Drugs of the Future 2006, 31(2): 151-162

CCC: 0377-8282/2006
DOI: 10.1358/dof.2005.031.02.963107

Review Article

The $GABA_A$ receptor as a potential target for the treatment of cognitive dysfunction

Karen A. Maubach
BTG International, 10 Fleet Place, Limeburner Lane, London EC4M 7SB, UK. e-mail: karen.maubach@btgplc.com

CONTENTS

Abstract

Drug treatment of Alzheimer's disease and other dementias represents a major unmet medical need, with no preventive or curative therapies available. The currently approved therapies are directed toward symptomatic relief of impaired cognition but have modest utility. In the quest for novel cognition enhancers, the observation that classical benzodiazepines (BZ) are amnesic highlighted the $GABA_A$ receptor as a potential therapeutic target. In contrast to the amnesic BZ agonists which potentiate $GABA_A$ receptor function, the BZ inverse agonists, which attenuate $GABA_A$ receptor function, have been shown to improve performance in animal models of memory formation. Unfortunately, such nonselective ligands are also anxiogenic and proconvulsant and therefore cannot be used clinically to treat dementia. More recently, novel ligands have been developed that demonstrate functional selectivity for and high inverse agonism at the α5 $GABA_A$ receptor subtype. These α5-selective inverse agonists enhance learning and memory in animal models but are devoid of the adverse effects associated with other $GABA_A$ receptor subtypes. In a preliminary clinical study, an α5-selective inverse agonist was reported to prevent alcohol-induced memory deficits in healthy volunteers. If the preclinical efficacy and safety profiles continue to translate into the clinic, these recently developed, functionally selective α5 inverse agonists may present attractive new therapeutic options for dementia.

Current treatment of dementia and the need for novel therapies

Alzheimer's disease (AD) is the most common neurodegenerative disease and cause of dementia in the elderly, with estimates indicating that 40% of people over 80 years of age are afflicted. Given the aging of the population, it is predicted that the incidence of AD will increase 3-fold within the next 50 years if no therapeutic advances are made (1). AD is characterized clinically by global cognitive dysfunction, including memory loss, behavior and personality changes, and impairment in the performance of activities of daily living. In addition to AD, millions of patients worldwide suffer from mild cognitive impairment (MCI), with a single specific area of impairment in cognition, and are at high risk of developing AD and other dementias (2). Consequently, these dementias represent a major public health and economic problem, stressing the importance of therapeutic intervention.

Significant research efforts are focusing on an extensive array of diverse approaches to tackle such dementias. These include antioxidants, drugs for improving neurotransmission, hormonal replacement, γ- and β-secretase inhibitors, β-amyloid (Aβ)-clearing agents (Aβ immunization, disruption of Aβ fibrils, modulation of cholesterol-mediated Aβ transport), nonsteroidal antiinflammatory drugs (NSAIDs), microtubule-stabilizing drugs and kinase inhibitors (3). Despite the great progress made in this field in the last few decades, currently there are no neuroprotective therapies for AD or MCI that slow progression or delay the onset of the disease.

At present, two types of palliative therapies have been developed which are directed toward the symptomatic improvement of impaired cognition. The first of these are the cholinesterase inhibitors, such as donepezil, which increase acetylcholine activity by inhibiting the enzyme that hydrolyzes acetylcholine, acetylcholinesterase (4). This approach is based on the cholinergic hypothesis that the cognitive symptoms of AD derive from reductions in acetylcholine synthesis, degeneration of cholinergic neurons and depletion of muscarinic and nicotinic receptors (5).

The Cochrane Dementia Group has conducted meta-analyses on the efficacy and safety of the cholinesterase inhibitors donepezil, rivastigmine and galantamine (6-8).

In these studies, data were extracted from randomized, double-blind, parallel-group, placebo-controlled trials in patients with mild to moderate AD. Each drug had a similar effect at 6 months on global and cognitive rating scales. These findings were supported by previous trials reporting benefits with donepezil (9, 10). More recently, a 24-week, multicenter, randomized, double-blind, placebo-controlled study that enrolled patients with early-stage AD showed significant treatment benefits for donepezil (11). In addition, rivastigmine treatment has been demonstrated to improve cognitive performance for up to 2 years in patients with AD compared to no treatment or placebo treatment (12). The main disadvantages of cholinesterase inhibitors are that they produce minimal improvements in cognition and global function in a limited number of patients, are potentially beneficial only where there is a cholinergic deficit, and are associated with cholinergic side effects such as anorexia, nausea, diarrhea and vomiting.

Last year, the U.S. Food and Drug Administration approved the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine for the treatment of dementia in patients with moderate to severe AD (13), although memantine has actually been prescribed for the treatment of neurodegenerative conditions in Europe since 1982. Memantine is a noncompetitive, moderate-affinity, phencyclidine-site NMDA receptor antagonist that is thought to protect neurons from glutamate-mediated excitotoxicity without preventing physiological activation of NMDA receptors (14). The reduction in NMDA activation by memantine has been shown to prevent the loss of cholinergic neurons (15) and amyloid neurotoxicity (16), and to be neuroprotective during acute ischemia (17). However, the precise mechanism of action has yet to be determined, since the published literature from preclinical studies with NMDA antagonists is confusing. Although low doses of memantine promote synaptic plasticity and preserve mnemonic function in animal models of AD (18), another NMDA receptor antagonist, MK-801, has been shown to inhibit synaptic plasticity and impair learning (19). Furthermore, memantine has also been reported to impair spatial learning in animal models (20). Memantine is reported to block other ionotropic receptors, including $5\text{-}HT_3$ and acetylcholine (nicotinic) receptors, indicating that it has a plethora of biological effects.

In a randomized, double-blind, placebo-controlled, parallel study in 166 patients with severe dementia, memantine improved both primary outcome measures (the Clinical Global Impression of Change and the Behavioral Rating Scale for Geriatric Patients) (21). Another randomized, placebo-controlled trial in 252 patients with advanced AD showed that memantine treatment was associated with less deterioration in cognitive measures compared with placebo (22). Furthermore, preliminary results from a 6-month, multicenter, randomized, controlled trial of memantine in combination with donepezil in 400 patients with moderate to severe AD showed a significant benefit over a donepezil-placebo combination (23). Despite these encouraging results, more studies are necessary to determine the overall benefits of memantine in the treatment of cognitive dysfunction, either as monotherapy or in combination therapy.

The development of a compound with a favorable benefit/risk profile, greater or at least equivalent efficacy, and improved safety and tolerability compared to the currently available therapies would provide significant benefit to patients with dementia. Therefore, the search for novel therapies to reliably and safely enhance memory formation in humans continues.

Modulators of the $GABA_A$ receptor influence cognitive performance

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian central nervous system. It is estimated that over 20-50% of all central synapses utilize GABA as their transmitter, depending on the brain region (24). Three main pharmacologically and structurally distinct GABA receptor subtypes have been identified: the ionotropic bicuculline-sensitive $GABA_A$ receptor, the metabotropic baclofen-sensitive $GABA_B$ receptor and the ionotropic $GABA_C$ receptor found primarily in the retina (25).

$GABA_A$ receptors are ligand-gated chloride ion channels which contain allosteric sites in addition to the agonist binding site, through which other agents can modulate receptor function (26). Positive modulators of the $GABA_A$ receptor, such as the classical benzodiazepines (BZ; *e.g.*, diazepam, lorazepam), the neuroactive steroids (*e.g.*, pregnenolone, allopregnenolone) and the barbiturates (*e.g.*, pentobarbital), are prescribed as sedatives, muscle relaxants, anxiolytics and anticonvulsants. Conversely, negative $GABA_A$ modulators, such as BZ inverse agonists (*e.g.*, the β-carbolines DMCM **[1]** and FG-7142 **[2]**; Fig. 1) and the neuroactive steroids (*e.g.*, pregnenolone sulfate, dehydroepiandrosterone sulfate), have anxiogenic and convulsant effects (27-30). Neutral modulators (*e.g.*, the BZ-site antagonist flumazenil) bind to the $GABA_A$ receptor but have no intrinsic activity, although flumazenil antagonizes the effects of both positive and negative $GABA_A$ modulators that act via the BZ site.

