GABA and Sleep

Molecular, Functional and Clinical Aspects

Bearbeitet von
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1. Auflage 2010. Buch. xxiv, 486 S. Hardcover
ISBN 978 3 0346 0225 9
Format (B x L): 15,5 x 23,5 cm
Gewicht: 1024 g

Development of Subtype-Selective $GABA_A$ Receptor Compounds for the Treatment of Anxiety, Sleep Disorders and Epilepsy

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Abstract There is little doubt regarding the therapeutic possibilities of modulation of $GABA_A$ receptor function as exemplified by the clinical utility of benzodiazepines for half a century. The emerging understanding of the role of different $GABA_A$ receptor subtypes in mediating different physiological functions and pathological processes continues to offer opportunities for novel therapeutics. However, the challenge remains in turning the increased understanding of molecular pharmacology of $GABA_A$ receptors into clinically efficacious drugs. Probably the most active area of research has been the search for a non-sedating anxiolytic that acts via the benzodiazepine binding site. Unfortunately, the clinical development of a number of drugs with promising pre-clinical profiles, such as ocinaplon, SL65.1498, pagoclone, MRK-409, TPA023 and TPA023B has halted for a variety of reasons. Therefore, the underlying hypothesis that sub-type-selective compounds are non-sedating anxiolytics in man remains to be adequately tested. As regards hypnotics, the benzodiazepine site compounds adipiplon and indiplon, as well as the $GABA_A$ $\alpha4\beta\delta$-preferring agonist gaboxadol, are no longer in clinical development, leaving EVT-201, which has demonstrated efficacy in Phase II studies in primary insomnia, as the single, novel $GABA_A$-mediated hypnotic currently under evaluation. Benzodiazepines remain the first-line treatment for status epilepticus, an indication for which, therefore, there would appear little need for $GABA_A$ subtype-selective compounds. With respect to epilepsy, modulation of $GABA_A$ receptor function via the neurosteroid recognition site is the mechanism of action of ganaxolone, which is currently being evaluated in Phase III studies in adult partial seizures and children with infantile seizures. The continually evolving understanding of the structure and function of not only the $GABA_A$ receptor but also the variety of diverse binding sites that it harbours should continue to provide the molecular basis for designing strategies to selectively modulate the function of

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J.M. Monti et al. (eds.), *GABA and Sleep*,
DOI 10.1007/978-3-0346-0226-6_2,

distinct subtypes of the $GABA_A$ receptor and thereby provide novel therapies for the treatment of anxiety, sleep disorders, and possibly also epilepsy.

1 Introduction

1.1 $GABA_A$ Receptor Structure and Function

The $GABA_A$ receptor is a member of the Cys-loop ligand-gated ion channel family, which also includes the inhibitory glycine, ionotropic serotonin ($5HT_3$), and nicotinic acetylcholine receptors [1, 2]. The structural characteristics of these receptors are relatively well understood and is based upon homology modelling derived from the structure of the nicotinic acetylcholine receptor-binding protein [3, 4]. Hence, the Cys-loop family of receptors are homo- or heteropentameric assemblies of subunits arranged around a membrane-spanning pore. Each subunit has a large extra-cellular N-terminal domain, which contains the Cys-loop and is involved in agonist binding, as well as four membrane-spanning domains (TM1–4). The respective intracellular and extracellular loops between TMs 1–2 and 2–3 are relatively short whereas the intracellular loop between TMs 3 and 4 is comparatively long [1, 5], and the TM2 region is orientated such that it lines the ion channel pore (Fig. 1a; [6, 7]).

In the case of the $GABA_A$ receptor, there are 16 related subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π) that comprise the "classical" $GABA_A$ receptor plus an additional

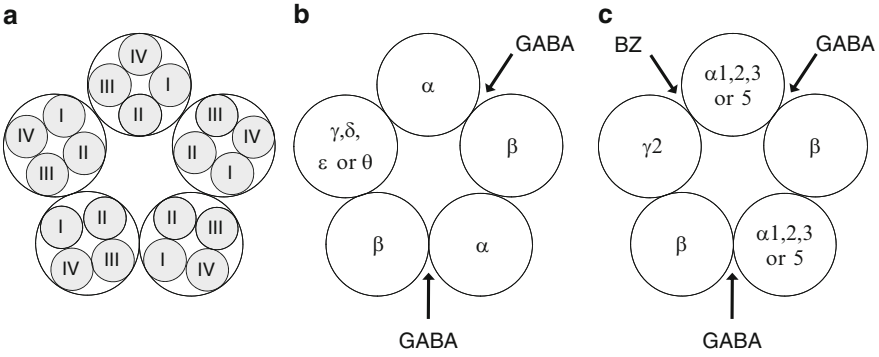

Fig. 1 Schematic representation of the arrangement of subunits in the $GABA_A$ receptor as viewed from the synapse. (**a**) The $GABA_A$ receptor is a heteropentameric arrangement of subunits, with each subunit containing four membrane-spanning regions (I–IV), of which the transmembrane region II lines the pore. (**b**) The most common arrangement of subunits is two α, two β and one γ, of which the latter may be replaced by either an δ, ε or θ subunit. The GABA binding site occurs at the interface of the α and β subunits. (**c**) When the γ subunit is γ2 and the α subunit is either an α1, α2, α3 or α5 (but not α4 or α6), a benzodiazepine recognition site is formed at the interface of the these subunits

three subunits (ρ1–3) that form the so-called $GABA_C$ receptor [8–10]. The most common arrangement is α, β, and γ subunits in a 2:2:1 stoichiometry [11], although the γ subunit can be replaced by a δ, ε or θ subunit (Fig. 1b). The GABA recognition site occurs at the interface of the α and β subunits (Fig. 1b; [12]) and when a γ2 subunit is adjacent to either an α1, α2, α3 or α5 subunit, a benzodiazepine recognition site is formed (Fig. 1c; [12]). Given the very large number of potential pentameric arrangements of the different $GABA_A$ receptor subunits, it is perhaps surprising that to date only 26 subtypes fulfil the criteria for either "identified" (11 subtypes), "existence with high probability" (six subtypes) or "tentative" (nine subtypes) [10].

1.2 $GABA_A$ Receptor Pharmacology

A number of different classes of pharmacological agents exert their effects on the $GABA_A$ receptor by binding to recognition sites that are distinct from the endogenous ligand (GABA) binding site. Hence, barbiturates, ethanol, certain anaesthetics and convulsants as well as a variety of other compound classes act either via direct activation of the $GABA_A$ receptor or allosteric modulation of the inhibitory effects of GABA [13, 14]. An understanding of how these various compounds interact with different subtypes of the $GABA_A$ receptor forms the basis for developing compounds with novel pharmacological profiles that selectively interact with specific subtypes. In this regard, the benzodiazepine recognition site is the best understood based upon not only the proven clinical efficacy of compounds acting at this site but also the availability of pharmacological tool compounds as well as genetically modified mice. Accordingly, the focus of the subtype-selective $GABA_A$ modulators described in the present article will be on compounds that act via the benzodiazepine binding site. Nevertheless, as the understanding of structure and pharmacology of the non-benzodiazepine binding sites increases (for example, the neurosteroid binding site [15–17]) , it is probable that novel molecular targets on the $GABA_A$ receptor will emerge. A brief overview of some of these different recognition sites is presented below.

1.2.1 GABA Binding Site

Based upon the structure of GABA, a number of structurally-related agonist or partial agonist analogues have been described (Fig. 2a) that are either conformationally restricted and/or contain a bioisosteric replacement of the carboxylic acid group found in GABA [18, 19]. These include the natural product muscimol, a constituent of the mushroom *Amanita muscaria*, which contains a 3-isoxazolol carboxylic acid bioisostere. Further conformational restriction of muscimol by incorporating the amino group of muscimol into a piperidine ring results in THIP

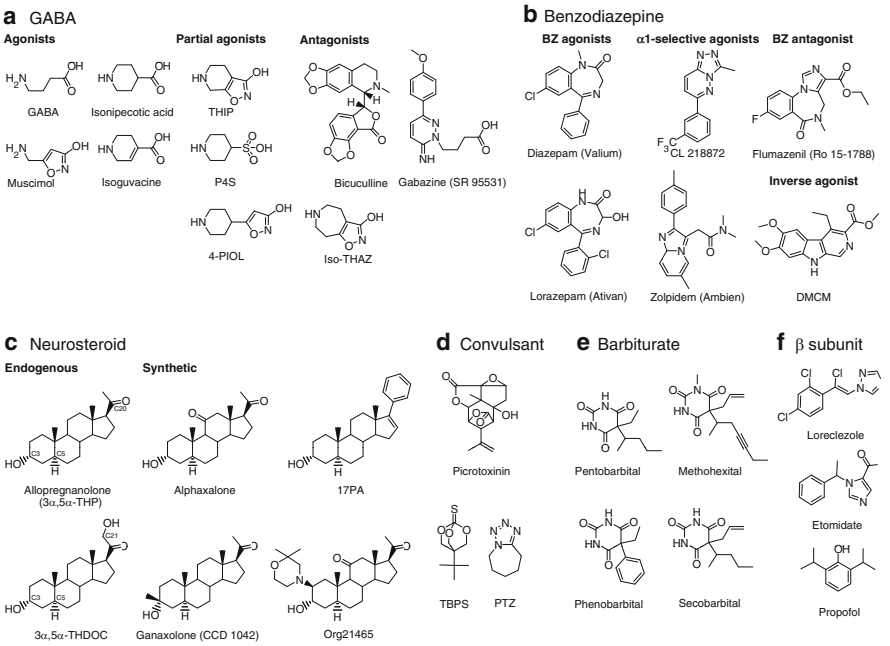

Fig. 2 Structures of different classes of compounds known to bind to the various recognition sites associated with the $GABA_A$ receptor. (**a**) At the agonist (GABA) binding site, the endogenous ligand GABA and its structural analogue muscimol acts as agonists whereas bicuculline is the prototypic antagonist. (**b**) The 1,4-benzodiazepines as well as the non-benzodiazepine, α1 subtype-preferring agonists CL 218872 and zolpidem bind to the benzodiazepine recognition site. At this same site, flumazenil (Ro 15-1788) and DMCM are prototypic antagonists and inverse agonists, respectively. (**c**) At the neurosteroid binding site, allopregnanolone and 3α,5α-THDOC are endogenous ligands and a number of structurally related synthetic ligands have also been described, of which alphaxalone and ganaxolone are most noteworthy since the former is the active component of the anaesthetic Althesin and the latter is currently undergoing clinical trials as a treatment for epilepsy. (**d**) Picrotoxinin, TBPS and PTZ bind within the ion channel pore itself and in doing so block GABA-mediated inhibitory transmission thereby causing seizures, and consequently, this recognition site is known as the convulsant binding site. (**e**) Numerous barbiturates have been described, which are all based upon the structure of barbituric acid. The recognition site for these compounds is less well described than other binding sites on the $GABA_A$ receptor. (**f**) The anti-convulsant loreclezole and the anaesthetics etomidate and propofol bind to recognition sites associated with the β subunit. *Abbreviations*: 17PA 3α,5α-17-phenylandrost-16-en-3-ol; 3α,5α-THDOC 3α,5α-tetrahydrodeoxycorticosterone; *4-PIOL* 5-(4-piperidyl)isoxazole-3-ol; *DMCM* methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate; *iso-THAZ* 5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol; *P4S* piperidine-4-sulphonic acid; *PTZ* pentylenetetrazole; *TBPS* *t*-butylbicyclophosphorothionate; *THIP* gaboxadol, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol

[20], which has subsequently become known as gaboxadol. By analogy with THIP, the incorporation of the amino group in GABA into a piperidine was used to produce the monoheterocyclic agonist, isonipecotic acid, and the introduction of a double bond yielded isoguvacine. Replacement of the carboxylic acid group of

isonipecotic acid resulted in compounds such as P4S and 4-PIOL. As regards GABA site antagonists, the prototypic compound is the alkaloid bicuculline [21]. However, additional structural classes of antagonists include the arylaminopyridazine analogues of GABA, typified by Gabazine (SR 95531; [22]) and iso-THAZ, a bicyclic 5-isoxazolo analogue of THIP (Fig. 2a).

