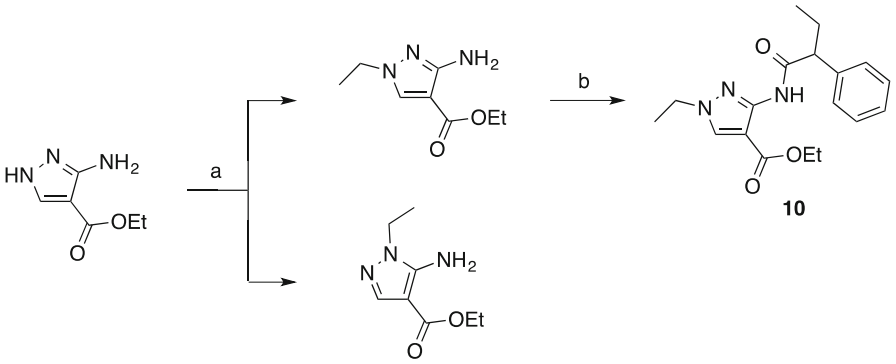
Fig. 3.6 Chemical structure of five-membered heterocyclic amides



Scheme 3.8 Reagents and conditions: (*a*) 4-fluoroaniline, EtOH, reflux; (*b*) *tert*-butyl bromoacetate, K_2CO_3; (*c*) MeONa; (*d*) 2,4,6-trifluorobenzoyl chloride, PS-DIPEA, rt to 50 °C

3-amino-1*H*-pyrazole-4-carboxylate to give the alkylated derivative as a mixture of isomers, which was used without separation in the subsequent acylation step with 2-phenylbutyryl chloride. Purification of the reaction mixture afforded the expected amido derivative **10**.

Compounds **11** and **12** were prepared from the common intermediate ethyl 2-[(2-methyl)propionylamino]cyanoacetate (Scheme 3.10) in turn obtained by



Scheme 3.9 Reagents and conditions: (*a*) EtI, NaH, acetonitrile, rt; (*b*) 2-phenylbutyryl chloride, TEA, THF, rt

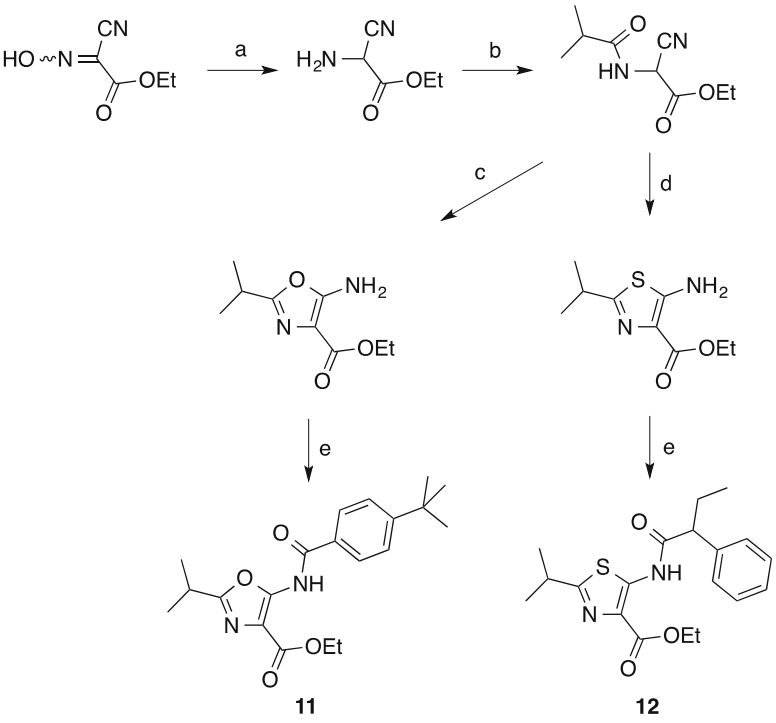
reduction and subsequent acylation of easily available ethyl 2-(hydroxyimino)cyanoacetate. Treatment of the common intermediate with hydrogen chloride or Lawesson's reagent led to ethyl 5-amino-4-ethoxycarbonyl-2-isopropyl-oxazole or thiazole, respectively, which were then converted into the desired amides by acylation of the amino group with the appropriate acyl chloride.

An extensive survey of 19 patents of the different scaffolds for positive allosteric modulators of $GABA_B$ receptors and of the major players in the field was published by Froestl (2010b). This paper gives a relevant overview of several indications that can be addressed with $GABA_B$ positive allosteric modulators.