Positive $GABA_A$ modulators have utility in inducing not only sedation and muscle relaxation prior to surgical procedures but also amnesia (31). The amnesic effects of the positive modulators in animals and man are well established (32-34), and thus it has been hypothesized that the negative modulators may have the opposite effect, being procognitive (35, 36). Indeed, improvements in learning and memory dysfunction have been reported after dehydroepiandrosterone administration to individuals with low dehydroepiandrosterone sulfate levels (37), but further studies have failed to demonstrate a significant effect of these steroids on cognitive abilities (38, 39). Thus, the therapeutic use of neurosteroids for cognitive dysfunction is of uncertain value (40). Similarly, nonselective BZ inverse agonists cannot be used to treat neurological disorders associated with cognitive impairment because of their profound anxiogenic and proconvulsant liabilities (41).

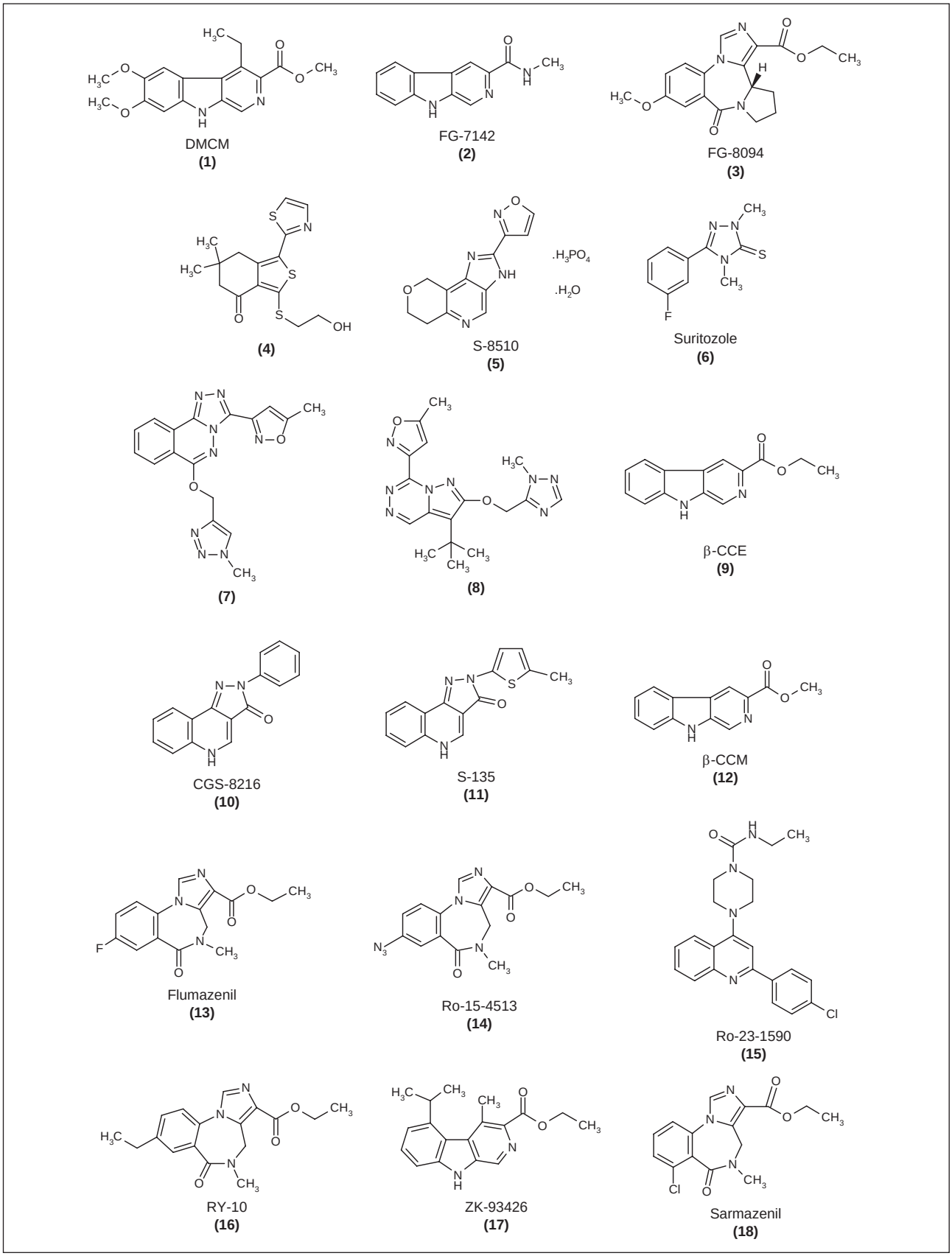

Fig. 1. $GABA_A$ benzodiazepine-site ligands.

α5 Inverse agonists for cognitive enhancement

The discrete localization of the different BZ-sensitive $GABA_A$ receptor subtypes provides restricted targets within the brain, offering the potential to improve the therapeutic window for negative $GABA_A$ receptor modulators.

At present 19 $GABA_A$ receptor subunits have been identified, including α1-α6, β1-β3, γ1-γ3, δ, ε, θ, ρ1-ρ3 and π (42). The vast majority of $GABA_A$ receptors in the brain contain α, β and γ subunits and the BZ binding site occurs at the interface of a γ2 and either an α1, α2, α3 or α5 subunit, with the α subunit being of particular importance in determining the pharmacological effects (24). Studies using molecular genetic or pharmacological approaches have indicated that $GABA_A$ receptors containing an α1 subunit account for the sedative and muscle relaxant effects of nonselective BZ agonists, whereas those with an α2 or α3 subunit mediate the anxiolytic and anticonvulsant effects (43-45).

In situ hybridization and immunocytochemistry studies have shown that the α5 $GABA_A$ receptor subtype is preferentially expressed at high levels in the hippocampus, although it is also found to a lesser degree within the cortex and the olfactory bulb (46, 47). The receptors are thought to be located extrasynaptically (48) and play a role in tonic inhibition of hippocampal CA1 pyramidal neurons (49). Furthermore, the hippocampal α5-mediated tonic conductance is highly sensitive to anesthetics, which are known to produce a pronounced amnesic effect *in vivo* (50). Visualization of the α5 subunit by [^{11}C]-Ro-15-4513 (51) using positron emission tomography (PET) in monkey brain has confirmed a relatively high accumulation in the fronto-temporal limbic regions (52). Rich immunostaining for the α5 subunit protein has also been demonstrated in the hippocampal formation in human brain (53). Although immunoprecipitation studies have shown that α5-containing receptors comprise only 5% of the total brain $GABA_A$ receptors, up to 20% of $GABA_A$ receptors in the rat hippocampus are of the α5 subtype (54). Thus, α5 subtype-selective inverse agonists may enhance neuronal activity in the hippocampus, but have limited effects in other brain regions.

Although many regions of the CNS are involved in cognitive functions, the hippocampus clearly plays a key role (55). This claim is supported by studies indicating that hippocampal size is correlated with memory performance (56), and lesions of this brain region can impair spatial memory in animal models (57) and humans (58). Furthermore, it has been suggested that BZ agonists may exert their amnesic effects by modulating hippocampal function, since the anterograde rather than retrograde amnesia is similar to deficits induced by hippocampal lesions in animals and man (59).

It has therefore been hypothesized (35, 60) that α5 subtype-selective inverse agonists could be procognitive but devoid of anxiogenic and proconvulsant effects associated with activity at other $GABA_A$ receptor subtypes.