Despite the variety of possible combinations of α1–6 and β1–3 subunits that could theoretically comprise the GABA recognition site, most full agonists, such as GABA, muscimol and isoguvacine, and antagonists, for example, bicuculline and Gabazine, demonstrate little difference in affinity between recombinant $GABA_A$ receptor sub-types [18, 19, 23, 24]. Moreover, the very defined requirements for binding at the GABA recognition site mean that relatively few different structural classes of compound binding at this site have been reported. In contrast to full agonists and antagonists, certain partial agonists appear to have a degree of subtype selectivity. Such compounds include 4-PIOL, P4S and THIP [18, 19] and whereas non-selective GABA site agonists and antagonists would appear to be of limited therapeutic potential, partial agonists that posses a degree of subtype-selectivity, such as THIP (Gaboxadol), may be of therapeutic benefit, for example, in sleep disorders (see below). A 3D-pharmacophore model for the GABA binding site has been described [25, 26].

1.2.2 Benzodiazepine Binding Site

Since their introduction half a century ago [27], the therapeutic utility of benzodiazepines as hypnotics, anticonvulsants, muscle relaxants [28, 29] and, particularly, anxiolytics [30] has become well established. However, the choice of particular benzodiazepines as, for example, either anxiolytics or hypnotics, is related more to pharmaceutical and pharmacokinetic properties and commercial considerations than to intrinsic differences in pharmacology [31]. With respect to pharmacokinetics, benzodiazepines can be categorised as short-, intermediate- and long-acting in a manner analogous to the classification of barbiturates as short-intermediate- and long-acting that dictated their use as anaesthetics, hypnotics and anxiolytics, respectively. The prototypic benzodiazepine is diazepam (Fig. 2b), which is more specifically described as a 1,4-benzodiazepine referring to the position of the nitrogens in the diazepine core. Multiple other analogues of diazepam have been used in the clinical, of which lorazepam (Fig. 2b) is but one example.

In addition to their clinical efficacy, benzodiazepines are a particularly attractive class of drugs since they have low levels of toxicity, especially compared to the barbiturates, which they superseded in clinical practise [32, 33]. The safety of benzodiazepines is based primarily upon the fact that they cannot directly activate the $GABA_A$ receptor; rather, they modulate the inhibitory functions of GABA by allosterically increasing the frequency of GABA-induced channel opening events. Such compounds are described as benzodiazepine site agonists (or positive

allosteric modulators) whereas those that reduce GABA-mediated chloride flux are known as inverse agonists (negative allosteric modulators). Compounds such as flumazenil, which bind with high affinity but do not affect GABA-induced chloride currents, exert no physiological effect on the $GABA_A$ receptor but can block, or antagonise, the effects of benzodiazepine site agonists or inverse agonists and are therefore described as benzodiazepine site antagonists.

Non-benzodiazepine chemical structures also bind to this recognition site and the differential affinity of the triazolopyridazine CL 218872 (Fig. 2b) for $GABA_A$ receptors in different parts of the brain was the initial indication for the heterogeneity of benzodiazepine binding sites [34]. The advent of molecular cloning and the detailed pharmacological characterisation of recombinant receptors further refined the heterogeneity of $GABA_A$ subtypes [13]. It is now apparent that the benzodiazepine binding site occurs at the interface of the α and $\gamma 2$ subunits of $GABA_A$ receptors with either an $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$ or $\alpha 5\beta\gamma 2$ subunit composition [35], a combined $GABA_A$ receptor population that accounts for around 75% of total brain $GABA_A$ receptors [36, 37]. Most notably, receptors with an $\alpha 4\beta\gamma 2$ or $\alpha 6\beta\gamma 2$ composition have no affinity for classical benzodiazepines such as diazepam, and this can be attributed to a single amino acid, which is a histidine in $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunits, but an arginine in $\alpha 4$ and $\alpha 6$ subunits [38].

The fact that the single histidine to arginine switch in the α subunit confers diazepam insensitivity to the $GABA_A$ [38] has been exploited to generate a number of point-mutated mice in which particular populations of $GABA_A$ receptor (i.e. $\alpha 1$-, $\alpha 2$-, $\alpha 3$-, or $\alpha 5$-containing receptors) retain their normal physiological GABA-gated chloride flux function but are insensitive to the pharmacological effects of diazepam [39]. Using this strategy, Mohler, Rudolph and colleagues plus, to a lesser extent, the group at Merck have begun to dissect which of the pharmacological features of diazepam in particular, but also certain other benzodiazepine site modulators, are associated with which specific subtypes of $GABA_A$ receptor [40–46]. These studies have been complemented by observations in α subunit-deleted (knock-out) mice as well as the use of $GABA_A$ subtype-selective pharmacological tool compounds. Collectively, these data show that the $\alpha 1$ subtype is associated with sedation whereas the $\alpha 2$ and/or $\alpha 3$ subtypes are the "anxiolytic" subtypes [40, 41, 47, 48] and the $\alpha 5$ subtype is associated with aspects of cognition [49–54]. Although the delineation of $GABA_A$ subtypes into $\alpha 1$ = sedation, $\alpha 2/\alpha 3$ = anxiety, and $\alpha 5$ = cognition is undoubtedly a gross over-simplification, it does nevertheless form the basis for the hypotheses that compounds that have (1) preferential agonist activity at the $\alpha 1$ subtype should be sedative-hypnotics; (2) agonist or partial agonist activity at the $\alpha 2$ and $\alpha 3$ subtypes but have reduced or, preferably, no activity at the $\alpha 1$ subtype should be non-sedating anxiolytics [55, 56]; and (3) $\alpha 5$-selective inverse agonism should improve cognitive function [52]. There is already a degree of clinical validation for the hypothesis that the $\alpha 1$ subtype is associated with sedation in so far as the sedative-hypnotic zolpidem (Ambien) is $\alpha 1$ subtype-preferring (see below). As regards anticonvulsant activity, there is no specific "anticonvulsant subtype", with data suggesting that efficacy at

more than one subtype is required, with the α3 and α5 subtypes possibly playing a lesser role [44].

1.2.3 Neurosteroid Binding Site

Neuroactive steroids (Fig. 2c) are endogenous neuromodulators that are synthesised both in the brain as well as in the adrenal glands, ovaries and testes [57, 58]. The recognition that they are potent modulators of $GABA_A$ receptor function was based upon observations with alphaxalone (3α-hydroxy-5α-pregnan-11,20-dione; [59]), a component of the steroid anaesthetic Althesin, which was introduced for clinical use as an anaesthetic in Europe in the 1970s but was withdrawn due to adverse events associated with the vehicle in 1985 [60]. These observations were extended to include the naturally occurring neurosteroids allopregnanolone (3α-hydroxy-5α-pregnan-20-one or 3α,5α-THP) and pregnanolone (3α-hydroxy-5β-pregnan-20-one or 3α,5β-THP), both of which also enhanced GABAergic neurotransmission [61]. Additional endogenous neurosteroids that interact with the $GABA_A$ receptor include a variety of 3α,5α- and 3α,5β-reduced metabolites of deoxycorticosterone, dihydroepiandrosterone and testosterone [57, 58].

At relatively low concentrations (<1 μM), neurosteroids act by increasing both the frequency and the duration of GABA-induced channel opening events, whereas at higher concentrations (>1 μM), neurosteroids, like barbiturates, can directly activate the $GABA_A$ receptor [62, 63]. These data suggest that there may be different recognition sites which mediate the potentiating and direct activating actions of neurosteroids. This has been confirmed by using structural homology modelling plus chimaeras of mouse α1 and β2 subunits and the neurosteroid-insensitive Drosophila *Rdl* (resistance to dieldrin) GABA receptor. Along with mutagenesis experiments, these studies have identified the α1 subunit TM1 Q241 as well as TM3 N407 and Y410 residues as crucial for conferring sensitivity to the modulatory aspect of neurosteroid function, whereas there is a separate binding site formed by the TM1 αT236 and TM3 βY284 residues that mediates the direct activation of neurosteroids [15–17]. Furthermore, there may also be one or possibly two additional binding sites on mammalian $GABA_A$ receptors associated with the inhibitory sulphated steroids [16]. Although neurosteroids modulate most $GABA_A$ receptor isoforms, they have a modestly higher affinity for the αβδ-containing subtypes [63–65]. However, the δ subunit does not appear to contribute to the binding site but is more likely associated with the degree of potentiation [15–17].

The realisation that endogenous and synthetic neurosteroids acted via the $GABA_A$ receptor triggered the search for additional synthetic neurosteroids based upon, for example, tricyclic analogues of steroids and unnatural enantiomers of endogenous neuroactive steroids [60, 66, 67]. More recently, 17PA (3α,5α-17-phenylandrost-16-en-3-ol) has been described as a neurosteroid antagonist that does not affect GABA-mediated responses but does block the effects of 3α,5α-THP (but not, interestingly 3α,5β-THP) [68]. Pre-clinical data indicate that synthetic neurosteroids might be of therapeutic utility based upon their anxiolytic,

anaesthetic, hypnotic, antimigraine and anticonvulsant properties [67], although this potential has yet to be fulfilled. For example, despite initial clinical studies being promising, a double-blind, placebo-controlled Phase II study of a tablet formulation of ganaxolone did not show significant efficacy in acute migraine [67], although this may have been a consequence of the slow T_{max} for this compound. In addition, the development of the water-soluble neurosteroid anaesthetic Org21465 was discontinued in 1996 due to side-effects [67]. Furthermore, CoCensys (now Purdue Neuroscience) selected the neurosteroids CCD-3693 [69] and Co 2-6749 (also known as GMA-839 or WAY-141839; [70]) for clinical development as a hypnotic and anxiolytic, respectively [67]. However, by the beginning of 2003, development of the former was reported to have been halted, whereas there has been no update on the development of Co 2-6749 since 1999 [67]. Nevertheless, ganaxolone (CCD 1042; [71]) is currently under evaluation for uncontrolled adult partial seizures and infantile spasms (see below).

The role of different $GABA_A$ receptors in mediating not only the physiological functions of endogenous neurosteroids but also the pharmacological effects mediated via this site (which include anxiolytic, anaesthetic, hypnotic and anticonvulsant properties; [67]) remains poorly understood, and it is therefore difficult to predict the possible therapeutic benefits of selectively modulating $GABA_A$ receptor subtypes via the neurosteroid recognition site. Nevertheless, the identification of specific neurosteroid recognition sites [15–17] should permit the generation of, for example, α subunit Q241 point-mutated neurosteroid-insensitive knock-in mice [72, 73] that will help elucidate the role of specific $GABA_A$ receptor subtypes in mediating the different functions of neurosteroids in a manner analogous to the methods used to dissect the subtype-selective functions of benzodiazepines and anaesthetics [43, 74]. Moreover, the identification of the different binding sites that mediate the modulatory and direct activation aspects of neurosteroid function may provide the basis for identifying compounds that selectively modulate, but do not directly activate, $GABA_A$ receptors. Such compounds might therefore be expected to have an improved therapeutic index relative to neurosteroids that not only modulate but also directly activate the receptor.

1.2.4 Convulsant Binding Site

A number of structurally unrelated compounds bind to the $GABA_A$ receptor and block GABA-gated chloride flux in a non-competitive manner, resulting in convulsions in animals [75]. Compounds that bind to this convulsant binding site (Fig. 1d) include picrotoxinin (the active component of picrotoxin), pentylenetetrazole, a number of insecticides (including dieldrine) and TBPS. This latter compound can be radiolabelled and the modulation of [^{35}S]TBPS or, alternatively, [^{3}H]TBOB or [^{3}H]EBOB binding can used as an indicator of the efficacy of compounds that allosterically modulate $GABA_A$ receptor function [76, 77]. However, given that compounds that bind at this site produce convulsions, there is little apparent therapeutic benefit for drugs acting at this particular site on the $GABA_A$

receptor. Nevertheless, sub-convulsant doses of picrotoxin and pentylenetetrazole have been described as alleviating the cognitive deficit associated with a mouse model of Down syndrome [78]. Although there does appear a degree of heterogeneity in the agonist-induced modulation of TBPS binding to the convulsant site of different $GABA_A$ receptor populations [79–81], it is unclear how this might be exploited to pharmacological advantage.

