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Synthesis of chiral $GABA_A$ receptor subtype selective ligands as potential agents to treat schizophrenia as well as depression

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This paper is dedicated to Professor Gordon Gribble for his outstanding contributions to heterocyclic chemistry

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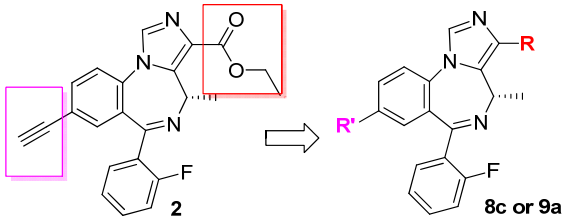
Abstract

A series of novel imidazobenzodiazepine analogs of the lead chiral ligand SH-053-2'F-*S*-CH_3 (**2**), an α2/α3/α5 (Bz)GABA (A)ergic receptor subtype selective ligand, which reverses PCP-induced prepulse inhibition (PPI) of acoustic startle, were synthesized. These chiral (*S*)-CH_3 ligands are targeted for the treatment of schizophrenia and depression. These new ligands were designed by modifying the labile ester functionality in **2** to improve the metabolic stability, cytotoxicity, and activity as compared to **2**. Based on the data to date, the most promising ligands are the *N*-cyclopropyl amide GL-I-55 (**8c**) and the methyl bioisostere GL-I-65 (**9a**). The *in vitro* metabolic stability, cytotoxicity and *in vivo* locomotor effects are described in this report. Based on these results, **8c** and **9a** are the most promising for further *in vivo* pharmacology.



Keywords: Schizophrenia, depression, $GABA_A$ receptor, SH-053-2'F-*S*-CH_3, bioisosteres, metabolism

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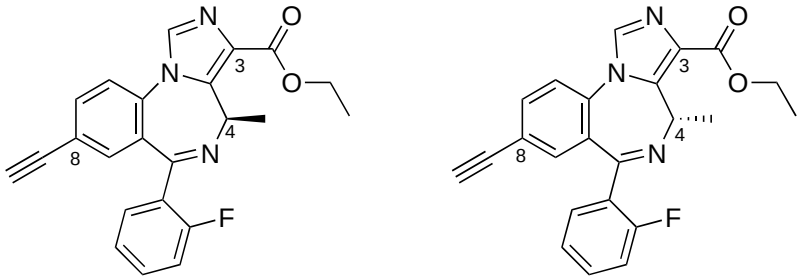
Introduction

Schizophrenia is one of the most complex central nervous system (CNS) disorders known. Approximately 1% of the population worldwide is affected by this disease. The symptoms fall into three broad categories: positive symptoms including hallucinations and delusions; negative symptoms such as the blunted affect, social dysfunction and lack of motivation or pleasure in daily life, and cognitive abnormalities. For instance, in the disruption of cognition, symptoms such as impairments in working memory, verbal memory, attention and executive deficits, which can lead to severe emotional distress, are prominent.[1] The treatments commonly used to manage positive symptoms are typical and atypical antipsychotic drugs, which target and block the over-activation of dopamine receptors[2] in patients with schizophrenia. In fact, the drugs prescribed, to date, only reduce the positive symptoms in the majority of schizophrenia patients. They are often not effective, especially in patients with negative symptoms and cognitive abnormalities.[3] Moreover, antipsychotics in many patients induce adverse effects such as tolerance, addiction, sedation, drug-resistance, weight gain and liver toxicity.[4] The lack of proper medications and limited efficient psychosocial therapy together increase the psychological and financial burden rapidly not only for the patients and their family but also for society as a whole. Consequently, there is an urgent clinical need for novel drug candidates that can address all aspects of schizophrenia including the alleviation of the negative and cognitive symptoms with decreased side effects.