Utility of transgenic mouse models in elucidating α5 $GABA_A$ receptor function

Gene-targeting approaches in mice have proved to be very useful to gain clues about receptor function, and this is indeed the case for the $GABA_A$ receptor family (61). The gene disruption approach has been utilized to investigate the function of α5-containing $GABA_A$ receptors (62). Homozygous $\alpha5^{-/-}$ mice display no overt phenotypic abnormalities, have a normal life span and breed normally. Since 17% of flumazenil binding sites are lost in the $\alpha5^{-/-}$ mice, indicating no compensatory upregulation by other α subunits, the $\alpha5^{-/-}$ mice provide a useful tool to validate the role of the α5 subunit in cognition. Indeed, a reduced tonic inhibitory current amplitude was observed in the CA1 region of hippocampal brain slices and cultured neurons from $\alpha5^{-/-}$ mice compared to wild-type littermates, confirming the involvement of the α5 $GABA_A$ receptor subtype in hippocampal neurophysiology (49). Furthermore, a decrease in the amplitude of the inhibitory postsynaptic GABAergic currents was observed in the same region of brain slices from $\alpha5^{-/-}$ mice (62), indicating that although α5-containing $GABA_A$ receptors are predominantly reported to be expressed extrasynaptically (48), they may also be localized within the synapse.

Long-term potentiation (LTP) is a stimulus frequency-dependent form of synaptic plasticity found in several brain regions, particularly the hippocampus (63). LTP, induced by brief tetanic stimulation followed by a theta burst or a theta burst alone, was not altered in $\alpha5^{-/-}$ mice (62). However, another form of synaptic plasticity associated with memory function, paired-pulse facilitation of field excitatory postsynaptic potential (EPSP) amplitudes, was significantly enhanced in these mice.

There is also evidence of enhanced power and increased stability in the frequency domain of 20-80 Hz (gamma) oscillations in hippocampal slices of $\alpha5^{-/-}$ mice, suggesting that α5 $GABA_A$ receptors are associated with hippocampal gamma frequency network activity (64). Such temporal characteristics of network rhythms have been proposed to underlie the coordination of neuronal activity, and more specifically, cognitive processes (65). It is possible that α5 $GABA_A$ receptors might affect the dynamic response of such rhythms to changes in network drive and thereby influence cognitive performance.

The Morris water maze is a model of spatial learning that focuses on hippocampus-dependent cognition (66). In this task, the animal is required to locate the position of a submerged platform in a pool and the improvement in memory is quantified by calculating the difference between the time taken to find the hidden platform on trial 1 compared with subsequent trials. Performance in this task is disrupted by BZ agonists (67), and temporal gene expression profiling 1 h after spatial learning has revealed many changes, including the downregulation of α5 subunits (68). The $\alpha5^{-/-}$ mice show significantly improved performance in the water-maze model of spatial learning in comparison to wild-type controls. Furthermore, performance in non-hippocampus-dependent learning tasks and the elevated plus maze model of anxiety was unal-

tered and the $\alpha5^{-/-}$ mice do not exhibit spontaneous seizures, indicating that selective reduction in activity at the $\alpha5$ $GABA_A$ receptor subtype is not anxiogenic or convulsant (62).

To further clarify the *in vivo* relevance of the $\alpha5$ $GABA_A$ receptor and its modulation by BZ-site ligands, a histidine-to-arginine point mutation was introduced in position 105 of the murine $\alpha5$ subunit gene, which renders the $\alpha5$ $GABA_A$ receptors diazepam-insensitive. The sedative, anticonvulsant and anxiolytic-like activity of diazepam was not impaired in these $\alpha5$(H105R) mice, although, surprisingly, the point mutation resulted in a selective reduction of $\alpha5$ $GABA_A$ receptors in hippocampal pyramidal cells (> 30%). Furthermore, a form of hippocampus-dependent learning, trace fear conditioning, was facilitated in these mutant mice (69). In contrast, but consistent with findings in the $\alpha5^{-/-}$ mice, behaviors that are unaffected by hippocampal lesions, such as delayed fear conditioning and unconditioned freezing, were unaltered in the mutant mice.

These data suggest a central role for $\alpha5$-containing $GABA_A$ receptors in hippocampus-dependent cognitive processes, and further support the rationale that the $\alpha5$ $GABA_A$ receptor represents a promising therapeutic target for the treatment of dementia.

Functionally selective α5 inverse agonists

In addition to BZs, a variety of different chemical classes have been shown to bind to the BZ site on the $GABA_A$ receptor. These include the β-carbolines such as DMCM **(1)** and FG-7142 **(2)** (Fig. 1) (70), pyridinoindoles (71, 72), pyrazoloquinolinones (73, 74), triazolophthalazines (75), imidazopyridines (76) and imidazobenzodiazepines (77). Selective $\alpha5$ inverse agonism may be achieved in either of two ways: by binding selectivity (*i.e.*, a high-affinity $\alpha5$ inverse agonist which has very low affinity for the other $GABA_A$ receptor subtypes) or through functional selectivity (*i.e.*, an inverse agonist at the $GABA_A$ $\alpha5$ receptor with low efficacy at the other $GABA_A$ receptor subtypes) (78).

Of the many different structural classes of $GABA_A$ receptor ligands, relatively few are reported to exhibit binding selectivity for $\alpha5$-containing $GABA_A$ receptors compared to the other receptor subtypes. These include the imidazobenzodiazepines, such as FG-8094 **(3)** (Fig. 1) (79-81), and some diazepam analogues (82). Such compounds are presumed to exert their *in vivo* actions via the $\alpha5$ receptor population, although the efficacy of these compounds at the $\alpha1$, $\alpha2$ and $\alpha3$ subtypes has not been thoroughly examined. In the absence of such an analysis, it is not possible to attribute the *in vivo* effects of $\alpha5$-selective compounds solely to the $\alpha5$-containing $GABA_A$ receptors. However, a series of thiophenes have been identified which display a significant degree of binding selectivity for the $\alpha5$ subtype compared to the other $GABA_A$ subtypes, and several analogues exhibit high $\alpha5$ receptor inverse agonism (83). The binding affinities of these compounds have been determined using displacement of [^{3}H]-flumazenil binding from mammalian fibroblast L(tk) cells stably expressing human $GABA_A$ receptors, and the efficacy profiles have been determined as the modulation of an EC_{20} response to GABA using whole-cell patch-clamp recording in the same expression system. In particular, compound **4** (Fig. 1) displays high affinity for the $\alpha5$ receptor, with a K_i of 1.6 nM and 10-13-fold binding selectivity over the other subtypes. Furthermore, it shows functional selectivity, being a full inverse agonist at the $\alpha5$ receptor (–51%), a low-efficacy inverse agonist at the $\alpha1$ subtype (–21%) and an antagonist at $\alpha2$ (–1%) and $\alpha3$ (–3%) subtypes (83, 84).

Several compounds which do not display binding selectivity have been shown to have weak inverse agonist activity at the $\alpha5$ $GABA_A$ receptor subtype. S-8510 **(5)** (Fig. 1) has been characterized as a partial inverse agonist at the $\alpha5$ subtype. MDL-26479 (suritozole **[6]**; Fig. 1) inhibited [^{3}H]-flumazenil binding in mouse cortex (0.22 ± 0.05 mg/kg), indicating that it binds to BZ sites in this region. The functional selectivity of these compounds for the $\alpha5$ subtype is suggested by the lack of mechanism-based liabilities (proconvulsant, convulsant or anxiogenic effects) associated with other subtypes (85, 86).

Compounds possessing higher $\alpha5$-selective inverse agonist efficacy have now been described (87-89). These include compound **7** (88) and compound **8** (Fig. 1). The binding affinities and efficacy profiles of these compounds were again determined using displacement of [^{3}H]-flumazenil binding and whole-cell patch-clamp recording, respectively, in L(tk) cells expressing human $GABA_A$ receptors. Inhibition of [^{3}H]-flumazenil binding showed that **7** binds with equivalent subnanomolar affinity (0.6-0.9 nM) to the BZ binding site in recombinant $GABA_A$ receptors containing $\alpha1$, $\alpha2$, $\alpha3$ and $\alpha5$ subunits. Compound **7** has high efficacy at the $\alpha5$ subtype (–40%) and lower efficacy at the $\alpha1$, $\alpha2$ and $\alpha3$ subtypes, showing modest inverse agonism at the $\alpha1$ subtype (–18%) and very weak efficacy at the $\alpha2$ and $\alpha3$ subtypes (+13% to –7%) (90).