1.2.5 Barbiturate Binding Site

Although barbituric acid does not have any effects on the CNS, the barbiturates that derive from it have a pronounced pharmacology. The initial barbiturate, diethylbarbituric acid, was synthesised in 1903 and was made by substituting two hydrogens for ethyl groups at the same C-5 position. This compound, barbital (Veronal; Fig. 1e), was found to be hypnotic and has a relatively long half-life. Subsequently, phenobarbital (Luminal) was marketed as a sedative and anticonvulsant, and thereafter, additional barbiturates were introduced, including amobarbital (Amytal), pentobarbital (Nembutal), secobarbital (Seconal) and the ultra short-acting hexobarbital (Evipal), thiopental (Pentothal) and methohexital (Brevital). The clinical use of barbiturates is dictated to a large extent by their respective plasma half-lives. Hence, ultra-short to short-acting barbiturates are used for anaesthesia, the short-intermediate acting barbiturates may be used for insomnia and/or anxiety and the long-acting barbiturates, such as phenobarbital, have utility as anticonvulsants, particularly as a second line treatment for status epilepticus. Despite the decline in the use of barbiturates as hypnotics and anxiolytics, they have retained their clinical utility as anaesthetics and anticonvulsants, with thiopental and phenobarbital being respective examples [82, 83].

Barbiturates exert their effects via $GABA_A$-mediated inhibitory neurotransmission [84–86] with, for example, their functional effects at the $GABA_A$ receptor correlating with their anaesthetic potency [87]. More specifically, at lower concentrations (below ~0.1 mM), barbiturates modulate the effects of GABA, but at intermediate concentrations (~0.1–1 mM), they directly activate the $GABA_A$ receptor, and at high concentrations (>1 mM), they can block the ion channel [88, 89]. Although the barbiturate binding site(s) remains to be fully defined, it does appear to be associated with the β subunit [90, 91], and the allosteric changes produced by direct activation of the $GABA_A$ by pentobarbital are distinct from those produced by GABA [89].

1.2.6 β Subunit Binding Site(s)

The broad spectrum anticonvulsant loreclezole was shown to selectively activate β2- and β3-, but not β1-containing $GABA_A$ receptors [92]. This selectivity can be attributed to a single amino acid in the TM2 region of the β subunit, which is a serine in the β1 subunit and an arginine in the β2 and β3 subunits [93]. Similar data were observed using etomidate [94–96], which although used clinically as an intravenous anaesthetic has a structural resemblance to loreclezole (Fig. 2f).

In a manner analogous to the use of α subunit point-mutated mice to identify $GABA_A$ subtypes associated with the various pharmacological properties of benzodiazepines [43], mice containing point mutations of the β subunit, e.g., β2 (Asn265Ser) and β3(Asn265Met), have been used to attribute distinct aspects of the in vivo profile of β subunit-interacting compounds to specific $GABA_A$ subtypes. Such compounds include the intravenous anaesthetics etomidate and propofol [97–99], the anticonvulsant loreclezole [100], the general anaesthetic isoflurane [101] and the anaesthetic barbiturate pentobarbital [91].

Based upon the selectivity of enaminones [102] for β2- and β3- over β1-containing $GABA_A$ receptors, it has been proposed that such compounds might be a novel way of developing anxioselective compounds [103]. Although this is still an emerging area, it nevertheless highlights the possibility of developing $GABA_A$ receptor subtype-selective compounds via mechanisms distinct from the benzodiazepine binding site.

1.3 Subtype-Selective Affinity and Subtype-Selective Efficacy

In the search for $GABA_A$ receptor subtype-selective compounds, two different approaches may be employed, namely subtype-selective affinity and subtype-selective efficacy. The selective affinity strategy is probably the most intuitive and describes a compound that binds preferentially to the subtype of interest with much lower, or preferably negligible, affinity at the other subtypes (Fig. 3a). Although such a compound might possess equivalent efficacy at each of the subtypes to which it binds, particular the in vivo effects will be mediated predominantly via the high-affinity subtypes since the compound will preferentially occupy these receptors in vivo. The alternative approach is to design compounds with subtype-selective efficacy (Fig. 3b). Such compounds bind with equivalent affinity to all four subtypes but only have efficacy at certain of those receptors. The antagonist efficacy at other subtypes means that even though the compound might bind with very high affinity, there is no consequence for GABA-mediated receptor function. Although the difference between the selective affinity and selective efficacy approaches are illustrated (Fig. 3) in terms of the benzodiazepine recognition site (i.e. α1-, α2-, α3- and α5-containing $GABA_A$ subtypes), the principle is equally applicable to other binding sites on the $GABA_A$ receptor.

2 $GABA_A$ Receptor Compounds for Anxiety

2.1 Anxiety Disorders and Benzodiazepines

Anxiety disorders are a group of conditions [30] that include social anxiety disorder (also known as social phobia; which has an estimated lifetime prevalence in the region of 13%), phobias in general (11%), post-traumatic stress disorder (8%), generalised

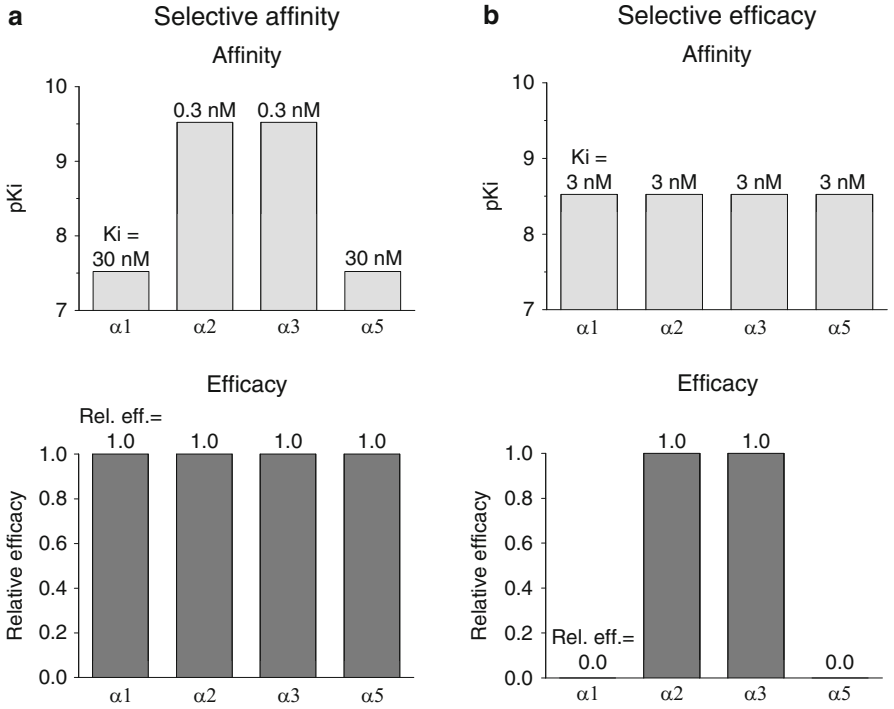

Fig. 3 Schematic representation of compounds with either subtype-selective affinity or subtype-selective efficacy for the benzodiazepine site of different $GABA_A$ receptors. (**a**) A compound with subtype-selectivity affinity binds to the benzodiazepine binding site of the $GABA_A$ receptor subtypes with differing affinities (in this example a 100-fold higher affinity for α2 and α3 versus α1 and α5 subtypes), yet when bound, the compound has equivalent (in this case full agonist) efficacy at each subtype, albeit with a 100-fold difference in the EC_{50} for agonist efficacy at the α2 and α3 versus α1 and α5 subtypes. (**b**) In contrast, a compound with subtype-selectivity efficacy binds to the benzodiazepine binding site of the $GABA_A$ receptor subtypes with equivalent affinity (in this example 3 nM), but the compound has differential efficacy at the various subtypes (in this case full agonist efficacy at the α2 and α3 subtypes and antagonist efficacy at the α1 and α5 subtypes). It is important to note that these are hypothetical examples since in practise it has not proved possible to achieve the levels of α2/α3 binding selectivity illustrated in *panel A* (with a maximal, approximately tenfold binding α3 versus α1 selectivity having been reported; [104]). Similarly, it has not been possible to demonstrate the level of efficacy selectivity illustrated in *panel B* with at best partial, or weak partial agonist efficacy at the α2 and α3 and antagonist efficacy at the α1 and α5 subtypes being described [104]

anxiety disorder (GAD; 5%), panic disorder (4%) and obsessive-compulsive disorder (2%). In the USA alone, the estimated total annual cost of anxiety disorders (including direct and indirect costs) is in the region of sixty billion dollars [30]. Although a variety of drugs are used to treat these disorders, the most common ones are antidepressants, particularly SSRIs and the $5HT_{1A}$ partial agonist buspirone, and benzodiazepines [30, 105–107]. Despite the undoubted anxiolytic efficacy of

benzodiazepines, major concerns related to the use of this class of drugs include abuse, dependence and withdrawal [33, 108–111]. In conjunction with the over-prescribing of benzodiazepines that occurred in the 1960s and 1970s, a negative public perception of this class of drugs developed [29, 112]; a perception that lingers to this day [107]. An additional liability associated with benzodiazepine use in the treatment of anxiety disorders is that of sedation which, although a desirable pharmacological effect when benzodiazepines are used as hypnotics, is clearly undesirable in the performance of activities of daily living, such as operating machinery or driving [113] and is also a risk factor associated with falls in the elderly [114].

Based upon the proven clinical efficacy and safety of benzodiazepines, as well as the pre-clinical rationale emerging from the use of transgenic mouse models and the extensive understanding of the benzodiazepine recognition site, efforts to develop the next generation of anxiolytic $GABA_A$ modulators have primarily focused upon compounds that modulate $GABA_A$ receptor function via the benzodiazepine binding site. More specifically, the hypothesis that $GABA_A$ modulators that modulate the α2 and α3 subtypes to a greater extent than α1-containing $GABA_A$ receptors, should be anxiolytic but with a reduced sedation liability [115], has resulted in the identification of a number of α2/α3-selective compounds that are either pre-clinical tool compounds or clinical development candidates, examples of which are discussed below.

2.2 *Benzodiazepine Site Modulators: Pre-clinical Compounds*

A number of subtype-selective benzodiazepine site $GABA_A$ modulators have been reported to be non-sedating anxiolytics in pre-clinical species. These include the prototypic α2/α3 efficacy-selective triazolopyridazine L-838417 [42, 116], which has partial agonist efficacy at the α2, α3 and α5 subtypes but, most notably, has antagonist efficacy at α1-containing $GABA_A$ receptors (Fig. 4a). This compound proved to be anxiolytic, but with a much reduced sedation liability in both rodent and primate species [42, 116]. However, pharmacokinetic issues restricted its use to pre-clinical species [118]. The Merck group also described the fluoroimidazopyridine TP003 (Fig. 4b; [48]) as an α3-selective partial agonist, and as such, it is one of the few examples where there is a marked separation of α2 and α3 efficacy since, generally speaking, the efficacy of a compound at these two subtypes is comparable [104]. Like L-838417, TP003 was also anxiolytic in rodent and primate species and provides support for the notion that the α3 subtype is an important contributor to the anxiolytic properties of non-selective benzodiazepines [48]. Unfortunately, poor pharmacokinetic characteristics once again prevented this compound being developed further. MRK-529 (Fig. 4c) is an α2/α3 efficacy-selective tricyclic pyridone, which has a non-sedating anxiolytic profile as well as favourable pharmacokinetic properties in pre-clinical species. However, progression of this compound into clinical studies was halted due to phototoxicity in pre-clinical safety and toxicity studies [104]. The Danish company NeuroSearch have described

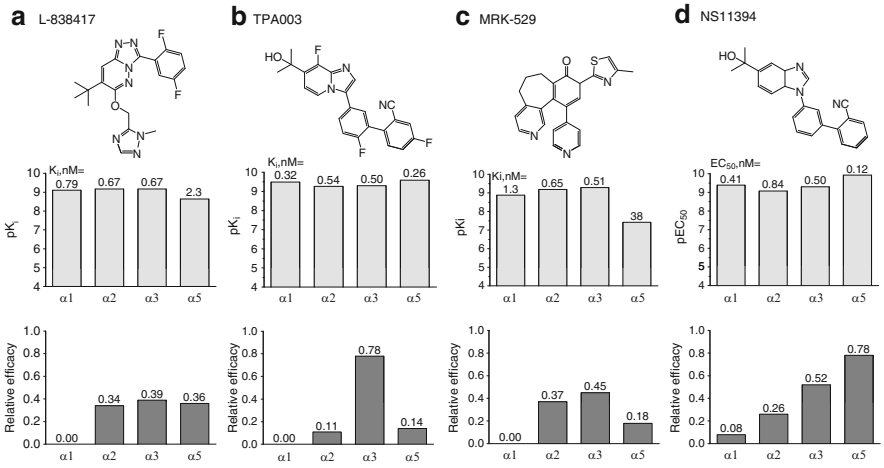

Fig. 4 Examples of the structure and in vitro binding and relative efficacy profiles of compounds that are non-sedating anxiolytics in pre-clinical species but did not progress into clinical development. For each compound, the upper panel represents the affinity, plotted as the pK_i or, where data are derived from functional assays, pEC_{50} (with values above bars showing the mean affinity expressed in nM). Efficacy data are expressed relative to a non-selective benzodiazepine agonist. Data are derived from: (**a**) L-838417, [42]; (**b**) TP003, [48]; (**c**) MRK-529, [104]; (**d**) NS11394, [117]

NS11394 (Fig. 4d) as a benzimidazole, which is structurally related to NS2710, a compound that was in Phase II clinical studies but whose development was halted due to skin rash [115]. This more recent compound was, like L-838417, selective for the α2 and α3 subtypes and was a non-sedating anxiolytic in rodents [117]. Moreover, this compound also possessed efficacy in a number of pre-clinical pain models [119].