Accumulating evidence has long suggested that the disruption of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA) activity may play a critical role in the pathophysiology of schizophrenia, as well as depression.[5,6] The $GABA_A$ receptor ($GABA_AR$) is a pentameric ligand-gated chloride ion channel,[7] which is located in the membrane of neuronal cells. It consists of a total of 19 different subunits in the human brain ($\alpha_{1–6}$, $\beta_{1–3}$, $\gamma_{1–3}$, δ, ε, π, θ, ρ_{1-3}),[8] the configuration of which determine ligand affinity, ion flux, membrane resistance, depolarization and other properties of the membrane.[9] The minimum requirement to provide a benzodiazepine receptor-mediated $GABA_AR$ ion channel is the combination of five subunits, which contain two α-, two β- and one γ- subunit.[10] $GABA_ARs$ contain the binding sites of a series of important clinical drugs, such as benzodiazepines, neurosteroids, and barbiturates.[11] The benzodiazepines, which include classical psychoactive drugs such as diazepam, bind at the extracellular interface of the $\alpha+\gamma_2$-subunits which results in increasing efficacy of GABA regarding chloride ion flux.[12] Subtype-specific ligands exhibit different CNS effects depending on the specific $\alpha_{1-6}\beta_{2/3}\gamma_2$ subtype involved.[13] Principally, the α1-containing $GABA_ARs$ are associated with the sedative, ataxia, anticonvulsant, anterograde amnesia, and addictive effects of benzodiazepine allosteric modulators of $GABA_ARs$.[14] The α2/3-subunit-containing $GABA_ARs$ mediate the anxiolytic, anticonvulsant, antinociceptive effects and perhaps muscle relaxant effect at higher doses.[15] Whereas the relatively minor population of α5-containing $GABA_ARs$, which have been described in the previous investigation, are implicated in cognition, learning and memory processes without motor effects or anxiolysis.[16-19] The ligands have also been implicated in reversing the adverse effects in animal models of schizophrenia and depression. Therefore, novel α5 or α2/3/5 subtype selective benzodiazepine ligands that are nearly silent or exhibit no efficacy at α1 $GABA_ARs$ may have the potential to reduce the negative symptoms and improve cognitive brain function with little or no sedative, amnesic or ataxia effects for patients with schizophrenia, as well as effects to alleviate the symptoms of depression.

It is well known that asymmetry plays an essential role in physiological processes; therefore, a large number of drugs are chiral molecules, in which one enantiomer may have the desired beneficial effect, while the other enantiomer may cause serious adverse effects.[20,21] This was observed with thalidomide.[22] Based on our earlier established $BZD/GABA_A$ pharmacophore model[23] that was developed by ligand binding data (see Supplementary material), a series of chiral molecules have been designed, synthesized and studied. Ligand SH-

053-2'F-*R*-CH_3 (**1**) was the lead stereospecific $GABA_AR$ α5-subtype selective positive allosteric modulator (PAM) because of the (*R*)-methyl group at the C-4 position of imidazobenzodiazepine (IBZD), as shown in Figure 1. This resulted in increased potency at α5 $GABA_A$ receptor subtypes accompanied by a concomitant decrease at an α1, α2, and α3 subtypes. This α5-selective ligand has been reported to successfully alleviate the hyperactivity of the dopamine system induced by amphetamine in the methylazoxymethanol acetate model (MAM) of schizophrenia.[24,25] Moreover, ligand **1** in an animal behavioral model of depression exhibited anxiolytic and antidepressant effects in female mice that were exposed to unpredictable chronic mild stress (UCMS) in a sex-dependent manner. The effects on male mice in the same paradigm were very weak or none at all.[26] Furthermore, analogs of 4(*R*)-CH_3 enantiomer **1**, such as the methyl amide MP-III-022,[27] the carboxylic acid SH-053-2'F-*R*-CH_3-acid[28], etc., which are more potent at the α5-subtype than **1**, have also been investigated and undergone a number of behavioral studies.[29,30]



1 (SH-053-2'F-*R*-CH_3) **2** (SH-053-2'F-*S*-CH_3)

Figure 1. Structures of enantiotopic chiral lead imidazobenzodiazepine (IBZD) ligands **1** and **2**.