The binding affinity of **8** was also similar at $\alpha1$, $\alpha2$, $\alpha3$ and $\alpha5$ subtypes, with K_i values ranging from 0.8 to 1.4 nM. Compound **8** has efficacy at the $\alpha5$ subtype approaching full inverse agonism (–55%). In contrast, the efficacy at the $\alpha1$, $\alpha2$ and $\alpha3$ subtypes is much less, compound **8** being essentially an antagonist at the $\alpha2$ subtype (+6%) and a weak partial inverse agonist at the $\alpha1$ and $\alpha3$ subtypes (–16 and –9%, respectively) (89).

Profile in preclinical models of cognition

The cellular mechanism underlying hippocampally mediated cognitive processes may involve long-term changes in synaptic efficacy, such as LTP (63). The induction of LTP in the mammalian hippocampus has been shown to be disrupted by BZ receptor agonists such as diazepam (91). The nonselective BZ-site inverse agonists DMCM, β-CCE **(9)**, CGS-8216 **(10)**, S-135 **(11)**, MDL-26479 and S-8510 (Fig. 1) have been shown to enhance LTP of the Schaffer collateral/commissural fiber-CA1 synapses in mouse, guinea pig or rat hippocampal

slices (85, 86, 92). The augmentation of LTP produced by such inverse agonists can be antagonized by concomitant application of flumazenil, confirming that this effect is mediated via a specific action on the BZ $GABA_A$ receptor complex (93). Although α5-containing receptors are enriched within the hippocampus, they are still outnumbered by the combined population of α1-, α2- and α3-containing $GABA_A$ receptors, which the nonselective inverse agonists will also affect. However, more recently, the functionally selective α5 inverse agonist **7** has also been demonstrated to robustly enhance LTP (90). It is therefore tempting to conclude that the α5 subtype is solely responsible for the enhanced LTP observed with nonselective compounds. Finally, similar effects have been observed *in vivo*; chronic treatment with the inverse agonist β-CCM **(12)** (Fig. 1) has been shown to induce LTP of evoked synaptic responses recorded from the dentate gyrus of the hippocampus of freely moving rats (94).

Conditioned learning tasks are often used in rodents because of ease of control of the conditioned stimulus. These tasks, in which rodents are trained to associate a number of diffuse cues (odor, lights and sounds) that together make up the conditioning context with a brief aversive stimulus (electric shock), are a reliable hippocampus-dependent test thought to model human explicit memory (95). Furthermore, contextual memory is also an aspect of memory affected in various cognitive disorders including AD (96). The BZ-site inverse agonists DMCM, flumazenil **(13)**, Ro-15-4513 **(14)**, Ro-23-1590 **(15)**, RY-10 **(16)** and ZK-93426 **(17)** (Fig. 1) have all been shown to attenuate scopolamine-induced contextual memory impairment in this task (97). However, as yet, the effects of compounds showing greater selectivity for the α5 $GABA_A$ receptor subtype have not been reported using this task.

Spatial memory is particularly sensitive to AD and the normal aging process (98), and therefore spatial learning tasks, such as the Morris water maze, are among the most commonly used tests for detecting potential cognition enhancers (99). Rats treated with the muscarinic cholinergic antagonist scopolamine show a longer latency to find the platform, signifying a retention deficit, compared to animals treated with saline. The cholinesterase inhibitor donepezil can reverse behavioral deficits induced by either cholinergic depletion (basal forebrain lesions) or systemic scopolamine (100). Furthermore, coadministration of a $GABA_A$ inverse agonist (a 1,5-naphthyridine) with a noneffective dose of donepezil attenuates scopolamine-induced deficits in this model, indicating the potential benefit of combining these two therapeutic agents to enhance memory (101). The BZ-site ligands DMCM, ZK-93426, flumazenil, Ro-15-4513 and S-8510 were able to reverse learning or retention deficits in this model in the absence of a cholinesterase inhibitor (86, 102-104). In addition, these ligands did not impair locomotor function at the doses tested (97).

A limitation of using scopolamine to model cognitive disorders is that the impairment is likely induced by the postsynaptic blockade of muscarinic acetylcholine M_1 receptors, while in AD cholinergic contributions to cognitive dysfunction are likely due to the destruction of presynaptic cholinergic neurons (105). Furthermore, scopolamine antagonizes muscarinic receptors throughout the brain and periphery, in contrast to the anatomically selective deficits associated with AD. Nonselective BZ inverse agonists, for example CGS-8216 and β-CCM, have been shown to enhance spatial learning in the water maze in the absence of a cholinergic deficit (106). These effects were blocked by the administration of flumazenil, providing evidence that the BZ site is involved in these effects (107).

The effectiveness of α5-selective inverse agonists to enhance performance in this model has been investigated. Rats dosed with compound **4** at 0.3 mg/kg i.p. showed a significant improvement in performance between trial 1 and trial 2 compared with vehicle-treated animals ($p < 0.05$), with no effect on swim speed. At this dose the occupancy of $GABA_A$ α5 receptors ($[^3H]$-L-655708 binding) was 40%. Furthermore, compound **4** had no proconvulsant or convulsant effects in mice at a 10-fold higher dose (3 mg/kg i.p.), where 91% of α5 $GABA_A$ receptors were occupied (84).

Similarly, compound **7** also produced a significant enhancement in performance in the water maze in two separate experiments. In the low-dose experiment, **7** had a minimal effective dose of 0.3 mg/kg, which corresponded to a BZ site occupancy of ~25%, whereas in the high-dose experiment, the compound was effective at all doses tested (0.3, 1 and 3 mg/kg). In addition, the ability of **7** (3 mg/kg p.o.) to increase the mean difference score was prevented by the nonselective BZ antagonist flumazenil (10 mg/kg i.p.) given 30 min prior to trial 1, confirming that these behavioral effects of compound **7** are mediated via the BZ site of $GABA_A$ receptors (90). Moreover, **7** was not convulsant or proconvulsant and did not produce kindling upon chronic dosing, even at doses producing > 90% occupancy. Furthermore, compound **7** did not show an anxiogenic-like profile in the elevated plus maze, nor did it impair motor behaviors in mice as measured by performance on the rotarod assay (90). Finally, compound **8** has similarly been reported to enhance performance in this task in a dose-dependent manner (89), supporting the suggestion that α5-containing $GABA_A$ receptors play a role in hippocampus-dependent cognitive processes.

Clinical efficacy

Profile in healthy volunteers

To date, a very limited number of clinical investigations have been conducted with BZ inverse agonists. Many studies with nonselective ligands have been terminated due to unacceptable side effects. For example, FG-7142, a nonselective partial inverse agonist that is anxiogenic in animals, was reported to cause intense unrest and intolerable tension when administered orally to

volunteers. They subjects experienced tachycardia and were profoundly agitated, and these symptoms were rapidly reversed by an intravenous BZ agonist (30, 41). Similarly, Ro-15-3505 (sarmazenil **[18]**; Fig. 1), another nonselective partial inverse agonist, caused anxiety symptoms (restlessness and fear) in a placebo-controlled study in 12 healthy volunteers (108). These adverse effects have clearly hampered the investigation of the procognitive properties of nonselective inverse agonists in man.

However, the β-carboline ZK-93426, which is often referred to as a BZ receptor antagonist (109), has been shown to reverse the cognitive impairments that result from cholinergic hypofunction and enhance cognition when given alone in healthy volunteers. This intrinsic activity of ZK-93426 is thought to result from a weak partial inverse agonist component. In a study in 36 healthy volunteers, scopolamine significantly impaired performance on attentional and visual vigilance tasks, in psychomotor tasks assessed by measuring tapping speed (related to gross motor abilities) and a pegboard task (related to fine motor abilities), and in a task to measure working memory, the Pauli test. ZK-93426 (0.04 mg/kg i.v.) partially antagonized most of these effects of scopolamine on memory and attention (110). In another study in healthy volunteers, ZK-93426 (0.04 mg/kg i.v.) was well tolerated and exhibited no side effects. At this dose, ZK-93426 improved performance in two cognitive tasks that estimate concentration and attentional processes (the logical reasoning task and the pictures difference task), while also increasing vigilance. ZK-93426 also produced a slight improvement in some memory processes, especially long-term retrieval of a visual memory test and verbal list learning (111).