Importantly, these compounds collectively demonstrate that not only can α2/α3-selective efficacy be established with a variety of different chemotypes (in this case using triazolopyridazine, fluoroimidazopyridine, pyridone and benzimidazole scaffolds) but also that such compounds consistently behave as non-sedating anxiolytics in pre-clinical species. Moreover, α2/α3-selective compounds have also been reported in a variety of other structural classes, including imidazopyrimidine, imidazotriazine, imidazopyrazinone, pyrazolotriazine, pyridazine and pyrazolopyridinone [56, 104].

2.3 Benzodiazepine Site Modulators: Clinical Compounds

Although the α2/α3-selective compounds described in the previous section clearly highlight the non-sedating anxiolytic behaviour of such compounds in pre-clinical

species, the critical issue is the translation of the pre-clinical pharmacology profile into clinical utility. The difficulty in making this transition is best exemplified by the non-selective partial agonist bretazenil, which despite demonstrating a clear separation between anxiolytic and sedating doses in pre-clinical studies did not show the corresponding separation in man [120]. Although the primary focus is to demonstrate a non-sedating anxiolytic profile in man, consideration should also be given to the abuse potential of such compounds since the scheduling (or ideally non-scheduling) of a drug for an indication such as GAD has a marked influence on the prescribing habits of doctors. In the present section, the fate of a variety of compounds that act at the benzodiazepine binding site (some of which are not particularly α2/α3 subtype-selective) and progressed into clinical development are summarised.

2.3.1 Ocinaplon

Ocinaplon (also known as CL 273,547; [121, 275]) is a pyrazolopyrimidine (Fig. 5a) that is structurally related to zaleplon (Sonata®, CL 284,846) and indiplon (see below). It has low affinity for $GABA_A$ receptors (IC_{50} >5 μM at each subtype) but has higher efficacy at the α1 compared to α2, α3 and α5 subtypes [121, 128]. However, despite its high efficacy at the "sedation" (α1) $GABA_A$ subtype, it has an anxioselective profile in pre-clinical species with, for example, an approximately 30-fold difference between the doses that produced anxiolytic-like activity and sedation in rhesus monkey [121, 129]. In a short, 2-week, 3-arm (placebo and 180 or 240 mg/day ocinaplon; $n = 31–35$/group), proof-of-concept study in GAD, ocinaplon produced a significantly greater reduction in the Hamilton Anxiety (HAM-A) scale when compared to placebo-treated subjects at a dose of 240 mg/day (120 mg twice a day) without significant signs of sedation [121]. This was followed up by a 4-week, 2-arm study (placebo and 270 mg ocinaplon – 90 mg three times a day, $n = 29$ and 31, respectively), which again demonstrated that ocinaplon was more effective than placebo at reducing the HAM-A score, albeit with the caveat that out of the 60 subjects initially assigned to a treatment group, only 23 completed the entire 4 weeks since the study was terminated early due to a serious adverse event that was considered to be possibly drug-related [130]. More specifically, a single subject had a significant elevation of serum liver alanine and aspartate transaminase enzymes, with a peak of >50-fold higher than the upper limit of normal followed by icterus (jaundice). Nevertheless, and as in the initial proof-of-concept study, ocinaplon did not demonstrate signs of sedation. However, as the Phase III study of ocinaplon had to be halted, development was discontinued at the end of 2005.

Clearly, the behaviour of ocinaplon as a non-sedating anxiolytic is at odds with the prevailing α1 = sedation hypothesis and the reason for its lack of sedation in pre-clinical species and in man remain uncertain [121, 129, 130]. Furthermore, DOV 51892, which is structurally related to ocinaplon, is essentially a "super-agonist"

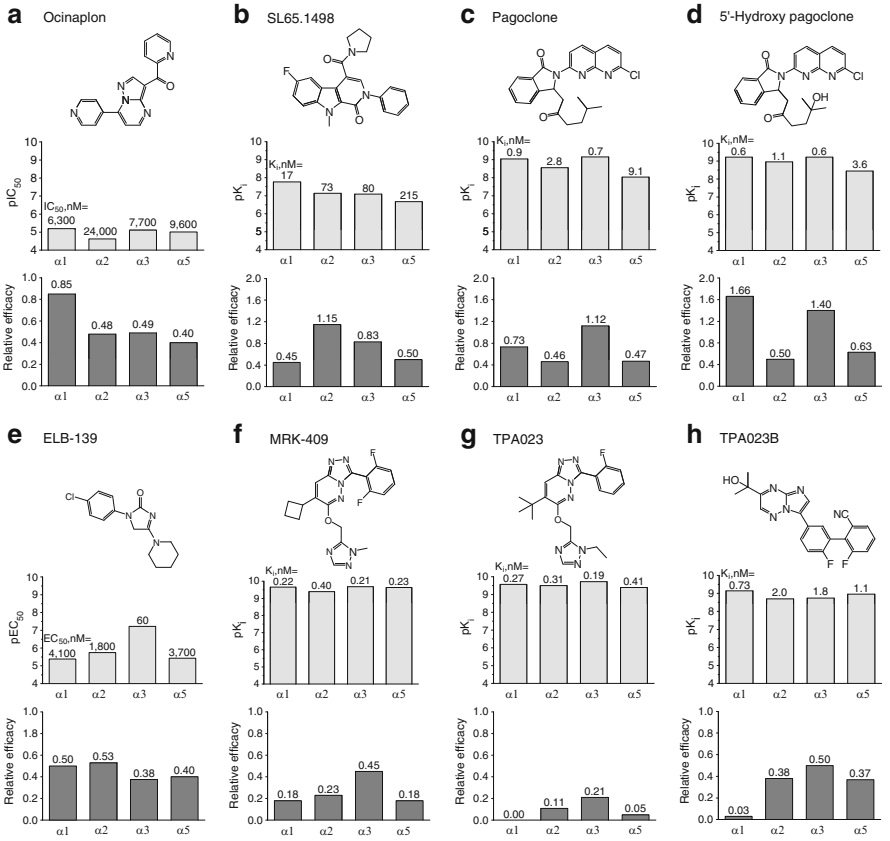

Fig. 5 The structure and in vitro binding and relative efficacy profiles of compounds that modulate $GABA_A$ receptor function via the benzodiazepine binding site, are non-sedating anxiolytics in pre-clinical species and have progressed into clinical development. For each compound, the upper panel represents the affinity, plotted as the pK_i, pIC_{50} or, where data are derived from functional assays, pEC_{50} (with values above *bars* showing the mean affinity expressed in nM). Efficacy data are expressed relative to a non-selective benzodiazepine agonist. Data are derived from: (**a**) Ocinaplon, [121]; (**b**) SL65.1498, [115, 122]; (**c** and **d**). Pagoclone and 5′-hydroxy pagoclone, [123]; (**e**) ELB-139, [124]; (**f**) MRK-409, [125]; (**g**) TPA023, [126]; (**h**) TPA023B, [127]

at the α1 subtype yet pre-clinically possesses a non-sedating anxiolytic profile [131], suggesting that, and for whatever reason, efficacy at the α1 subtype may not per se be a reliable indicator of sedation liability in man [132].

2.3.2 SL65.1498

Sanofi-Synthelabo (now Sanofi-Aventis) described SL65.1498 as a pyridoindole with full agonist efficacy at the α2 and α3 subtypes and lower efficacy at the α1

and α5 subtypes (respective relative efficacy values of 1.15, 0.83, 0.45 and 0.50; Fig. 5b). However, the α2/α3 versus α1-selective efficacy is offset, to a certain extent, by the approximately fivefold higher affinity of SL65.1498 for α1- compared to α2- and α3-containing $GABA_A$ receptors. Nevertheless, in a pharmacodynamic study in normal volunteers, SL65.1498 did not show overt signs of sedation even at a dose of 25 mg, despite possessing significant α1 efficacy and a slight α1 binding selectivity [133]. Moreover, although SL65.1498 did decrease saccadic peak velocity at a dose of 25 mg (but not 2.5 or 7.5 mg), the extent of this reduction was much less than that observed not only with lorazepam [133] but also TPA023 and MRK-409 [134, 135]. Although the maximum dose used for SL65.1498 (25 mg) was much higher than that used for either TPA023 or MRK-409 (1.5 mg and 0.75 mg, respectively; [134, 135]), this is probably related more to the lower affinity of SL65.1498 at the α1, α2 and α3 subtypes (17–80 nM; [122]) compared to either TPA023 (K_i values ranging from 0.19–0.41 nM and 0.21–0.40 nM, respectively) rather than reflecting differences in tolerability. SL65.1498 was described in February of 2004 as undergoing Phase IIb studies for the indications of anxiety and muscular spasms. However, following the merger of Sanofi-Synthelabo and Aventis in August 2004, the drug did not appear in the March 2005 pipeline of the newly formed Sanofi-Aventis pipeline.

2.3.3 Pagoclone

Pagoclone is a member of the cyclopyrrolone series (Fig. 5c), that also includes suriclone and zopiclone [136]. It is the active enantiomer of the racemate RP 59037 [137], which was originally described as possessing partial agonist efficacy [138] and an anxioselective profile in pre-clinical animal models [139], although others have reported little separation between anxiolytic and sedative doses in rat [123]. It has equivalent affinity at the α1, α2 and α3 subtypes with 3–10-fold lower affinity at the α5 subtype (Fig. 5c). The active metabolite, 5′-hydroxypagoclone, has a similar binding profile compared to its parent but has increased efficacy at α1-containing receptors (Fig. 5d).

In man, a dose of 0.4 mg pagoclone resulted in $GABA_A$ receptor occupancy, as measured using [^{11}C]flumazenil positron emission tomography (PET), of 15% whereas 1 mg lorazepam produced 6% occupancy. However, despite its greater occupancy, pagoclone produced changes in saccadic eye movements that were comparable to lorazepam, suggesting that it behaves in man as a partial agonist [140]. Consistent with this, pagoclone produced relatively mild and transient effects on learning and memory at doses of 0.15, 0.3 and 0.6 mg [141]. On the other hand, in a comparative study in healthy recreational drug users, pagoclone (4.8 mg) was rated as being similar to diazepam [142], suggesting that its efficacy profile may not be too dissimilar from that of a non-selective full agonist and that the abuse potential of pagoclone might be comparable to that of diazepam.

Pagoclone was originally licensed from Rhone-Poulenc Rorer (which became Aventis and then Sanofi-Aventis) to Interneuron (which became Indevus and then Endo Pharmaceuticals) and was being co-developed by Warner-Lambert (which became part of Pfizer) for the treatment of panic attacks and GAD [136, 143, 144]. However, development for these disorders was discontinued due to lack of robust efficacy [55]. Nevertheless, pagoclone is still being evaluated as a treatment for stuttering. Hence, an initial 8-week, placebo controlled, double-blind, multi-centre Examining Pagoclone for Persistent Developmental Stuttering Study (EXPRESS) study demonstrated proof-of-concept in developmental stuttering in patients given escalating doses of pagoclone from 0.3 to 0.6 mg/day. As a result, Teva Pharmaceutical, in collaboration with Endo, has evaluated pagoclone in additional Phase II studies of developmental and adult stuttering (www.ClinicalTrials.gov, identifiers NCT00830154 and NCT00239915). The drug has also been evaluated as a potential treatment for premature ejaculation. However, the interim analyses of phase II data in September 2006 showed that pagoclone had insufficient efficacy for this indication and development for premature ejaculation was discontinued.