In order to study how the chirality at the C-4 position of IBZD could affect the pharmacological response, the enantiomer of **1**, SH-053-2'F-*S*-CH_3 (**2**) was synthesized.[18] As expected, these two enantiomers exhibited vastly different GABAergic efficacy and binding affinity at Bz/GABA(A) receptor sites. Ligand **2** was found to be an α2-, α3-, α5-β3γ2 subtype selective, while **1** was an α5-subtype selective ligand. Since **1** and **2** both target the α5-subunit, they have been assayed in several behavioral models. Examination of the results revealed that **2** exerted anxiolytic effects in both rhesus monkeys[31] and rats,[32] while **1** had only a minimal anxiolytic effect when much higher doses were applied. Interestingly, **2** also successfully mitigated the amphetamine-induced hyperactivity in a poly (I:C) animal model of schizophrenia.[33]

The results of these studies indicated that both enantiomers may be useful in the treatment of the symptoms of schizophrenia, while the α2/3/5 agonist **2** exhibited the potential for treatment of conditions comorbid with schizophrenia, such as anxiety, which commonly coexists in patients with schizophrenia. Unfortunately, during other studies, ligand **2** was shown to be metabolized at a very high rate *in vitro* in the presence of human liver microsomes (HLM),[34] only 3% of **2** remaining after incubation at 37 ^oC for 30 minutes, while ligand **1** was stable in HLM with 100% remaining under the same conditions, as presented in Table 1. The principal aim of this research was to improve the metabolic stability of the ester functional group while retaining the properties potentially useful for the treatment of schizophrenia and depression. Schizophrenia and some forms of depression (bipolar I disorder and perhaps major depressive disorder) are linked both genetically and etiologically. Herein we present the synthesis and characterization of novel analogs of SH-053-2'F-*S*-CH_3 (**2**) including esters, thioesters, amides, carboxylic acids and the ester bioisostere oxadiazoles at the C(3) position together with modifications at the C(8) position. The newly designed ligands were prepared and investigated in *in vitro* metabolism studies, cytotoxicity assays and *in vivo* in mice with a rotarod study to

identify ligands with a lower systematic clearance rate, low toxicity, little or no sedative/ataxia activity, which might be useful in the management of the symptoms schizophrenia[23] and depression.[25] This go/no-go point is important for the development of a safe treatment for schizophrenia and depression that can occur at any age.

Table 1. *In vitro* liver microsomal stability of **1** and **2**

Ligand	Liver microsomal stability (%-remaining) *Conditions*: 4 μM compound, 37 °C, 30 minutes			
	HLM[a]	DLM[b]	MLM[c]	RLM[d]
SH-053-2'F-*R*-CH_3 (**1**)	100.0	90.3	59.1	5.4
SH-053-2'F-*S*-CH_3 (**2**)	2.3	90.7	66.5	6.0

[a] Human liver microsomes (HLM), [b] dog liver microsomes (DLM), [c] mouse liver microsomes (MLM), and [d] rat liver microsomes (RLM)

Results and Discussion

Previously, evidence from the ligand-based $GABA_AR$ pharmacophore/receptor model and SAR[23,35] have indicated that subtle changes in the IBZD substituents affect the BzR/GABA(A)R subtype selectivity dramatically. For instance, loss of the methyl group at the C(4) position decreases the α5-subtype efficacy while the efficacy at α2/α3-β3γ2 GABA(A)ergic subtypes increases dramatically. Efficacy at the α1 subtype increases with a 2'-F substituent on the C(6) pendent phenyl ring, but the 2'-N and 2'-H analogs remain α2/α3 subtype selective.[30] It has also been shown that the α5 selectivity can be modified by the presence of different lipophilic groups at the C(8) position.[19] Analogs, which have been optimized at the C(3) position, demonstrated higher affinity and potency at α5-[36] or α2/3-[37-39] subtypes leading to better and longer physiological effects. For these reasons, the C(3) and C(8) positions are considered to be the optimal regions for ligand improvement. To evaluate the modification of the lead compound **2** at these two positions, a series of novel analogs were designed, synthesized and characterized, as summarized in Figure 2.