Utility against alcohol-induced memory deficits in healthy volunteers

The α5-containing $GABA_A$ receptor has also been implicated in the effects of alcohol in animal models, including reinforcement, motor impairment and sedation (112, 113). Alcohol has been shown to indirectly increase the actions of GABA and other agents that act on the $GABA_A$ receptor complex, such as BZ-site modulators (114), by increasing the allosteric coupling between the $GABA_A$ receptor and the chloride ion channel (115, 116). Alcohol has been shown in preclinical models to inhibit LTP (117). The BZ-site inverse agonist Ro-15-4513 has been shown to block the anxiolytic-like effects of ethanol and attenuate the behavioral intoxication produced by alcohol (118, 119). In addition, a functionally selective α5 inverse agonist has been shown to dose-dependently decrease ethanol (10%) self-administration at high doses, where 90% receptor occupancy was achieved (120).

Recently, the ability of compound **7** to reverse the effects of alcohol intoxication on memory was investigated in a double-blind, placebo-controlled, crossover study in 12 normal male volunteers who regularly drank between 20 and 40 units of alcohol a week. Two hours after dosing with either **7** (4 mg) or placebo, subjects were given alcohol (0.8 g/kg). An hour later, they were given a printed list of 20 words, given 2 min to memorize them and told that they would be asked to recall them after 30 min. The number of words recalled correctly was significantly larger after **7**, indicating that this compound reversed the effects of alcohol-induced memory impairment. Furthermore, the subjects reported no adverse effects except for mild dizziness compared to placebo; compound **7** did not cause anxiety or other significant side effects (121).

Potential utility in patients with dementia

Numerous pharmaceutical companies are believed to be pursuing the $GABA_A$ receptor as a target for the treatment of various dementias. The improvement in performance in healthy volunteers offers hope that these inverse agonists will also be effective in patients with cognitive deficits. α5-Containing $GABA_A$ receptors display an unaltered pharmacological profile for $GABA_A$ receptor ligands in rodent models of senile dementia of the Alzheimer's type produced by chemically or electrolytically lesioning the basal forebrain (122). PET scans of the brains of living Alzheimer's patients provide evidence for the preservation of cortical BZ binding sites (123). Furthermore, *post mortem* studies of brains from AD patients indicate that $GABA_A$ α5 receptor subtypes are present in the hippocampus at levels similar to those in non-AD patients, even at terminal stages of the disease (124), while many other neurotransmitter receptors are clearly reduced (125). More recently, *in situ* hybridization and quantitative densitometry techniques have shown that hippocampal α5 subunit mRNA was reduced by 32% between no cognitive impairment and moderate cognitive impairment groups, and by 35% between no cognitive impairment and probable AD groups (126). These data, in contrast to previous reports, suggest that reductions in α5 $GABA_A$ receptor subunit mRNAs occur early in AD and perhaps implicate this subtype in the progression of cognitive decline. Thus, further studies are necessary to establish whether the anatomic target for α5 subtype-selective inverse agonists is sufficiently preserved in patients suffering from cognitive impairment.

Conclusions

The most widely used palliative treatments for AD and other dementias are the cholinesterase inhibitors and memantine. However, sales of these compounds have been relatively low given the potential market size, emphasizing the limitations of drugs with minimal efficacy and prominent adverse effects.

This manuscript briefly reviews the status of an alternative effort to enhance cognition through the negative modulation of the $GABA_A$ receptor ion channel with BZ inverse agonists. Nonselective inverse agonists of the BZ site do enhance cognitive performance but cannot be used therapeutically because of profound anxiogenic and

convulsant adverse effects. However, it is hoped that inverse agonists that demonstrate selectivity for the $\alpha 5$ receptor subtype may offer an improved safety window. The more novel approach of identifying subtype-selective compounds based on selective efficacy rather than selective affinity appears to be paying off. Preclinical results are highly supportive, with functionally selective compounds (such as **7**) enhancing memory in animal models while minimizing potential side effects. Preliminary clinical studies where this $\alpha 5$-selective inverse agonist has been demonstrated to prevent deficits in word recall induced by alcohol in healthy volunteers are also encouraging.

However, it remains to be seen whether such inverse agonists will enhance cognitive performance where the deficit results from a pathological condition, and whether they will address the most appropriate phases of memory formation to provide benefit in AD and other dementias. Furthermore, studies to date have only used acute treatments and therefore it is not known whether chronic treatment protocols would increase or decrease drug efficacy.

Given that multiple combinations of co-factors produce variants of age-related cognitive decline (127), combination therapies may have greater efficacy than single-drug treatments. The demonstration that a GABA inverse agonist and an acetylcholinesterase inhibitor have a synergistic effect on cognitive ability when administered concomitantly supports this view. Furthermore, lower doses of each agent may result in efficacy similar to or greater than that observed with higher doses of either agent alone, thereby reducing adverse effect liability, a key factor when treating aged individuals, where only drugs with an excellent safety profile may be considered.

In summary, if the efficacy and safety profiles of the $\alpha 5$-selective $GABA_A$ inverse agonists observed in animal models are reflected in the clinical situation, the $GABA_A$ receptor would appear to represent an attractive novel target for the treatment of dementia.

Acknowledgements

The author wishes to thank Mark Chambers for his contribution to the preparation of Figure 1.

References

1. Sisodia, S.S. *Alzheimer's disease: Perspectives for the new millennium*. J Clin Invest 1999, 104: 1169-70.

2. Almkvist, O., Basun, H., Backman, L. et al. *Mild cognitive impairment—An early stage of Alzheimer's disease?* J Neural Transm Suppl 1998, 54: 21-9.

3. Pereira, C., Agostinho, P., Moreira, P.I. et al. *Alzheimer's disease-associated neurotoxic mechanisms and neuroprotective strategies*. Curr Drug Targets – CNS Neurol Disord 2005, 4: 383-403.

4. Grutzendler, J., Morris, J.C. *Cholinesterase inhibitors for Alzheimer's disease*. Drugs 2001, 61(1): 41-52.

5. Sunderland, T. *Alzheimer's disease, cholinergic therapy and beyond*. Am J Geriatr Psychiatry 1998, 6: 56-63.

6. Birks, J.S., Grimley Evans, J., Iakovidou, V. et al. *Rivastigmine for Alzheimer's disease*. Cochrane Database Syst Rev 2000, 4: CD001191.

7. Birks, J.S., Melzer, D., Beppu, H. *Donepezil for mild and moderate Alzheimer's disease*. Cochrane Database Syst Rev 2000, 4: CD001190.

8. Olin, J., Schneider, L. *Galantamine for Alzheimer's disease*. Cochrane Database Syst Rev 2002, 3: CD001747.

9. Winblad, B., Engedal, K., Soininen, H. et al. *A 1-year, randomized, placebo-controlled study of donepezil in patients with mild to moderate AD*. Neurology 2001, 57: 489-95.

10. Feldman, H., Gauthier, S., Hecker, J. et al. *A 24-week, randomized, double-blind study of donepezil in moderate to severe Alzheimer's disease*. Neurology 2001, 57: 613-20.

11. Seltzer, B., Zolnouni, P., Nunez, M. et al. *Efficacy of donepezil in early-stage Alzheimer disease: A randomized placebo-controlled trial*. Arch Neurol 2004, 61: 1852-6.

12. Grossberg, G., Irwin, P., Satlin, A. et al. *Rivastigmine in Alzheimer disease: Efficacy over two years*. Am J Geriatr Psychiatry 2004, 12: 420-31.

13. Molinuevo, J.L., Llado, A., Rami, L. *Memantine: Targeting glutamate excitotoxicity in Alzheimer's disease and other dementias*. Am J Alzheimers Dis Other Demen 2005, 20(2): 77-85.

14. Scarpini, E., Scheltens, P., Feldman, H. *Treatment of Alzheimer's disease: Current status and new perspectives*. Lancet Neurol 2003, 2: 539-47.

15. Wenk, G.L., Zajaczkowski, W., Danysz, W. *Neuroprotection of acetylcholinergic basal forebrain neurons by memantine and neurokinin B*. Behav Brain Res 1997, 83(1-2): 129-33.