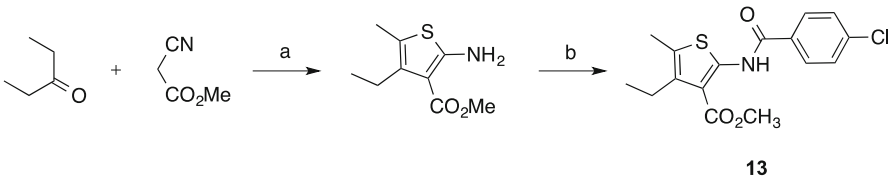
In 2012, Corelli and coworkers identified, by means of a virtual screening protocol, two new 2-(acylamino)thiophene derivatives, referred to as COR627 and COR628 (Fig. 3.6), as novel $GABA_B$ receptor positive allosteric modulators (Castelli et al. 2012). In an attempt to optimize this structural motif and obtain SAR information, a number of congeners were synthesized by acylation of methyl 2-amino-4-ethyl-5-methylthiophene-3-carboxylate, in turn obtained by Gewald synthesis from methyl cyanoacetate and 3-pentanone in the presence of sulfur and a base (Scheme 3.11). These compounds showed an interesting positive allosteric modulator profile (Mugnaini et al. 2013). Although less potent than GS39783, used as a reference compound, some of them, such as compound **13**, were found to be more active in vivo, even after intragastric administration, and displayed low cytotoxicity.

3.3 Negative Allosteric Modulators of $GABA_B$ Receptors

In an attempt to find novel and more potent $GABA_B$ receptor positive allosteric modulators starting from the structure of CGP7930, Nan and coworkers reported, in 2014, the discovery of the first negative allosteric modulator (NAM) of the $GABA_B$ receptor (compound **14**, later on named CLH304a, Fig. 3.7) (Chen et al. 2014).



Scheme 3.10 Reagents and conditions: (*a*) $Na_2S_2O_3$, H_2O, $NaHCO_3$; (*b*) isobutyryl chloride, base; (*c*) HCl, dioxane, reflux; (*d*) Lawesson's reagent, toluene, reflux; (*e*) appropriate acyl chloride, TEA or PS-DIPEA, THF, rt to 50 °C



Scheme 3.11 Reagents and conditions: (*a*) S_8, morpholine, EtOH, reflux; (*b*) 4-chlorobenzoyl chloride, dioxane, 100 °C

Compound **14**, characterized by a di-(*tert*-butyl)phenol scaffold reminiscent of CGP7930 and *rac*-BHFF, was synthesized according to the procedure depicted in Scheme 3.12. 3,5-Bis-(*tert*-butyl)-4-hydroxybenzaldehyde was converted into the corresponding dimethyl acetal and this was reacted, in the presence of boron trifluoride etherate, with the silyl enol ether obtained from ethyl 2-oxopropanoate.

Compound **14** decreased GABA-induced IP3 production with an IC_{50} of 37.9 μM and had no effect on other GPCR Class C members, such as mGluR1, mGluR2, and

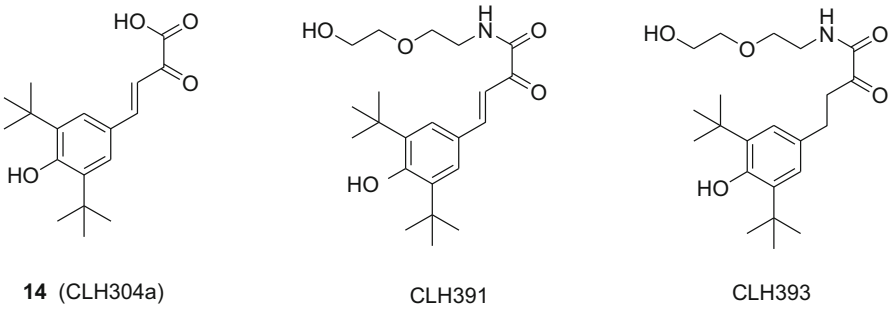
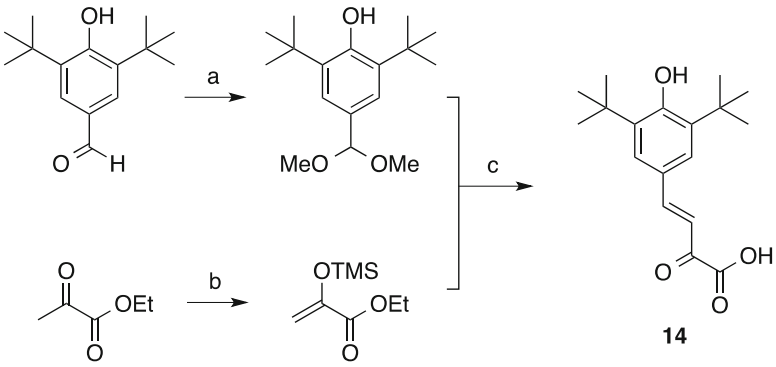


Fig. 3.7 Chemical structure of the first $GABA_B$ NAMs



Scheme 3.12 Reagents and conditions: (*a*) $HC(OEt)_3$, NH_4Cl, MeOH, reflux; (*b*) TMSCl, DMAP, Et_3N, toluene, reflux; (*c*) $BF_3{\cdot}Et_2O$, DCM, –78 to 0 °C

mGluR5. Moreover, it was shown that **14** does not bind to the orthosteric binding site of the receptor, demonstrating that it negatively modulates $GABA_B$ receptors activity as a NAM through the heptahelical domain of the $GABA_{B2}$ receptor subunits (Sun et al. 2016).