As mentioned earlier, the C(8)-acetylene and C(4) methyl function were found to enhance the α5-subtype selectivity, therefore a cyclopropyl group, which is commonly incorporated into biologically active materials, was introduced into the parent compound **2** as a substituent to extend the SAR. As the result of its flexible rotation at the C(8) position, it should fill a similar volume of electron density as the ethynyl moiety. Consequently, it might exhibit parallel physiological effects and enhanced subtype selectivity due to similar interactions in the lipophilic binding pocket (L2, see Figure S1 in Supplementary material for the model).[22] Moreover, metabolism by liver microsomes would be expected to be different for a cyclopropyl substituent as compared to an ethynyl functional group. Hence, the C(8)-substituted cyclopropyl analog GL-I-78 (**3**) was prepared based on this hypothesis. The cyclopropyl ligand **3** was synthesized from cyclopropyl boronic acid *via* a Suzuki cross-coupling reaction[40] with the aryl bromide SH-I-48B (**4b**), the synthesis of which had been previously described. [17] The coupling reaction was carried out in the presence of potassium phosphate and 10 mol% Pd-catalyst, bis(triphenylphosphine)palladium(II) diacetate, in toluene at 100 °C for 12 hours in good yield (82%), as shown in Scheme 1.

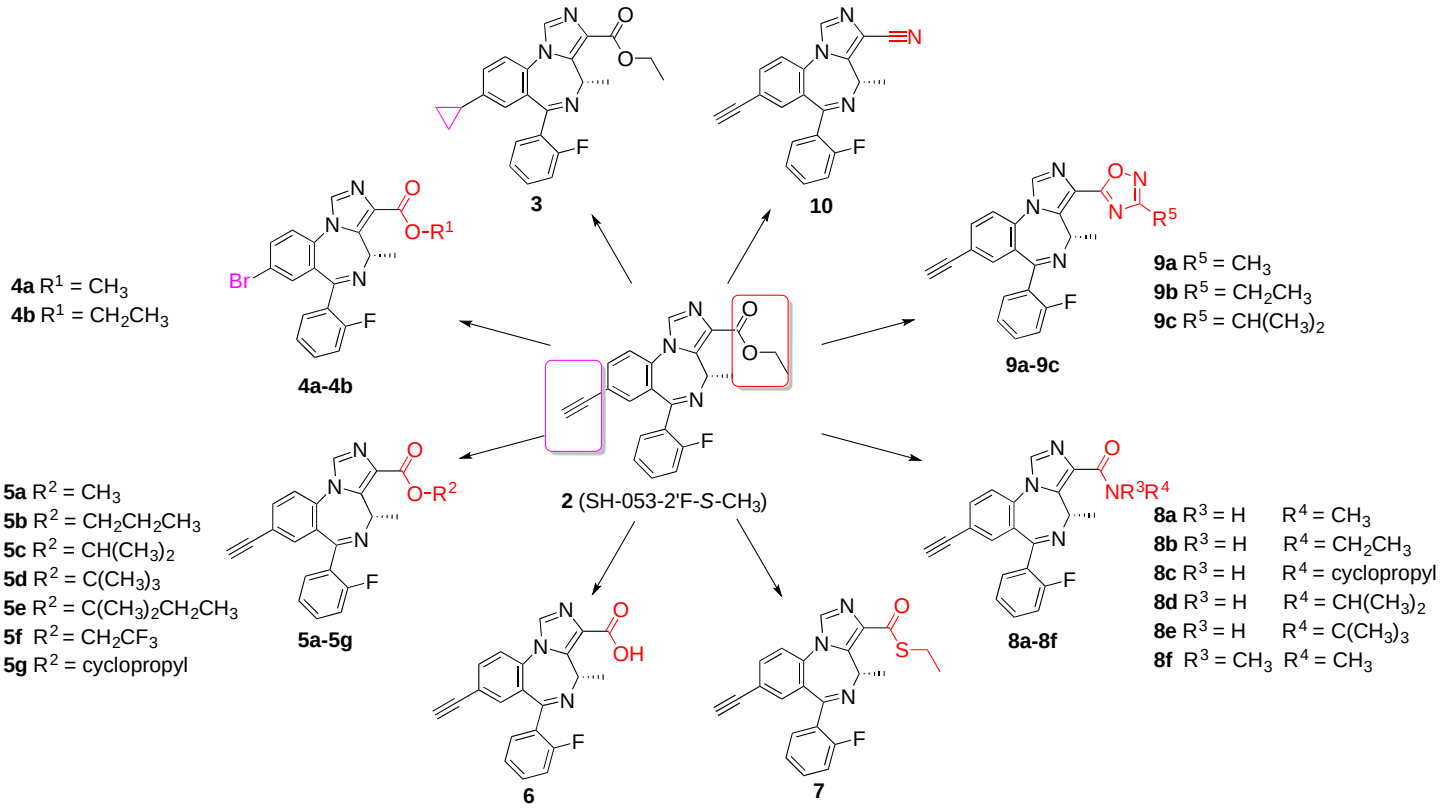
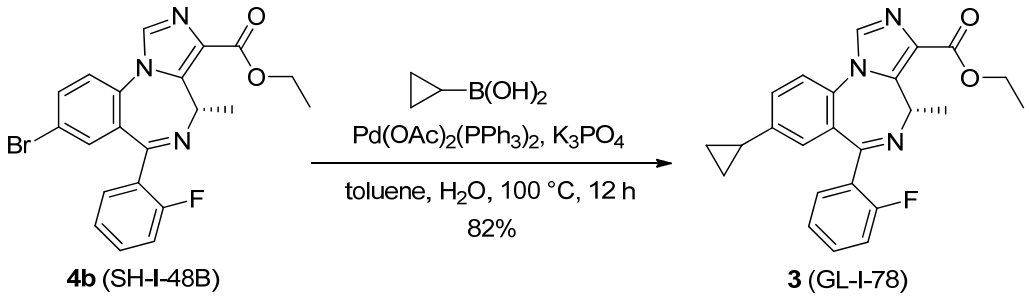