16. Miguel-Hidalgo, J.J., Alvarez, X.A., Cacabelos, R. et al. *Neuroprotection by memantine against neurodegeneration induced by beta-amyloid(1-40)*. Brain Res 2002, 958(1): 210-21.

17. Chen, H.S., Wang, Y.F., Rayudu, P.V. et al. *Neuroprotective concentrations of the N-methyl-D-aspartate open-channel blocker memantine are effective without cytoplasmic vacuolation following post-ischaemic administration and do not block maze learning or long-term potentiation*. Neuroscience 1998, 86(4): 1121-32.

18. Rogawski, M.A., Wenk, G.L. *The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease*. CNS Drug Rev 2003, 9(3): 275-308.

19. Spangler, E.L., Bresnahan, R.L., Garofalo, P. *NMDA receptor channel antagonism by dizocilpine (MK-801) impairs performance of rats in aversively motivated complex maze tasks*. Pharmacol Biochem Behav 1991, 40(4): 949-58.

20. Creeley, C.E., Olney, J.W., Taylor, G.T. *Memantine impairs retention of a spatial learning task at low doses in adult rats*. Soc Neurosci Abst 2004, Abst 219.5.

21. Winblad, B., Poritis, N. *Memantine in severe dementia: Results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine)*. Int J Geriatr Psychiatry 1999, 14(2): 135-46.

22. Reisberg, B., Doody, R., Stoffler, A. et al. *Memantine in moderate-to-severe Alzheimer's disease*. New Engl J Med 2003, 348(14): 1333-41.

23. Tariot, P.N., Farlow, M.R., Grossberg, G.T. et al. *Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: A randomized controlled trial*. JAMA – J Am Med Assoc 2004, 291(3): 317-24.

24. Sieghart, W. *Structure and pharmacology of gamma-aminobutyric acid A receptor subtypes*. Pharmacol Rev 1994, 47: 181-234.

25. Bormann, J. *The 'ABC' of GABA receptors*. Trends Pharmacol Sci 2000, 21: 16-9.

26. Smith, G.B., Olsen, R.W. *Functional domains of GABA-A receptors*. Trends Pharmacol Sci 1995, 16: 162-8.

27. Argyropoulos, S.V., Nutt, D.J. *The use of benzodiazepines in anxiety and other disorders*. Eur Neuropsychopharmacol 1999, 9(6): 391-2.

28. Park-Chung, M., Malayev, A., Purdy, R.H. et al. *Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites*. Brain Res 1999, 830(1): 72-87.

29. Rupprecht, R., Holsboer, F. *Neuroactive steroids: Mechanisms of action and neuropsychopharmacological perspectives*. Trends Neurosci 1999, 22: 410-6.

30. Dorow, R. *FG7142 and its anxiety inducing effects in humans*. Br J Clin Pharmacol 1987, 23: 781-2.

31. Williams, T.J., Bowie, P.E. *Midazolam sedation to produce complete amnesia for bronchoscopy: 2 Years' experience at a district general hospital*. Respir Med 1999, 93: 361-5.

32. Stewart, S.A. *The effects of benzodiazepines on cognition*. J Clin Psychiatry 2005, 66: 9-13.

33. Lister, R.G. *The amnestic action of benzodiazepines in man*. Neurosci Biobehav Rev 1985, 9: 87-94.

34. Ghoneim, M.M., Mewaldt, S.P. *Benzodiazepines and human memory: A review*. Anaesthesiology 1990, 72: 926-38.

35. MacLeod, A.M., Dawson, G.R., Pillai, G. et al. *Discovery of $GABA_A$ $\alpha5$ subtype selective inverse agonists as cognition enhancers*. 221st ACS Natl Meet (April 1-5, San Diego) 2001, Abst MEDI 187.

36. Sarter, M., Bruno, J.P., Berntson, G.G. *Psychotogenic properties of benzodiazepine receptor inverse agonists*. Psychopharmacology 2001, 156: 1-13.

37. Wolkowitz, O.M., Reus, V.I., Roberts, E. et al. *Antidepressant and cognition-enhancing effects of DHEA in major depression*. Ann NY Acad Sci 1995, 774: 337-9.

38. Bloch, M., Schmidt, P.J., Danaceau, M.A. et al. *Dehydroepiandrosterone treatment of midlife dysthymia*. Biol Psychiatry 1999, 45: 1533-41.

39. Wolf, O.T., Koster, B., Kirschbaum, C. et al. *A single administration of dehydroepiandrosterone does not enhance memory performance in young healthy adults, but immediately reduces cortisol levels*. Biol Psychiatry 1997, 42: 845-8.

40. Vallée, M., Mayo, W., Le Moal, M. *Role of pregnenolone, dehydroepiandrosterone and their sulfate esters on learning and memory in cognitive aging*. Brain Res Rev 2001, 37: 301-12.

41. Dorow, R., Horowski, R., Paschelke, G. et al. *Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors*. Lancet 1983, 2: 98-9.

42. Simon, J., Wakimoto, H., Fujita, N. et al. *Analysis of the set of $GABA_A$ receptor genes in the human genome*. J Biol Chem 2004, 279: 41422-35.

43. McKernan, R.M., Rosahl, T.W., Reynolds, D.S. et al. *Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor $\alpha1$ subtype*. Nat Neurosci 2000, 3: 587-92.

44. Rudolph, U., Möhler, H. *Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics*. Annu Rev Pharmacol Toxicol 2004, 44: 475-98.

45. Atack, J.R., Hutson, P.H., Collinson, N. et al. *Anxiogenic properties of an inverse agonist selective for $\alpha3$ subunit-containing $GABA_A$ receptors*. Br J Pharmacol 2005, 144: 357-66.

46. Wisden, W., Laurie, D.J., Monyer, H. et al. *The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon*. J Neurosci 1992, 12: 1040-62.

47. Thompson, C.L., Bodewitz, G., Stephenson, F.A. et al. *Mapping of $GABA_A$ receptor $\alpha5$ and $\alpha6$ subunit-like immunoreactivity in rat brain*. Neuroscience 1992, 144: 53-6.

48. Brünig, I., Scotti, E., Sidler, C. et al. *Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro*. J Comp Neurol 2002, 443: 43-55.

49. Caraiscos, V.B., Elliott, E.M., You-Ten, K.E. et al. *Tonic inhibition in hippocampal pyramidal neurons is regulated by $\alpha5$ subunit containing GABA-A receptors*. Proc Natl Acad Sci USA 2004, 101: 3662-7.

50. Caraiscos, V.B., Newell, J.G., You-Ten, K.E. et al. *Selective enhancement of tonic GABAergic inhibition in murine hippocampal neurons by low concentrations of the volatile anesthetic, isoflurane*. J Neurosci 2004, 24: 8454-8.

51. Hadingham, K.L., Wingrove, P., Le Bourdelles, B. et al. *Cloning of cDNA sequences encoding human $\alpha2$ and $\alpha3$ gamma-aminobutyric acid A receptor subunits and characterization of benzodiazepine pharmacology of recombinant $\alpha1$, $\alpha2$, $\alpha3$ and $\alpha5$ containing human gamma-aminobutyric acid A receptors*. Mol Pharmacol 1993, 43: 970-5.

52. Maeda, J., Suhara, T., Kawabe, K. et al. *Visualization of $\alpha5$ subunit of $GABA_A$/benzodiazepine receptor by [^{11}C]Ro15-4513 using positron emission tomography*. Synapse 2003, 47: 200-8.

53. Wainwright, A., Sirinathsinghji, D.J.S., Oliver, K.R. *Expression of $GABA_A$ receptor $\alpha5$ subunit-like immunoreactivity in human hippocampus*. Mol Brain Res 2000, 80: 228-32.

54. McKernan, R.M., Quirk, K., Prince, R. et al. *GABA-A receptor subtypes immunopurified from rat brain with alpha subunit-specific antibodies have unique pharmacological properties*. Neuron 1991, 7(4): 667-76.

55. Gilman, S., Newman, S. *The hypothalamus and limbic system.* In: Essentials of Clinical Neuroanatomy and Neurophysiology. J. Manter and A. Gatz (Eds.). Davis, Philadelphia, 1992, 219-25.