2.3.4 ELB-139

ELB-139 is an imidazolone (Compound 3, [145]) that was evaluated as part of the NIH-sponsored in vivo screening programme to identify novel anticonvulsants [146]. It was being developed by the German company Elbion AG, which was derived from Arzneimittelwerk Dresden GmbH's (AWD) drug discovery business. This compound has low affinity for the α1, α2 and α5 subtypes ($\geq$1.8 μM) but reportedly has a 30-fold higher affinity for the α3 subtype (EC_{50} = 60 nM; Fig. 5e; [124]), which is intriguing given that the structural analogue ELB-138 demonstrates no such α3 selectivity (see below). It has essentially equivalent, partial agonist efficacy at each subtype (relative efficacy values ranging from 0.38 to 0.53). The compound was anxiolytic in the rat elevated plus-maze, light and dark box, and Vogel conflict tests [147] yet showed no signs of sedation. In addition, the compound retained its anxiolytic efficacy on the elevated plus maze following 6 weeks of treatment [147]. Furthermore, there were no signs of tolerance developing to the anticonvulsant effects of ELB-139 in the rat amygdala kindling model [148, 149]. Finally, ELB-139 increased the concentrations of extracellular 5-HT in the striatum and pre-frontal cortex of rats without altering striatal dopamine concentrations, an effect that was blocked by the benzodiazepine site antagonist flumazenil [150].

In healthy male volunteers, ELB-139 was well tolerated either as single doses (ranging from 200 to 1,200 mg) or multiple doses (200–600 mg three times a day) with no signs of overt sedation [149]. EEG recording were used as a pharmacodynamic readout and ELB-139 dose-dependently reduced the α band and enhanced the β band, whilst changes in the δ and θ bands were limited. Effects were observed even at the lowest multiple-dose administration (200 mg; [149]). Elbion were reportedly evaluating the efficacy of ELB-139 following a

35% CO_2 challenge in subjects with a diagnosis of panic disorder in Germany and The Netherlands with a study start date of May 2006 (ClinicalTrials.gov, identifier NCT00322803) although results from this study have yet to be disclosed. Following the merger of Elbion AG with 4 AZA in December 2006 to form Elbion NV and the subsequent acquisition of this company by BioTie Therapies in November 2008, the drug was no longer listed as part of the company pipeline. Consequently, development of ELB-139 is presumed to have stopped.

2.3.5 Merck Compounds

Of the new generation of α2/α3 subtype-selective partial agonists, only clinical data for the triazolopyridazines MRK-409 and TPA023 and the imidazotriazine TPA023B have been published [125–127]. These compounds all have high and equivalent affinity for each of the four $GABA_A$ subtypes with MRK-409 possessing weak partial agonist activity at the α1 subtype whereas TPA023 and TPA023B both lack efficacy at this subtype (Fig. 5f–h). All three compounds produced non-sedating anxiolytic-like profiles in rodent and non-human primate (squirrel monkey) pre-clinical species [125–127, 151].

MRK-409 was progressed into man based upon its pre-clinical anxioselective profile [125]. However, in single-dose, healthy volunteer studies, the drug surprisingly produced sedation at relatively low doses (1.5 and 2 mg). [^{11}C]Flumazenil PET studies confirmed that this sedation occurred at relatively low levels of $GABA_A$ receptor occupancy (~10%; Fig. 6a). Hence, although this compound (also known as MRK-0343) clearly has a pharmacodynamic profile that distinguishes it from lorazepam [135], these effects were observed at doses of 0.25 and

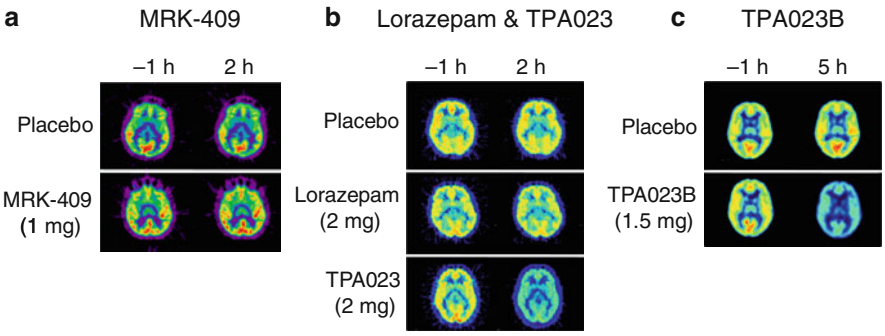

Fig. 6 Representative pseudo-colour positron emission tomography images representing the uptake of [^{11}C]flumazenil in the human brain prior to (−1 h) or either 2 h (MRK-409, lorazepam and TPA023) or 5 h (TPA023B) after oral administration of single doses of either (**a**) 1 mg MRK-409; (**b**) 2 mg lorazepam or 2 mg TPA023; or (**c**) 1.5 mg TPA023B. The times of the first scan after dosing were chosen so they roughly correspond to the T_{max} for each compound [125, 127, 152]

0.75 mg that are assumed to be too low to be of therapeutic relevance for anxiety disorders [125].

The development of TPA023 for the treatment of GAD was halted due to preclinical toxicity (cataract formation) in long-term dosing studies [153]. Nevertheless, combining the data from the three Phase II studies underway at the time development was halted showed that TPA023 gave a significantly greater decrease in the HAM-A score relative to placebo [153]. These clinical studies employed flexible dosing schedules of either 1.5–4.5 mg or 3–8 mg total doses of an extended release (gel extrusion module) formulation, with the main adverse event observed in Phase I studies of the 8-mg dose being dizziness. The corresponding C_{max} achieved using an 8 mg dose of this formulation (25 ng/ml) corresponds to an occupancy in the region of 70% based upon a single-dose normal volunteer PET study (Fig. 6b; [152]). The lack of overt sedation-like adverse events was confirmed in pharmacodynamic studies, in which TPA023 did not affect parameters such as body sway that are associated with sedation [134]. This compound has been subsequently evaluated in a small population of schizophrenia patients, in which it produced a consistent trend towards improved cognitive function [154]. Like TPA023, the back-up compound TPA023B was also able to achieve significant levels of occupancy in the absence of overt sedation [127, 155]. However, development of this compound was halted for as yet undisclosed reasons.

2.4 Additional Properties Of Non-Sedating Anxiolytics

In terms of developing novel, non-sedating anxiolytics, it is pertinent to discuss how a compound could differentiate itself from generic benzodiazepines. Clearly the lack of, or at the very least a much reduced, sedation liability whilst retaining the anxiolytic efficacy of non-selective compounds would be a minimal requirement. However, an additional key factor would be that of abuse liability. Hence, the lack of abuse potential to support a claim for a drug being non-scheduled would clearly be advantageous. In this regard, no compounds have progressed far enough in clinical development for this issue to be addressed. Nevertheless, in drug discrimination studies, the interoceptive cues associated with α2/α3-selective compounds differentiate them from non-selective benzodiazepines [116, 156]. Furthermore, it is encouraging to note that self-injection of TPA023 in baboons does not differ from that of vehicle, even at doses equivalent to complete $GABA_A$ receptor benzodiazepine site occupancy [157]. Moreover, there were no euphoria-like adverse events noted during Phase I studies with this drug [153]. Collectively, these data suggest that subtype-selective compounds may have a lower abuse potential relative to non-selective, full agonist benzodiazepine site modulators [33, 110, 156, 157], although the extent to which this is attributable to a generally lower partial rather than full efficacy profile or is more

related to reduced modulation of a specific $GABA_A$ receptor subtype remains to be clarified.

2.5 *Summary of $GABA_A$ Receptors as a Target for Anxiety Disorders*

There is a strong pre-clinical rationale to support the hypothesis that selective modulation of α2 and/or α3-containing $GABA_A$ receptors should be non-sedating anxiolytics. Moreover, it would appear that even relatively modest efficacy at the α1 subtype translates into sedation in man, based upon observations with bretazenil [120], MRK-409 [125] and EVT-201 (see below). From a medicinal chemistry point of view, α2/α3-preferring compounds can be identified using a selective efficacy rather than a selective affinity approach [104, 117] and although the development of the Merck compounds TPA023 and TPA023B had to be halted due to pre-clinical toxicity issues, other companies are continuing the search for anxioselective compounds acting via the benzodiazepine binding site, although the structures and in vitro and pre-clinical in vivo profiles of these compounds have yet to be disclosed.

NSD-721 is a $GABA_A$ subtype-selective modulator being developed by Neuro-Search in collaboration with GSK for the treatment of social anxiety disorder, with Phase I studies commencing in August 2009. AstraZeneca are evaluating AZD7325 in two proof-of-concept Phase II studies in GAD (ClinicalTrials.gov identifiers NCT00808249 and NCT00807937), both of which have been completed although no data has yet been disclosed. However, a separate study to assess the absorption, distribution, metabolism and excretion of AZD7325 after intravenous and oral administration (ClinicalTrials.gov identifier NCT00940641) has been suspended with the "study withdrawn prior to enrolment due to AZ business decision unrelated to safety" (clinicaltrials.gov). In addition, AstraZeneca have completed Phase I studies in healthy male volunteers using a further compound, AZD6280. These include a comparison of the pharmacodynamic effects (sedation, cognition and EEG) of AZD6280 and lorazepam (NCT00750802) as well as a [^{11}C]flumazenil PET study to measure the occupancy of human brain GABAA receptor by AZD6280 (NCT00681746).

3 $GABA_A$ Receptor Compounds for Sleep Disorders

3.1 *Insomnia*

It has been estimated that around 10–15% of adults suffer from chronic insomnia with an additional 25–35% having occasional difficulties sleeping [158]. In addition,

up to around 40% of individuals with insomnia also having a co-existing psychiatric disorder [159]. The primary goals for insomnia treatments should be an improvement in sleep quality and/or total sleep time along with a reduction in sleep latency and wakefulness after sleep onset. In addition, a decrease in insomnia-related daytime impairments such as attention difficulties, cognitive dysfunction and fatigue are also desirable [160, 161]. The guidelines for the development of hypnotic drugs tend to focus on primary insomnia and use sleep-related metrics. However, sleep disturbances are a common feature of the symptomatology of a variety of additional disorders, such as depression, anxiety and Alzheimer's disease. Accordingly, daytime functional readouts (e.g., depression, anxiety or cognition rating scale) may be more useful parameter for examining efficacy in these disorders in which disrupted sleep is a co-morbidity [162].