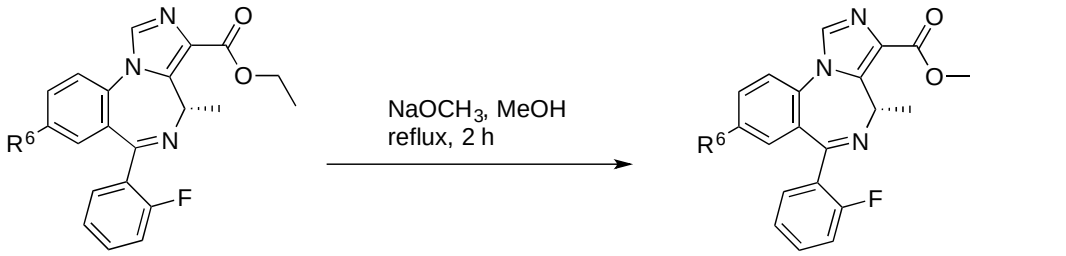
Figure 2. Chiral analogs and bioisosteres of the lead compound **2**.



Scheme 1. Synthesis of 8-cyclopropyl-4-(*S*-CH_3) imidazobenzodiazepine **3** from bromide **4b**.[17]

Based on the results described previously, the ethyl ester **2** was rapidly degraded in the HLM, which suggests that modification of the ester might play an essential role in metabolism, as well as efficacy, as compared to the lead compound **2**. Therefore, a series of esters, with different alkyl groups were synthesized. The synthesis of the C(8)-bromo methyl ester MP-III-059 (**4a**) and the C(8)-ethynyl methyl ester MP-III-021 (**5a**) were achieved in the presence of sodium methoxide in methanol in good yields, as depicted in Scheme 2. The majority of the reactions were carried out *via* a transesterification reaction[41] with pieces of freshly cut lithium rod in the presence of alcohols in THF at 40 °C for 0.5 hours to generate the corresponding alkoxides. To this solution, ester **2** was added**,** dissolved in dry THF, and full conversion was observed after 15 to 30 minutes. New ester analogs generated via this general route were: propyl ester GL-I-32 (**5b**), isopropyl ester GL-I-31 (**5c**), *t*-butyl ester GL-I-30 (**5d**) and 2-methylbutyl ester GL-I-33 (**5e**), respectively. The yields were good to excellent (77-97%), as illustrated in Scheme 3. However, the trifluoroethyl ester GL-I-36 (**5f**), and the cyclopropyl ester GL-I-38 (**5g**) were not obtained under these conditions. As a result, both analogs were