56. Golomb, J., Kluger, A., De Leon, M. *Hippocampal formation size in normal human aging: A correlate of delayed secondary memory performance*. Learn Memory 1994, 1: 45-54.

57. Kowalska, D. *Effects of hippocampal lesions on spatial delayed responses in dog*. Hippocampus 1995, 5: 363-70.

58. Corkin, S. *What's new with the amnesic patient H.M.?* Nat Rev Neurosci 2002, 3(2): 153-60.

59. McNaughton, N., Morris, R.G.M. *Chlordiazepoxide, an anxiolytic benzodiazepine, impairs place navigation in rats*. Behav Brain Res 1991, 24: 39-46.

60. Maubach, K. *GABA-A receptor subtype selective cognition enhancers*. Curr Drug Targets – CNS Neurol Disord 2003, 2: 233-9.

61. Rosahl, T. *Target validation/animal models*. Curr Drug Targets – CNS Neurol Disord 2003, 2: 207-12.

62. Collinson, N., Kuenzi, F.M., Jarolimek, W. et al. *Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the α5 subunit of the GABA-A receptor*. J Neurosci 2002, 22: 5572-80.

63. Bliss, T.V., Collingridge, G.L. *A synaptic model of memory: Long-term potentiation in the hippocampus*. Nature 1993, 361: 31-9.

64. Towers, S.K., Glovelli, T., Traub, R.D. et al. *α5 Subunit containing GABA-A receptors affect the dynamic range of hippocampal gamma frequency oscillations in vitro*. J Physiol 2004, 559: 721-8.

65. Mann, E.O., Paulsen, O. *Mechanisms underlying gamma ('40 Hz') network oscillations in the hippocampus–A mini-review*. Prog Biophys Mol Biol 2005, 87: 67-76.

66. Morris, R. *Developments of a water-maze procedure for studying spatial learning in the rat*. J Neurosci Methods 1984, 11: 47-60.

67. Arolfo, M.P., Brioni, J.D. *Diazepam impairs place learning in the Morris water-maze*. Behav Neural Biol 1991, 55(1): 131-6.

68. Cavallaro, S., D'Agat, V., Manickam, P. et al. *Memory-specific temporal profiles of gene expression in the hippocampus*. Proc Natl Acad Sci USA 2002, 99: 16279-84.

69. Crestani, F., Keist, R., Fritschy, J.M. et al. *Trace fear conditioning involves hippocampal α5 GABA-A receptors*. Proc Natl Acad Sci USA 2002, 99: 8980-5.

70. Lippke, K.P., Schunack, W.G., Wenning, W. et al. *Beta-carbolines as benzodiazepine receptor ligands. 1. Synthesis and benzodiazepine receptor interaction of esters of beta-carboline-3-carboxylic acid*. J Med Chem 1983, 26: 499-503.

71. Trudell, M.L., Basile, A.S., Shannon, H.E. et al. *Synthesis of 7,12-dihydropyrido[3,4-b:5,4-b']diindoles. A novel class of rigid, planar benzodiazepine receptor ligands*. J Med Chem 1987, 30: 456-8.

72. Trudell, M.L., Lifer, S.L., Tan, Y.-C. et al. *Synthesis of substituted 7,12-dihydropyrido[3,2-b:5,4-b']diindoles. Rigid planar benzodiazepine receptor ligands with inverse agonist/antagonist properties*. J Med Chem 1990, 33: 2412-20.

73. Yokoyama, N., Ritter, B., Neubert, A.D. *2-Arylpyrazolo[4,3-c]quinolin-3-ones: Novel agonist, partial agonist, and antagonist of benzodiazepines*. J Med Chem 1982, 25: 337-9.

74. Fryer, R.I., Zhang, P.M., Rios, R. et al. *Structure-activity relationship studies at the benzodiazepine receptor (BZR): A comparison of the substituent effects of pyrazoloquinolinone analogs*. J Med Chem 1993, 36: 1669-73.

75. Tarzia, G., Occelli, E., Toja, E. et al. *6-(Alkylamino)-3-aryl-1,2,4-triazolo[3,4-a]phthalazines. A new class of benzodiazepine receptor ligands*. J Med Chem 1988, 31: 1115-23.

76. Trapani, G., Franco, M., Ricciardi, L. et al. *Synthesis and binding affinity of 2-phenylimidazo[1,2-a]pyridine derivatives for both central and peripheral benzodiazepine receptors. A new series of high-affinity and selective ligands for the peripheral type*. J Med Chem 1997, 40: 3109-18.

77. Zhang, P., Liu, R., McKernan, R. M. et al. *Studies of novel imidazobenzodiazepine ligands at $GABA_A$/BzR subtypes: Effect of C(3) substituents on receptor subsite selectivity*. Med Chem Res 1995, 5: 487-95.

78. Atack, J.R. *The benzodiazepine binding site of $GABA_A$ receptors as a target for the development of novel anxiolytics*. Exp Opin Invest Drugs 2005, 14: 601-18.

79. Quirk, K., Blurton, P., Fletcher, S. et al. *[3H]L-655,708, a novel ligand selective for the benzodiazepine site of GABA-A receptors which contain the α5 subunit*. Neuropharmacology 1996, 35: 1331-5.

80. Liu, R., Hu, R.J., Zhang, P. et al. *Synthesis and pharmacological properties of novel 8-substituted imidazobenzodiazepines: High affinity, selective probes for α5-containing GABA-A receptors*. J Med Chem 1996, 39: 1928-34.

81. Huang, Q., He, X., Ma, C. et al. *Pharmacophore/receptor models for GABA(A)/BzR subtypes (α1β3γ2, α5β3γ2, and α6β3γ2) via a comprehensive ligand-mapping approach*. J Med Chem 2000, 43: 71-95.

82. Yu, S., Ma, C., He, X. et al. *Studies in the search for α5 subtype selective agonists for $GABA_A$/BzR sites*. Med Chem Res 1999, 9: 71-88.

83. Chambers, M.S., Atack, J.R., Bromidge, F.A. et al. *6,7-Dihydro-2-benzothiophen-4(5H)-ones: A novel class of GABA-A α5 receptor inverse agonists*. J Med Chem 2002, 45: 1176-9.

84. Chambers, M.S., Atack, J.R., Broughton, H.B. et al. *Identification of a novel selective GABA-A α5 receptor inverse agonist which enhances cognition*. J Med Chem 2003, 46: 2227-40.

85. Miller, J.A., Dudley, M.W., Kehne, J.H. et al. *MDL 26,479: A potential cognition enhancer with benzodiazepine inverse agonist-like properties*. Br J Pharmacol 1992, 107(1): 78-86.

86. Kawasaki, K., Eigyo, M., Ikeda, M. et al. *A novel benzodiazepine inverse agonist, S-8510, as a cognitive enhancer*. Prog Neuropsychopharmacol Biol Psychiatry 1996, 20(8): 1413-25.

87. Street, L.J., Sternfeld, F., Jelley, R.A. et al. *Synthesis and biological evaluation of 3-heterocyclyl-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-a]phthalazines and analogues as subtype-selective inverse agonists for the GABA-A α5 benzodiazepine binding site*. J Med Chem 2004, 47: 3642-57.

88. Sternfeld, F., Carling, R.W., Jelley, R.A. et al. *Selective, orally active gamma-aminobutyric acid-A α5 receptor inverse agonists as cognition enhancers*. J Med Chem 2004, 47: 2176-9.

89. Chambers, M.S., Atack, J.R., Carling, R.W. et al. *An orally bioavailable, functionally selective inverse agonist at the benzodiazepine site of GABA-A α5 receptors with cognition enhancing properties*. J Med Chem 2004, 47: 5829-32.

90. Dawson, G.R., Maubach, K.A., Collinson, N. et al. *An inverse agonist selective for α5 subunit-containing GABA-A receptors*

enhances cognition. J Pharmacol Exp Ther 2005, Dec 2 [E publication ahead of print].

91. Del Cerro, S., Jung, M., Lynch, G. *Benzodiazepines block long-term potentiation in slices of hippocampus and piriform cortex*. Neuroscience 1992, 49(1): 1-6.