A number of treatment options are available for insomnia, including psychological and behavioural therapies as well as pharmacological approaches [163]. With regard to the latter, FDA-approved treatments include several $GABA_A$ receptor benzodiazepine site modulators (Table 1) as well as the melatonin receptor antagonist ramelteon. Of the benzodiazepine site modulators, the short/intermediate half-life benzodiazepines temazepam and triazolam as well as the longer-lasting and less popular estazolam, flurazepam and quazepam are all approved for the treatment of insomnia. However, as regards flurazepam, Hollister commented that "I have always thought that flurazepam, a drug that requires biotransformation to become active, and the metabolite of which has a very long life-span, was badly miscast as an hypnotic" [31]. Other benzodiazepines not approved for insomnia, such as lorazepam or clonazepam, might also be used as hypnotics [161]. In addition to

Table 1 Summary of the principle pharmacokinetic parameters of FDA-approved and experimental $GABA_A$-mediated treatments for insomnia

Drug	Trade name	Status	Structure	Pharmacokinetic parameters[a]	
				T_{max} (h)	$T_{½}$ (h)
Estazolam	ProSom	FDA approved	Benzodiazepine	2	8–24
Flurazepam	Dalmane	FDA approved	Benzodiazepine	2	48–96[b]
Quazepam	Doral	FDA approved	Benzodiazepine	2	15–40
Temazepam	Restoril	FDA approved	Benzodiazepine	0.8	11
Triazolam	Halcion	FDA approved	Benzodiazepine	1.3	2.9
Zolpidem IR	Ambien	FDA approved	Imidazopyridine	1.0	1.0–2.6
Zaleplon	Sonata	FDA approved	Pyrazolopyrimidine	1.1	1.0
Zopiclone	Imovane	Approved outside US	Cyclopyrrolone	1.0	5.6
Eszopiclone	Lunesta	FDA approved	Cyclopyrrolone	1.6	6.5
Adipiplon	N/A	Experimental	Triazolopyrimidine	1	1.1
Indiplon	N/A	Experimental	Pyrazolopyrimidine	1	2–4
EVT-201	N/A	Experimental	Unknown	1–2	3–4
THIP (Gaboxadol)	N/A	Experimental	GABA site agonist	0.5–2	1–2

In addition, the melatonin receptor agonist ramelteon is also approved for the treatment of insomnia

[a]Pharmacokinetic data are derived from: [164–174]

[b]The half-life value for flurazepam refers to the active metabolite *N*1-desalkyl-flurazepam

non-selective benzodiazepines, the so-called "Z-drugs" are also FDA-approved hypnotics. These drugs include zolpidem (Ambien), zaleplon (Sonata) and (*S*)-zopiclone (eszopiclone; Lunesta), the latter of which is the active enantiomer of racemic zopiclone (Imovane), which is approved for use as a hypnotic outside the US. Although a variety of benzodiazepines have been evaluated as hypnotics [164, 175], the preferential use of the benzodiazepines temazepam and triazolam in the treatment of insomnia is based upon the relatively short half-life of these compounds rather than any differences in pharmacology relative to other non-selective benzodiazepines [161].

The Z-drugs (zolpidem, zaleplon, zopiclone and eszopiclone) are all non-benzodiazepine chemical structures but exert their actions by modulating $GABA_A$ receptor function via the benzodiazepine binding site. These compounds are all characterised by relatively short halves in man (~1–6 h, Table 1; [176]), and in the case of zolpidem, a higher affinity for $GABA_A$ receptors containing an $\alpha1$ rather than $\alpha2$, $\alpha3$ or $\alpha5$ subunit, which is of particular relevance to the pharmacology of zolpidem as this subtype is associated with the sedating properties of benzodiazepines [40, 42]. However, although $\alpha1$-preferring compounds are more selectively hypnotic compared to non-selective benzodiazepines, there are no conclusive data to suggest that such compounds have improved efficacy or reduced side effects relative to non-selective benzodiazepines [177–180].

Benzodiazepine and Z-drug hypnotics have the same dependence and abuse potential risks associated with the use of benzodiazepines in the treatment of anxiety disorders (see above). In addition, post-marketing surveillance has recently identified a number of relatively rare, and therefore poorly understood, sleep-associated behaviours related to the use of hypnotics, such as preparing and eating food, making phone calls and sleep-driving [181]. A further issue associated with the pharmacological effects of hypnotics acting via the benzodiazepine recognition site is that of residual daytime sedation ("hangover"), a side effect most noticeable with longer half-life benzodiazepines such as flurazepam or nitrazepam [182]. More specifically, the "hangover" effect relates to residual daytime sleepiness and impairment of cognitive and/or psychomotor functioning the day after drug administration prior to bedtime on the previous day [164]. As a consequence of these effects, patients may have an increased risk of falls and hip fractures, which is of particular concern in the elderly [114], or of traffic accidents. An epidemiological study showed that the risk of an accident increased as the half-life of the hypnotic increased, although even short half-life drugs were also associated with an increased risk [164]. Clearly, in order to minimise the degree of next-day effects, it is desirable to use drugs with a short half-life [182]. In addition, it could be argued that because relatively weak efficacy at the $\alpha1$ subtype is sufficient to produce sedation in man [125], full agonism at this subtype is not only more than sufficient to produce sedation but might also actually contribute to the "hangover" effects associated with such compounds. In this regard, a partial agonist hypnotic, such as EVT-201 (see below), might be of particular interest since it could well be devoid of some of the side effects associated with the full agonist hypnotics.

3.2 *Marketed Non-Benzodiazepine $GABA_A$-Modulating Hypnotics: The "Z-Drugs"*

In the present section, the properties of zolpidem, zaleplon, zopiclone and (*S*)-zopiclone are briefly discussed, particularly with respect to their in vitro $GABA_A$ receptor subtype selectivity profiles since this forms the basis for the comparisons with the newer drugs described in the subsequent section. However, more detailed reviews of these drugs are available elsewhere [165, 166, 183–188, 275–277].

Zolpidem: The imidazopyridine zolpidem (Ambien) is the prototypic α1-preferring sedative hypnotic [189, 190]. It has around 5–14-fold higher affinity for the α1 compared to α2 and α3 subtypes (Fig. 7a). Interestingly, it has very low affinity for the α5 subtype but this is not thought to play a major role in differentiating zolpidem from the non-selective benzodiazepines. Zolpidem is rapidly absorbed with a T_{max} of around 1 h and is cleared with a plasma half-life of 2.5 h or less (Table 1). It reduces the latency to sleep onset and the number of night-time awakenings as well as increasing the duration of sleep [191]. More recently, an extended-release formulation of (zolpidem CR) has been approved and is intended for patients with poor sleep maintenance [194]. This modified release formulation is a bilayer tablet comprising an immediate release portion, designed to induce sleep, as well as a slower release formulation intended to maintain sleep [195–197]. In additional to a bilayer tablet, zolpidem has also been reformulated in a number of other ways, including sublingual and spray formulations [198]. In human sedative drug abusers, neither zolpidem nor zaleplon differed from triazolam with respect to subjective ratings of drug liking [199].

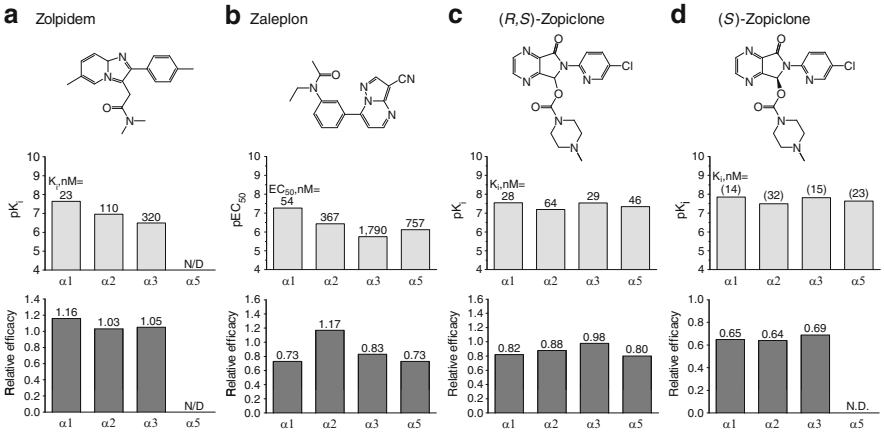

Fig. 7 Structure and in vitro and in vivo efficacy profiles of non-benzodiazepine, approved sedative-hypnotics. Data are taken from: zolpidem, [24]; zaleplon, [191]; (*R*,*S*)-zopiclone, [24]. For (*S*)-zopiclone (eszopiclone), the affinity was calculated based upon the affinity of the racemate (*panel C*) assuming that the (*S*)-enantiomer has an affinity approximately 2-fold higher than the racemate [192] whereas the relative efficacy was calculated by multiplying the relative efficacy values of the racemate (*panel C*) by the ratio of the increase in GABA currents produced by the (*S*)-enantiomer and the racemate [193]. *N.D.* not determined

Zaleplon: Zaleplon is a pyrazolopyrimidine, which, like zolpidem, has modestly higher affinity for the α1 compared to α2 and α3 subtypes (Fig. 7b), although the affinity at the α1 subtype is about twofold lower than zolpidem. Unlike zolpidem, zaleplon also has affinity for the α5 subtype [191, 278]. Interestingly, zaleplon has 10–20-fold higher affinity for γ3 compared to γ2 subunit-containing $GABA_A$ receptors [200], whereas zopiclone has similar affinity for the γ2 and γ3 subtypes and zolpidem has essentially no affinity for the γ3 subtypes. However, the pharmacological significance of the higher γ3 compared to γ2 affinity of zaleplon remains uncertain. It has a short T_{max} and elimation half-life, both of which are in the region of 1 h (Table 1).

Zopiclone and Eszopiclone: Zopiclone (Imovane) is a cyclopyrrolone which, although not approved for use within the US, has been available in Europe for many years. It has no selectivity for the different α subunit-containing $GABA_A$ subtype (Fig. 7c). Zopiclone is a racemate comprising *R*- and *S*-enantiomers with the latter possessing around 50-fold higher affinity for $GABA_A$ receptors than the former and the *S*-enantiomer being around twofold higher affinity than the racemate [192]. Accordingly, in animals, (*S*)-zopiclone (eszopiclone) produces sedation to a similar extent as (*R*,*S*)-zopiclone whereas (*R*)-zopiclone is much less potent [201]. Compared to racemic zopiclone, eszopiclone appears to have a reduction in next-day effects [176, 202] despite the fact that with a half-life in the region of 6 h (Table 1), significant amounts of drug would be predicted to be present at the beginning of the next day.

3.3 Non-Benzodiazepine $GABA_A$-Modulating Hypnotics in Clinical Development

3.3.1 Adipiplon

Adipiplon (NG2-73) is a triazolopyrimidine that was being developed as a hypnotic by Neurogen (recently acquired by Ligand Pharmaceuticals). Although claimed to be an α3-preferring compound [203], when efficacy values are normalised relative to a non-selective benzodiazepine full agonist, the compound has more of a non-selective partial agonist profile (Fig. 8a).

Based on rodent data, the minimally efficacious plasma concentration was predicted to be 2.6 ng/ml which, when corrected for plasma protein binding, gave a target human plasma concentration of 3.4 ng/ml [206]. In a transient insomnia model in healthy volunteers, a 1-mg dose of adipiplon produced a significant decrease in the latency to persistent sleep, with a C_{max} in the region of 2.1 ng/ml and a T_{max} of ∼1 h and a half-life of 1.1 h [167].

The compound was formulated into a bilayer tablet containing immediate- and controlled-release formulations, and the 6- and 9-mg doses were evaluated for efficacy in insomnia relative to zolpidem CR in a 60 patient double-blind Phase II/III study that commenced in July 2008. However, unwanted next-day effects were observed in a dose-dependent manner, and the trial was suspended pending

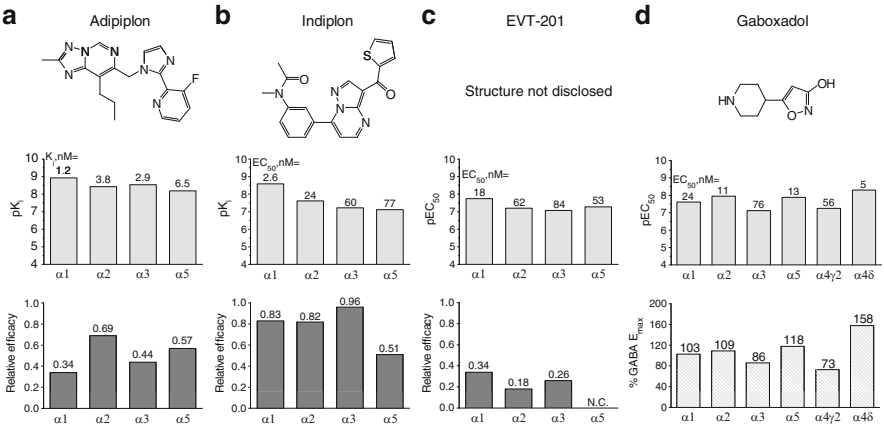

Fig. 8 Structure and in vitro binding and relative efficacy profiles of $GABA_A$ receptor-mediated sedative-hypnotics that exert their effects either via the benzodiazepine binding site (adipiplon, indiplon or EVT-201) or the GABA recognition site (gaboxadol). Data are taken from: adipiplon [203]; indiplon, affinity [204], efficacy [191]; EVT-201 [205]; Gaboxadol [191]. As regards EVT-201, the efficacy was calculated by multiplying the relative efficacy values of zolpidem (Fig. 7a) by the ratio of the increase in GABA currents produced by the EVT-201 and zolpidem [205]. N.C. not calculated

further studies of the bilayer tablet formulation. However, in November 2008, Neurogen announced that further studies with adipiplon were not planned. The Neurogen group have also described NDT 9530021 as an additional α3-preferring compound that is structurally related to adipiplon [207, 208]. This compound has an efficacy profile very similar to that of adipiplon [207] and was a potent hypnotic, anxiolytic and anticonvulsant but, in rats, there was little separation between the anxiolytic, anticonvulsant and motor-impairing doses [207, 208].