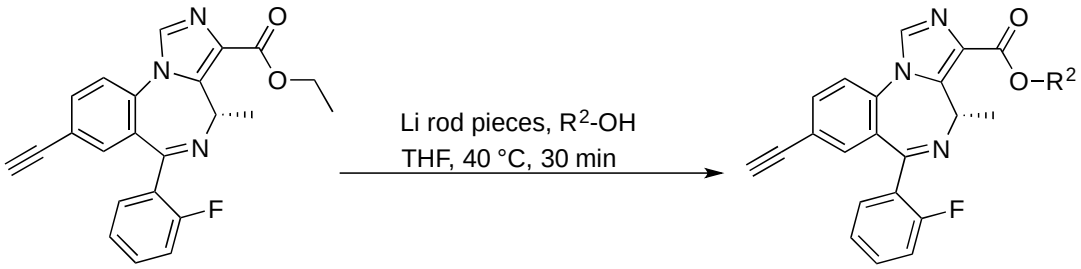
prepared by an alternate procedure. The ester **2** was saponified to form the carboxylic acid, SH-053-2'F-*S*-CH_3-acid (**6**), with sodium hydroxide in ethanol at reflux for 1 hour in nearly quantitative yield (99%) after acidic work-up, as illustrated in Scheme 4. The subsequent reactions for the synthesis of ester **5f** and **5g** were conducted with thionyl chloride in dichloromethane at reflux to give the acyl chloride, which was converted into esters **5f** and **5g** with 2,2,2-trifluoroethanol and cyclopropanol, respectively. Additionally, it must be pointed out that the acid **6** was synthesized not only because it was an important intermediate for the majority of the analogs, but also because the enantiomer (*R*)-CH_3 carboxylic acid in the C(4)*R*-isomer series significantly increased the α5-subtype selectivity and potency.[27] It was hoped that the acid **6** in the C(4)*S*-isomer series might affect a similar increase on the selectivity and potency at the α2/α3/α5-subtype.



4b R^6 = Br (SH-I-48B)
2 R^6 = C≡CH (SH-053-2'F-*S*-CH_3)

4a R^6 = Br (MP-III-059), 87%
5a R^6 = C≡CH (MP-III-021), 81%

Scheme 2. Synthesis of methyl esters **4a** and **5a** from ethyl ester **4b** or **2**, respectively.[17]



2 (SH-053-2'F-*S*-CH_3)