92. Seabrook, G.R., Easter, A., Dawson, G.R. et al. *Modulation of long-term potentiation in CA1 region of mouse hippocampal brain slices by GABA-A receptor benzodiazepine site ligands*. Neuropharmacology 1997, 36(6): 823-30.

93. Yasui, M., Kawasaki, K., Matsushita, A. et al. *Benzodiazepine inverse agonists augment long-term potentiation in CA1 and CA3 of guinea pig hippocampal slices*. Neuropharmacology 1993, 32: 127-31.

94. Rössler, A.S., Rüthrich, H., Krug, M. et al. *Long-lasting potentiation effects induced in rats by kindling with an inverse agonist of the benzodiazepine receptor*. Exp Brain Res 2002, 146: 77-85.

95. Kim, J.J., Fanselow, M.S. *Modality specific retrograde amnesia of fear*. Science 1992, 256: 675-7.

96. McEwen, B.S. *Possible mechanism for atrophy of the human hippocampus*. Mol Psychiatry 1997, 2: 255-62.

97. DeLorey, T.M., Lin, R.C., McBrady, B. et al. *Influence of benzodiazepine binding site ligands on fear-conditioned contextual memory*. Eur J Pharmacol 2001, 426(1-2): 45-54.

98. Moore, T.E., Richard, B., Hood, J. *Aging and the coding of spatial memory*. J Gerontol 1984, 39: 210-2.

99. Merlini, L., Pinza, M. *Trends in searching for new cognition enhancing drugs*. Prog Neuro-Psychopharm Biol Psychiatry 1989, 13: 61-75.

100. Ogura, H., Kosasa, T., Araki, S. et al. *Pharmacological properties of donepezil hydrochloride (Aricept), a drug for Alzheimer's disease*. Nippon Yakurigaku Zasshi 2000, 115(1): 45-51.

101. Cassella, J.V., Villalobos, A., Rajachandran, L. (Pfizer Products Inc.; Neurogen Corp.). *Combination use of acetylcholinesterase inhibitors and $GABA_A$ inverse agonists for the treatment of cognitive disorders*. WO 2002032412.

102. Jensen, L.H., Stephens, D.N., Sarter, M. et al. *Bidirectional effects of beta-carbolines and benzodiazepines on cognitive processes*. Brain Res Bull 1987, 19: 359-64.

103. McNamara, R.K., Skelton, R.W. *Assessment of cholinergic contribution to chlordiazepoxide-induced deficits of place learning in the Morris water-maze*. Pharmacol Biochem Behav 1992, 41: 529-38.

104. Prather, P.L., Forster, M.J., Lal, H. *Learning and memory-enhancing effects of Ro 15-4513: A comparison with flumazenil*. Neuropharmacology 1992, 31: 299-306.

105. Sarter, M., Bruno, J.P. *Transsynaptic stimulation of cortical acetylcholine and enhancement of attentional functions: A rational approach for the development of cognition enhancers*. Behav Brain Res 1997, 83: 7-14.

106. McNamara, R.K., Skelton, R.W. *Benzodiazepine receptor antagonists flumazenil and CGS8216 and inverse agonist beta-CCM enhance spatial learning in the rat: Dissociation from anxiogenic actions*. Psychobiology 1993, 21: 101-8.

107. Venault, P., Chapouthier, G., Simiand, J. et al. *Enhancement of performance by methyl beta-carboline-3-carboxylate, in learning and memory tasks*. Brain Res Bull 1987, 19(3): 365-70.

108. Gentil, V., Tavares, S., Gorenstein, C. *Acute reversal of flunitrazepam effects by Ro15-1788 and Ro15-3505: Inverse agonism, tolerance and rebound*. Psychopharmcology 1990, 100: 54-9.

109. Jensen, L.H., Petersen, E.N., Braestrup, C. et al. *Evaluation of the beta-carboline ZK 93426 as a benzodiazepine receptor antagonist*. Psychopharmacology (Berlin) 1984, 83(3): 249-56.

110. Duka, T., Ott, H., Rohloff, A. et al. *The effects of a benzodiazepine receptor antagonist beta-carboline ZK-93426 on scopolamine-induced impairment on attention, memory and psychomotor skills*. Psychopharmacology 1996, 123(4): 361-73.

111. Duka, T., Edelmann, V., Schutt, B. et al. *Beta-carbolines as tools in memory research: Human data with the beta-carboline ZK 93426*. Psychopharmacol Ser 1988, 6: 246-60.

112. June, H.L., Harvey, S.C., Foster, K.L. et al. *$GABA_A$ receptors containing $\alpha 5$ subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: An extended ethanol reward circuitry*. J Neurosci 2001, 21: 2166-77.

113. Platt, D.M., Duggan, A., Spealman, R.D. et al. *Contribution of $\alpha 1$GABA-A and $\alpha 5$GABA-A receptor subtypes to the discriminative stimulus effects of ethanol in squirrel monkeys*. JPET 2005, 313: 658-67.

114. Korpi, E.R. *Role of GABA-A receptors in the actions of alcohol and in alcoholism: Recent advances*. Alcohol Alcohol 1994, 29(2): 115-29.

115. Harris, R.A. *Ethanol actions on multiple ion channels: Which are important?* Alcohol Clin Exp Res 1999, 23: 1563-70.

116. Olsen, R.W., Chang, C.S., Li, G. et al. *Fishing for allosteric sites on $GABA_A$ receptors*. Biochem Pharmacol 2004, 68: 1675-84.

117. Blitzer, R.D., Gil, O., Landau, E.M. *Long-term potentiation in rat hippocampus is inhibited by low concentrations of ethanol*. Brain Res 1990, 537: 203-8.

118. Suzdak, P.D., Glowa, J.R., Crawley, J.N. et al. *A selective imidazobenzodiazepine antagonist of ethanol in the rat*. Science 1986, 234: 1243-7.

119. Lingford-Hughes, A., Hume, S.P., Feeney, A. et al. *Imaging the GABA-benzodiazepine receptor subtype containing the $\alpha 5$-subunit in vivo with [^{11}C]Ro15 4513 positron emission tomography*. J Cereb Blood Flow Metab 2002, 22: 878-89.

120. Stephens, D.N., Pistovcakova, J., Worthing, L. et al. *Role of GABA-A $\alpha 5$-containing receptors in ethanol reward: The effects of targeted gene deletion, and a selective inverse agonist*. Eur J Pharmacol 2005, 526: 240-50.

121. Wilson, S.J., Besson, M., Lingford-Hughes, A. et al. *Antagonising alcohol's actions by an inverse agonist at the $\alpha 5$ subtype of the GABA-A receptor in humans*. Br Assoc Psychopharmacol 2005, 19(5): MD07.

122. Sarter, M., Steckler, T. *Spontaneous exploration of a 6-arm radial tunnel maze by basal forebrain lesioned rats: Effects of the benzodiazepine receptor antagonist beta-carboline ZK 93426*. Psychopharmacology (Berlin) 1989, 98: 193-202.

123. Meyer, M., Koeppe, R.A., Frey, K.A. et al. *Positron emission tomography measures of benzodiazepine binding in Alzheimer's disease*. Arch Neurol 1995, 52: 314-7.

124. Howell, O., Atack, J.R., Dewar, D. et al. *Density and pharmacology of α5 subunit containing GABA-A receptors are preserved in hippocampus of Alzheimer's disease patients*. Neuroscience 2000, 98(4): 669-75.

125. Mizukami, K., Ikonomovic, M.D., Grayson, D.R. et al. *Immunohistochemical study of $GABA_A$ receptor β2/3 subunits in the hippocampal formation of aged brains with Alzheimer-related neuropathologic changes*. Exp Neurol 1997, 147: 333-45.

126. Rissman, R.A., Bennett, D.A., Armstrong, D.M. *Subregional analysis of $GABA_A$ receptor subunit mRNAs in the hippocampus of older persons with and without cognitive impairment*. J Chem Neuroanat 2004, 28: 17-25.

127. McDonald, R.J. *Multiple combinations of co-factors produce variants of age-related cognitive decline: A theory*. Can J Exp Psychol 2002, 56(3): 221-39.