3.3.2 Indiplon

Indiplon (also known as NBI 34060) is a pyrazolopyrimidine (Fig. 8b) that was acquired by Neurocrine Biosciences in 1998 from DOV Pharmaceuticals, who themselves had earlier licensed the compound from American Cyanamid Co. (now Wyeth). Indiplon has a degree of selectivity for the α1 subtype of $GABA_A$ receptors [209] that varies from 10–30-fold [204] to a more modest 2–4-fold [191]. The compound has equivalent, full agonist efficacy at each of the α1, α2, α3 and α5 subtypes and accordingly has marked sedative effects in rodents [210]. Indiplon has a short half-life in rodents (1 h) [210] as well as in man (2–4 h) [168, 211]) and in human volunteers with a history of drug abuse, the abuse potential of indiplon did not differ from that of the non-selective benzodiazepine triazolam [212].

Indiplon was developed as both an immediate-release capsule and a modified-release tablet (indiplon-IR and indiplon-MR, respectively) with the latter being

designed to provide an initial dose of drug followed thereafter by a gradual release over an extended period of time. Indiplon-IR has been evaluated in ten studies, of which four were in the elderly whereas indiplon-MR was assessed in four studies, two of which involved the elderly [168, 211]. Hence, most clinical data is derived from the double-blind, placebo-controlled studies using the IR formulation (a total of 3,105 healthy volunteers and insomnia patients between the ages of 18–80) rather than the indiplon-MR formulation (536 subjects aged between 19 and 85) [168, 211]. As regards the MR formulation, in a two-week study in elderly subjects with insomnia, 15 mg indiplon-MR significantly decreased the self-reported latency to sleep onset and the time spent awake after sleep onset whilst increasing the rating of sleep quality and total sleep time [213]. However, the 15 mg MR tablet received a Not Approvable letter in May 2006 with the FDA requesting a long-term safety and efficacy trial in the elderly and it would appear that this formulation is no longer being pursued.

In May 2006, the US Food and Drug Administration (FDA) issued an Approvable letter for the 5 and 10 mg indiplon-IR capsules and requested a reanalysis of some data to support the indications of sleep initiation and middle of the night dosing [168]. In June 2007, a New Drug Application was submitted to the FDA and an Approvable Letter was issued for the 5 and 10 mg doses in December 2007 pending the outcome of a study in the elderly, a study comparing the adverse events of indiplon versus an approved insomnia treatment and finally, a pre-clinical study of indiplon to support its use in the third trimester of pregnancy [211]. At this time, indiplon-IR remains unapproved, and even if approved, it is uncertain if it will have a specific advantage or indication compared to other insomnia medications. Furthermore, although the use of indiplon-IR for post-bedtime or middle of the night dosing may represent a potential advantage [214], zaleplon is already used off-label in a similar manner [186].

3.3.3 EVT-201

EVT-201 was licenced from Roche to Evotec and was originally developed by Roche as a non-sedating anxiolytic based upon pre-clinical data yet the compound caused sedation in human Phase I studies. Although the structure of EVT-201 has not been publically disclosed, it is a partial agonist [205] with a slight, 2–4-fold higher functional affinity at the α1 compared to α2, α3 and α5 $GABA_A$ receptor subtypes (Fig. 8c). It has an active metabolite, desmethyl-EVT-201, which has a functional affinity very similar to the parent but with an efficacy that is around half of that of EVT-201, therefore making it a weak partial agonist [205]. In pre-clinical models, EVT-201 clearly differentiates from non-selective agonists with anxiolytic-like activity in pre-clinical species being observed at doses associated with no motor impairment, whereas in comparison there was an overlap of the anxiolytic and motor-impairing doses of alprazolam [169].

In a Phase II study in adults with primary insomnia, 1.5 and 2.5 mg doses of EVT-201 significantly decreased the wake after sleep onset and increased total sleep time and there was no subjective residual sedation [169]. A second study in elderly

subjects with primary insomnia and daytime sleepiness also demonstrated an increase in the total sleep time [215]. Most notably, EVT-201 differentiates itself from the existing FDA-approved non-selective benzodiazepines as well as the Z-drugs by virtue of having much lower efficacy at each of the four $GABA_A$ receptor subtypes. In addition, since the metabolite has lower efficacy than the parent, this may also further attenuate the effects of EVT-201. Despite this, EVT-201 retains clinical efficacy in adult and elderly patients with primary insomnia [169, 215] and is consistent with other observations that full agonist efficacy at the α1 subtype is not required to produce sedation in man [120, 125]. Furthermore, irrespective of which subtypes are associated with the next-day hangover effects produced by hypnotics that act via the benzodiazepine binding site, it is likely that a partial agonist, such as EVT-201, will have a lower propensity to cause such side effects. Currently, Evotec are seeking a partner for the further development of EVT 201.

3.3.4 Gaboxadol

Gaboxabol (THIP) was originally described in 1977 [20] and is a conformationally restricted analogue of muscimol (Fig. 2a). It readily crosses the blood-brain barrier and was first developed as a potential treatment of acute pain, anxiety, epilepsy, schizophrenia and/or Huntington's disease [216]. Although initially described as a partial agonist, gaboxadol actually has higher efficacy than GABA at the α4βδ subtype (Fig. 8d; [216–218]), which is of particular interest as this subtype of $GABA_A$ receptors is generally thought to be localised extrasynaptically [219–221]. Neuroanatomically, the in vivo effects of gaboxadol may in part be mediated via the neurons of the ventrobasal thalamic nucleus [65, 222]. Furthermore, based on rat data, it would appear that despite the relatively modest α4βδ binding and efficacy selectivity of gaboxadol (Fig. 8d), plasma drug concentrations at a hypnotic dose are consistent with a selective activation of this subtype compared to α1, α2-, α3-, α4- or α5β3γ2 receptors [179], although clearly such calculations encompass a multitude of assumptions and extrapolations that may or may not be applicable to man.

Gaboxadol is rapidly absorbed in man, achieving peak plasma concentrations between 0.5 and 2 h and has a short, 1.5–2 h, half-life [170, 171]. As regards sleep architecture, in man, as in rats, gaboxadol produces a significant increase in slow-wave sleep without suppressing REM [223–225], and the changes in the EEG sleep spectrum produced are markedly different from those associated with compounds acting via the benzodiazepine binding site [191]. The difference in the mechanism of action between gaboxadol and benzodiazepine site modulators is further emphasised by the failure of gaboxadol to produce the same interoceptive cues as either the non-selective benzodiazepines, the marketed hypnotics such as zolpidem, zopiclone, zaleplon, or the experimental hypnotic indiplon [226, 227].

In healthy elderly subjects, gaboxadol significantly decreased the subjective sleep-onset latency as well as increasing sleep intensity and quality as measured by self-assessment [225]. Moreover, there were no signs of next-day effects in this subject population [228]. In a phase-advance model of transient insomnia, gaboxadol decreased

the latency to persistent sleep as well as the wakefulness after sleep onset and increased the total sleep time [229, 230]. In an additional experimental medicine model, namely an impairment in night time sleep produced by a late afternoon nap in healthy young volunteers, gaboxadol increased total sleep time and the amount of slow wave sleep as well as enhancing the subjective sleep quality relative to placebo treated subjects [231].

In primary insomnia, two initial small-scale exploratory, polysomnographic studies in adults aged 18–65 years ($n = 23$ and $n = 38$; [232, 233], respectively) demonstrated efficacy of gaboxadol following short-term (2-day) treatment with either 5 or 15 mg [232] or 10 and 20 mg doses [233]. These observations were extended to a large 2-week Phase III study of 5, 10 and 15 mg doses of gaboxadol in 18–65 year old adults with insomnia ($n = 742$, $n = \sim$140–150/treatment group) with outcome measures (subjective self-assessments recorded using electronic diaries) being compared to placebo and 10 mg zolpidem [234]. Generally speaking, the 15 mg dose of gaboxadol significantly improved a variety of sleep onset and maintenance parameters over the 2-week period; an effect that was comparable to, or slightly less than that observed with zolpidem [234]. Furthermore, although zolpidem shows signs of rebound insomnia following cessation of treatment, gaboxadol did not [234].

In two additional large Phase III studies of efficacy in primary insomnia, wake after sleep onset and latency to persistent sleep were used as the primary end-points in separate 30-night polysomnographic studies to assess the efficacy of gaboxadol in either adults (18–64 years old) given placebo or 10 or 15 mg gaboxadol ($n = \sim$150/group) or elderly subjects ($\geq$65 years old) receiving either placebo or 5 or 10 mg gaboxadol ($n = \sim$150–175/group; [235]). Comparisons were made between parameters averaged over nights 1 and 2 and over nights 29 and 30. Although in both studies the higher dose of gaboxadol was effective at decreasing the wake after sleep onset, it had no effect on the latency to persistent sleep in the adult study, and although the higher dose improved the latency to persistent sleep in the elderly study on Nights 1/2, this was not maintained through to Nights 29/30 [235]. Based upon these equivocal Phase III data and observations of psychiatric side effects at supratherapeutic doses in an abuse liability study involving drug abusers [235], Lundbeck and Merck announced the discontinuation of their joint gaboxadol development programme in March 2007. However, a characteristic effect of gaboxadol is its pronounced effect on slow-wave sleep; yet this drug was being developed in for an indication of primary insomnia, a condition where disrupted slow-wave sleep is not a main feature [236]. Accordingly, the effects of gaboxadol on subjects with impairments in slow-wave sleep remain unknown [162].

3.4 *Summary of $GABA_A$ Receptors as a Target for Hypnotics*

The $GABA_A$ receptor is clearly a well validated target for hypnotics based on clinical experience with the non-selective full agonist benzodiazepines as well as the non-selective and α1-selective non-benzodiazepine "Z-drugs". Of the next-generation of

$GABA_A$-mediated hypnotics, development of the benzodiazepine site modulators adipiplon and indiplon as well as the α4βδ-preferring GABA-site agonist gaboxadol has halted, for a variety of reasons, leaving just EVT-201 in clinical development. EVT-201 differs from other marketed and experimental benzodiazepine site-mediated hypnotics in that it has relatively weak, partial agonist efficacy and only a modest 2–4-fold higher affinity for the α1 compared to α2, α3 and α5 subtypes. Nevertheless, the modest α1 efficacy is sufficient to demonstrate efficacy in insomnia patients [169, 215] and the low efficacy at the α2, α3 and α5 subtypes may well be associated with a reduction in side effects ("hangover").

4 Epilepsy

Epilepsy is defined as the occurrence of two or more seizures not provoked by any identifiable cause. It is not a single disease but rather reflects a variety of disorders arising from many different types of brain dysfunction [237]. Seizures may be either partial (focal), in which the seizure begins in a local area of the brain, or generalised, which involves the whole brain simultaneously. Collectively, the various forms of epilepsy have a lifetime incidence in the region of 15–50/100,000/year and a prevalence of active epilepsy of 3–18/1,000 [238, 239]. Status epilepticus is defined as a single seizure that lasts for over 30 min without a recovery of consciousness, although particularly for tonic–clonic seizures, a period of 5 min should be viewed as status epilepticus and such a seizure may or may not be associated with epilepsy [240, 241]. When not associated with an underlying epilepsy disorder a status epilepticus seizure may occur as a consequence of, for example, an acute illness or insult or as the result of intoxication or drug abuse. Status epilepticus has an average incidence of around 20/100,000/year in the industrialised world and short-term mortality rate in the region of 20% [241, 242]. It has a bimodal age distribution, with the highest incidences being during the first year of life, generally as a result of febrile seizures, and after the age of 60 when cerebrovascular diseases, including acute stroke and haemorrhage, are major risk factors [240, 241]. It has been estimated that 12–30% of adults with a new diagnosis of epilepsy first present with status epilepticus [82].