5b-5e

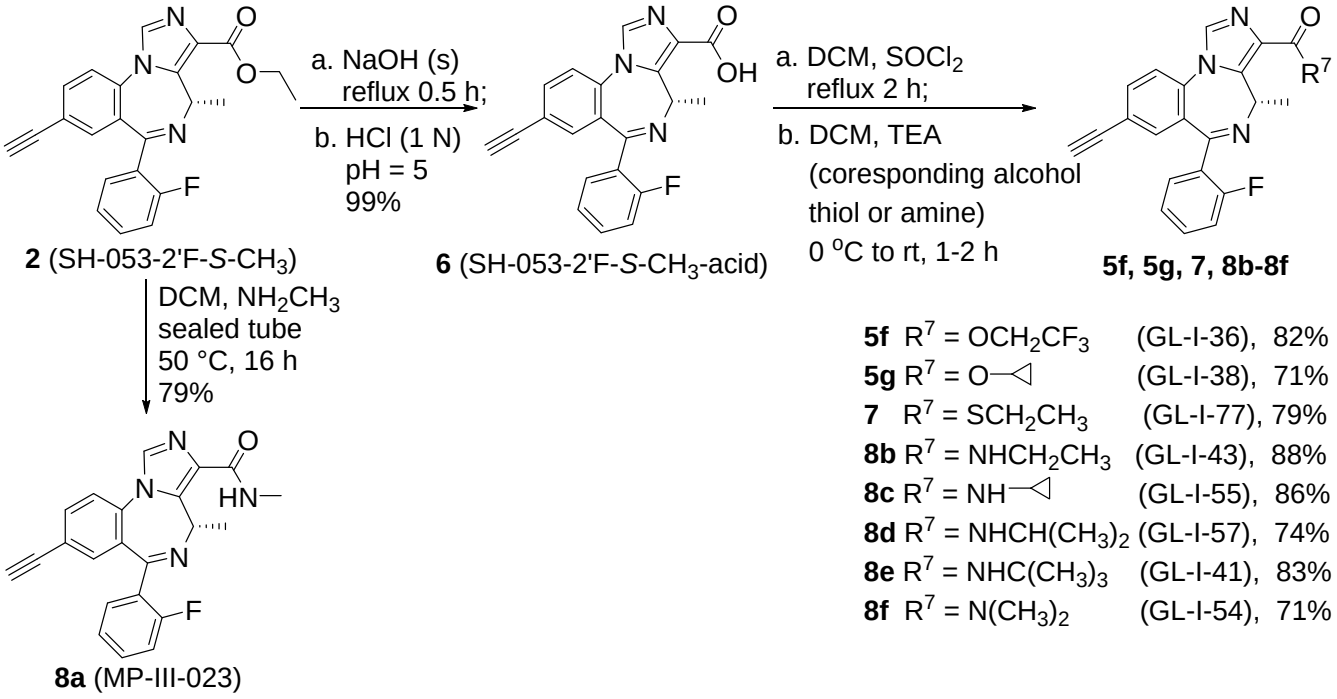
5b R^2 = $CH_2CH_2CH_3$ (GL-I-32), 82%
5c R^2 = $CH(CH_3)_2$ (GL-I-31), 97%
5d R^2 = $C(CH_3)_3$ (GL-I-30), 96%
5e R^2 = $C(CH_3)_2CH_2CH_3$ (GL-I-33), 77%

Scheme 3. Synthesis of esters **5b-5e**.[40]

In a previous study, it was shown that a heteroatom attached directly to the carbonyl at C(3) affects the $GABA_AR$ subtype selectivity. For example, a thioester at C(3) in the 2'N series exerted no efficacy at the α1-subtype even at a higher concentration in comparison with the corresponding oxygen substituted ester, but with a slightly higher preference for the α3-subtype.[42] Therefore, it was decided to replace the oxygen atom in lead compound **2** at the C(3) position with a sulfur atom. The thioethyl ester GL-I-77 (**7**) was synthesized from the carboxylic acid **6** following the general procedure mentioned below (Scheme 4) with ethanethiol as the nucleophile.

Besides the slight changes in steric effects as compared to lead ester **2**, amide analogs are well-known stable replacements for labile ester moieties. Amides were considered to be important replacements for ester functions to improve the *in vivo* stability, as well as the bioavailability when compared to the esters. To

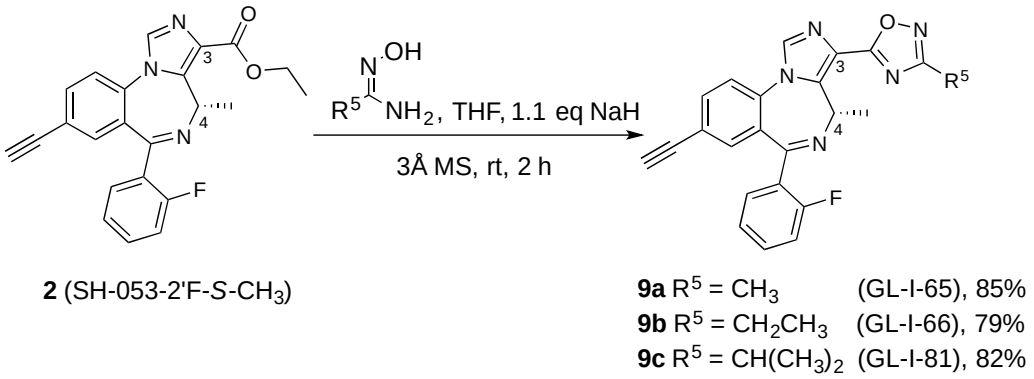
evaluate the potential activity of the amides in regard to future SAR, a series of amide analogs **8a-8f** were synthesized to enable a comparison between esters and amides in regard to *in vitro* metabolic stability, cytotoxicity and *in vivo* locomotor activity. The methyl amide MP-III-023 (**8a**) was prepared by the direct amidation of ethyl ester **2** in the presence of methylamine in dichloromethane in a sealed tube. As indicated in Scheme 4, the other amide analogs were synthesized from carboxylic acid **6** *via* an acyl chloride intermediate, using the corresponding primary or secondary amine. This process gave the ethyl amide GL-I-43 (**8b**), cyclopropyl amide GL-I-55 (**8c**), isopropyl amide GL-I-57 (**8d**), *t*-butyl amide GL-I-41 (**8e**) and dimethyl amide GL-I-54 (**8f**), respectively, in good to excellent yields (71-88%).