Two amide derivatives of CLH304a, namely, CLH391 and CLH393 (Fig. 3.7), showed comparable activity to that of the parent compound (Sun et al. 2016). However, these NAMs are not very attractive from the medicinal chemistry viewpoint. They were designed as derivatives of CGP7930, which was initially developed in 2001 as a pharmacological tool but then structurally elaborated by removing the phenol function in order to obtain more promising $GABA_B$ modulators, such as *rac*-BHFF. Conversely, the CLH compounds may show limitations in terms of pharmaceutical development, as they still possess an OH group on the aromatic ring, which might limit their pharmacokinetic properties, as well as a quite electrophilic alpha,beta-unsaturated ketone, which may be responsible for toxicological liability. Therefore, the efficacy and safety of these CHL compounds in vivo must be still carefully evaluated.

3.4 Challenges in the Discovery of New GPCRS Allosteric Modulators

The allosteric approach for GPCRs was impracticable until the late 1990s because of the lack of high-throughput screening technologies and functional assays able to identify molecules affecting target function irrespective of the site of binding. Nowadays, it is still very challenging considering that neither the binding modes nor the molecular mechanism of the drugs are known (Conn et al. 2012, 2014). Indeed, allosteric modulators show the phenomenon of functional (or mode) switching with a relatively high frequency, eliciting the propensity of structurally similar PAMs, NAMs and silent allosteric modulators (SAMs) to be generated by subtle structural changes of a given allosteric chemotype. Thus, during the lead-optimization process particular attention is required to avoid chemical series with a strong tendency to molecular switches because this can definitely complicate the SAR.

In addition, allosteric modulators very often suffer from extremely shallow or flat SAR. Very small electronic or steric modifications can have a dramatic impact on the biological response of the compound leading, in most of the cases, to a complete loss of activity. This behavior translates in a rate of success of around 10% during the chemical optimization phase. It is therefore necessary, when trying a chemical optimization of allosteric ligands, to use a focused iterative library synthesis based on the identification of regions of the hit that can tolerate modifications. To identify these particular sites of the molecule, the strategy of "walking" fluorine atoms has showed some potentiality (Conn et al. 2014).

Finally, CYP-mediated molecular switches are always possible. To study this phenomenon, major metabolites of allosteric ligands should be prepared and evaluated and, if necessary, metabolically labile sites should be blocked with deuterium. This strategy has emerged as an attractive one in order to improve metabolic stability without affecting potency and functional activity (Conn et al. 2014).

Functional switches and flat SAR have been widely observed for $GABA_B$ allosteric modulators. Since the discovery of the first $GABA_B$ PAM, the medicinal chemistry effort has allowed the identification of new structural motifs able to positively modulate the receptor with a significant expansion of our knowledge about SAR. Nevertheless, the attempt to rationalize biological outcomes on the basis of targeted structural modifications within a molecular class has often failed (Paterson et al. 2008; Mugnaini et al. 2013). Moreover, slight chemical manipulation of a PAM without affecting the central structural nucleus has led to the identification of a $GABA_B$ NAM (Chen et al. 2014).

If we add to this scenario the lack of structural information related to $GABA_B$ receptor, whose X-ray structure has been reported only in 2013 (Geng et al. 2013), it will come as no surprise that research in the area of allosteric modulators of the $GABA_B$ receptor has involved almost exclusively big pharma, with four companies (Novartis, Roche, AstraZeneca, and Addex) having invested substantial resources, and academia playing only a marginal role.

3.5 Conclusions

Since the discovery of the first $GABA_B$ positive allosteric modulator CGP7930 in 2001, a number of new scaffolds have been identified and, in some cases a lead optimization process has been undertaken with the aim of finding more potent ligands and getting new insight in the SAR of specific families of compounds. Because of the absence of structural knowledge about the allosteric site/sites on the receptor, this effort has not led to precise SAR information; on the contrary, besides shallow SAR, functional switches have been observed, driving to the discovery in 2014 of the first $GABA_B$ NAM, a molecule structurally related to CGP7930.

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