The goals for the treatment of epilepsy should be complete seizure control, thereby permitting a normal lifestyle, with minimal or preferably no side effects [243–245]. However, up to one-third of epilepsy patients continue to experience seizures or unacceptable side effects and are therefore refractory as defined by the failure of two appropriately chosen and used antiepileptic drugs to prevent seizures [246]. As GABA is the major inhibitory neurotransmitter in the CNS, the strategy of inhibition of the intracellular degrading enzyme GABA transaminase with vigabatrin, thereby increasing intracellular and secondarily extracellular GABA levels, or inhibition of the plasma membrane GABA transporter GAT-1 by tiagabine represent rationale approaches to the development of novel antiepileptic drugs. However, these strategies are relatively non-selective in so far as effects will be

mediated via both the ionotropic $GABA_A$ and metabotropic $GABA_B$ families of receptor [247]. An alternative and more selective approach for increasing GABA-mediated inhibition would be to enhance $GABA_A$ receptor function, especially given that mutations in genes that comprise this receptor family have been associated with idiopathic generalised epilepsies and thereby underscore the importance of these receptors in seizure activity [247–249]. Pharmacologically, the clinical use of barbiturates, particularly phenobarbital [83], and benzodiazepines (see below) in the treatment of epilepsy and status epilepticus further highlight the $GABA_A$ receptor as a pharmacological target for the development of novel antiepileptic drugs. The goal of such drugs should be to maintain efficacy yet at the same time reduce the various liabilities associated with modulating $GABA_A$ receptor function via either barbiturate, benzodiazepine or other recognition site.

4.1 Use of Benzodiazepines in the Treatment of Epilepsy

The anticonvulsant effects of benzodiazepines were reported shortly after their introduction [250], and although a number of new antiepileptic drugs have been launched during the past 15 years, benzodiazepines remain the first-line treatment for status epilepticus and seizures occurring following an anoxic insult [82, 251]. The most common benzodiazepines used in the treatment of status epilepticus are diazepam, lorazepam and midazolam, primarily due to the variety of formulations and dosing routes available for these particular drugs [251]. A comparison of the effectiveness of lorazepam and diazepam in the treatment of status epilepticus tends to favour lorazepam [252–254]. However, differences between benzodiazepines are more related to variations in pharmacokinetic rather than pharmacological properties [251], with lorazepam possibly being preferred as a consequence of its slower rate of distribution within the body [240]. Given the efficacy of non-selective, full agonist benzodiazepines in the treatment of status epilepticus plus their associated sedative and cognition impairing effects, which for the treatment of status epilepticus are actually very desirable features, there would appear to be little potential benefit of subtype-selective $GABA_A$ modulators for this indication.

Despite being a first-line choice for the treatment of status epilepticus, benzodiazepines are unsuitable for the prophylactic treatment of epilepsy due to the development of tolerance, which is defined as the decrease in the pharmacological effects of a drug over a period of time. In terms of antiepileptic drugs, tolerance is associated with an increase in the number and severity of seizures as well as an increased risk of seizures following cessation of treatment. Tolerance to the anticonvulsant effects of benzodiazepines has been demonstrated pre-clinically, and while this may be overcome to a certain extent by increasing the dose, benzodiazepines are, nevertheless, considered unsuitable for the long-term control of epilepsy [255, 256]. In pre-clinical species, the rate at which tolerance develops differs between benzodiazepines [257, 258], and while the mechanisms of tolerance are poorly understood [259, 260], they are thought to involve an uncoupling between

the GABA and benzodiazepine binding sites [261]. Hence, the challenge is to develop a $GABA_A$ receptor modulator that is devoid of the tolerance observed with the non-selective benzodiazepines and which is therefore appropriate for prophylactic use.

4.2 Novel Pre-clinical Benzodiazepine Site Modulator: ELB-138

ELB-138 (Compound 2, [145]) is an imidazolone that was originally evaluated as part of the NINDS Antiepileptic Drug Development Programme [146] and proved to have anticonvulsant activity in a number of mouse, rat and dog seizure models at doses below those associated with motor impairment. It has low affinity for $GABA_A$ receptors (K_i in rat cortex of 4.4 μM [145]) and at the different subtypes has efficacy relative to diazepam ranging from 0.27 to 0.43, with the EC_{50} of between 2.4 and 9.4 μM [262] (Fig. 9). However, it should be noted that these efficacy values may be an underestimate of the true relative efficacy since no apparent maximal effects were observed at a concentration if 10 μM and therefore the relative efficacy and EC_{50} values may well be higher than those reported [262].

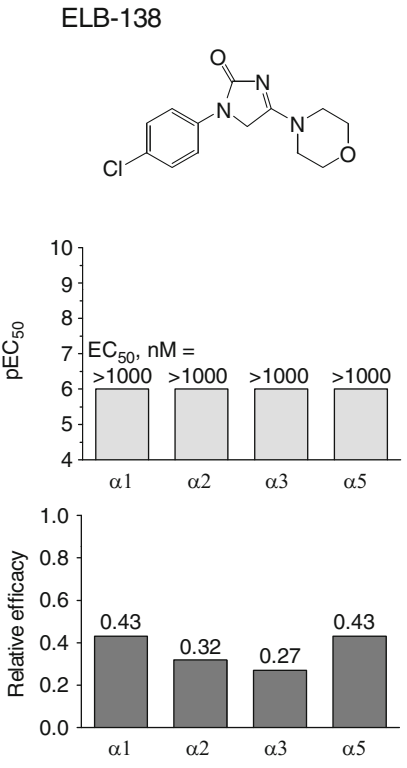

Fig. 9 Structure and in vitro binding and relative efficacy profile of ELB-138. Since the EC_{50} could not be accurately determined from the concentration-effect curves generated in *Xenopus laevis* oocytes, the EC_{50} at each subtype is stated as being >1,000 nM. Data are from [262]

This compound did not produce midazolam-like responding in a squirrel monkey drug-discrimination assay, and in the same species the rate of self-injection declined to vehicle levels when ELB-138 was substituted for cocaine in a self-administration assay, data which collectively suggest that ELB-138 has a low abuse liability [263].

ELB-138 was claimed to be in Phase I clinical development, but its fate in man is unclear. Based upon the observations that in dogs there is no tolerance to the anticonvulsant effects of ELB-138 [264], a pilot clinical study was performed in dogs with epilepsy. This study showed that ELB-138 was efficacious and had a reduced side-effect profile relative to conventional medications with the median plasma levels achieved in this study being in the region of 4,000–7,500 ng/ml 2 h after dosing of 10–15 mg/kg [265]. These plasma concentrations are in the regions of the concentrations (~5,000 ng/ml) required to produce around 20% occupancy of rat brain $GABA_A$ receptors (JA, unpublished observations).

4.3 Neurosteroids: Ganaxolone

Ganaxolone (CCD 1042, 3α-hydroxy-3β-methyl-5α-pregnan-20-one; Fig. 2c) is the 3β-methylated synthetic analogue of the progesterone analogue allopregnanolone (3α-hydroxy-5α-pregnan-20-one; [71]). It was originally developed as an anticonvulsant therapy by CoCensys (which was subsequently acquired by Purdue Pharma), but development was suspended in the late 1990s [266]. This compound has demonstrated anticonvulsant efficacy in a number of pre-clinical models [71, 266, 267] and, importantly, does not appear to demonstrate tolerance after dosing for 7 days [268]. There is evidence that neurosteroids may selectively modulate extrasynaptic, δ subunit-containing $GABA_A$ receptors [65], although the extent to which the anticonvulsant effects of ganaxolone can be attributed to this subtype is unclear. In a preliminary, placebo-controlled clinical trial in treatment-refractory patients with complex partial seizures and that were withdrawn from antiepileptic drugs during pre-surgical evaluation (placebo and ganaxolone group sizes = 28 and 24, respectively), ganaxolone (1,500 mg/day on Day 1 and 1,875 mg/day on Days 2–8) was shown to have antiepileptic-like activity [269]. Three Phase II, open-label, adjunctive studies have also been conducted in a total of 79 paediatric subjects (ranging from 6 months to 15 years old) with refractory seizures using 2–9 week titration followed by an 8 week maintenance period at doses of 12 mg/kg three times a day [270, 271]. There was an improvement in seizure frequency and behavioural improvement, which resulted in 29 subjects continuing ganaxolone treatment [272].

In September 2004, the rights to ganaxolone were acquired by Marinus Pharmaceuticals who are now actively developing this compound [273]. In early 2009, the company announced that ganaxolone (1–2 week escalation to an 8-week maintenance dose of 1,500 mg/day) was safe, well tolerated and efficacious as an adjunctive therapy in adults with partial onset seizures using a reduction in mean weekly

seizure frequency versus placebo as the primary endpoint. Moreover, efficacy was observed within the first week of dosing (www.marinuspharma.com/nr_positive_results.html). However, a common adverse event in clinical studies with ganaxolone is somnolence [273], which in healthy volunteers appears to occur at plasma concentrations greater than 300 ng/ml [271].

When complexed with β-cyclodextrin, ganaxolone showed a marked food-effect following oral dosing in man. More specifically, exposure was 5–15-fold higher when administered in the fed state compared to the fasted state [271], and consequently, Marinus Pharmaceuticals are currently investigating different non-cyclodextrin based formulations of ganaxolone in order to address the issue of variable absorption [245, 271], and a clinical study in infants and young children with infantile spasms is ongoing [271]. In addition, and based upon pre-clinical data, ganaxolone might be relevant to the treatment of catamenial epilepsy, a condition whereby women with epilepsy often report an increased incidence of seizures at the time of menstruation [266, 274].

4.4 Summary of $GABA_A$ Receptors as a Target for Epilepsy

Clearly, the $GABA_A$ receptor is a suitable target for novel anti-epileptic drugs with the challenges being the maintenance of the efficacy produced by the non-selective full agonist benzodiazepines while at the same time eliminating the tolerance associated with such drugs. The desirability of sedative properties is dependent upon the clinical use of the drug with sedation being advantageous for the treatment of status epilepticus whereas it is undesirable for prophylactic use as an antiepileptic drug. As regards modulation of the $GABA_A$ receptor via the benzodiazepine site, there does not appear to be a particular subtype associated with tolerance and it is unlikely that a compound targeting a single subtype would possess the anticonvulsant efficacy of a non-selective full agonist [44]. However, the variety of other recognition sites on the $GABA_A$ receptor provide alternative, non-benzodiazepine site-mediated opportunities for modulating receptor function as does the distinction between synaptic and extrasynaptic localisation; yet these avenues remain relatively unexplored in the context of anticonvulsant activities.

5 Overall Summary

The clinical use of drugs that alter $GABA_A$ receptor function, including barbiturates and, most notably, benzodiazepines as anxiolytics, hypnotics and anticonvulsants provides ample proof of concept that the $GABA_A$ receptor represents a validated target for the development of next-generation treatments for GAD, insomnia and epilepsy. Moreover, the multiple $GABA_A$ receptor subtypes that mediate these clinical effects suggests that targeting specific subtypes of this receptor population

may well lead to novel drugs with an improved clinical profile. Clearly, the desired profile varies according to the indication. Hence, a non-sedating anxiolytic is needed for the treatment of GAD, a drug with rapid onset and no next-day hangover is required for insomnia and other sleep disorders, and for epilepsy, a drug that does not develop tolerance, thereby permitting its use as a prophylactic, is desired. Some of these aspects are more related to pharmacokinetics rather than pharmacology (for example, the speed of onset for a hypnotic). However, as regards pharmacology, there is a strong pre-clinical rationale for the development of anxioselective compounds based upon selective activation of the α2/α3 subtypes. With respect to epilepsy, although the molecular mechanisms underlying the tolerance to the anticonvulsant effects of benzodiazepines remain poorly understood and represent a major obstacle to next generation antiepileptic drugs that act via this binding site, there are a variety of different binding sites on the $GABA_A$ receptor that may provide alternative mechanisms of anticonvulsant activity which might be devoid of tolerance (although this remains to be demonstrated). As the level of understanding of the non-benzodiazepine binding sites, such as the neurosteroid and loreclezole/anaesthetic sites develops [17, 74], so novel approaches to the modulation of the $GABA_A$ receptor will emerge [103] and should encourage further exploration of the pharmacological possibilities inherent in $GABA_A$ subtype-selective modulation.

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