Scheme 4. Synthesis of analogs **5f**, **5g**, **7**, **8a-8f**.

Ester bioisosteres have been utilized commonly in drug design in order to render the ester metabolically stable with less adverse effects and provide better clinical candidates.[43] The previous results indicated that replacement of ester functions by substituted 1,2,4-oxadiazoles[36,44] significantly increased the *in vitro* and *in vivo* stability, as well as slightly improved the hydrophilicity of the ligands.[19] Moreover, the efficacy at BZD receptors was enhanced as compared to the corresponding ester ligands.[19] Encouraged by this previous evidence, the substituted 1,2,4-oxadiazoles **9a-9c** were designed and synthesized from lead **2**. The condensation of the ethyl ester **2** and a corresponding amidoxime in the presence of sodium hydride in THF was employed to prepare the C(3)-substituted oxadiazole ligands. This route had been successfully applied previously with non-chiral ligands.[43] However, the desired oxadiazoles with a C(4) methyl group were obtained only in moderate yields (30-40%). The reason was the formation of a byproduct (50-60% yield), in which the C(5)-C(6) imine bond had isomerized to the C(4)-C(5) position. As a result, the chirality at the C(4) position was lost. To overcome this problem, the amount of sodium hydride, which would readily lead to isomerization of the imine bond, was reduced from 4 equivalents to 1.1 equivalents. In addition, the amidoxime was stirred longer with NaH before the ester was added to the mixture. This process gave methyl oxadiazole GL-I-65 (**9a**),

ethyl oxadiazole GL-I-66 (**9b**) and isopropyl oxadiazole GL-I-81 (**9c**) in improved yields of 79-85%, respectively, as depicted in Scheme 5.

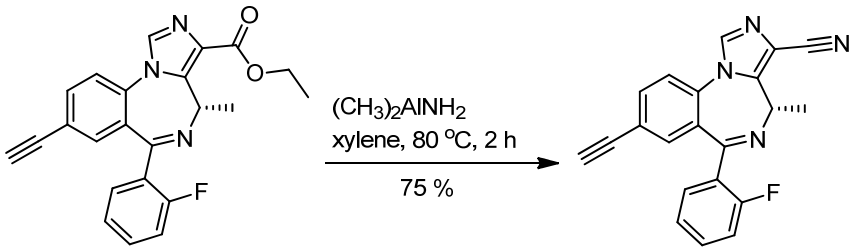


2 (SH-053-2'F-*S*-CH_3)

9a $R^5 = CH_3$ (GL-I-65), 85%
9b $R^5 = CH_2CH_3$ (GL-I-66), 79%
9c $R^5 = CH(CH_3)_2$ (GL-I-81), 82%

Scheme 5. Synthesis of oxadiazoles **9a-9c**.

To explore the extent of flexibility of the hydrogen-bond acceptor in the binding pocket designed H1 in the pharmacophore/receptor model (see Figure S1 in Supplementary material),[22,36] it was decided to synthesize a linear nitrile at the C(3) position, which has sometimes been employed to increase water solubility of a ligand and has also been used to reduce possible oxidative metabolism by the liver in clinical studies.[45] The nitrile MP-III-018.A (**10**) was produced from the ester **2** via reaction with amidodimethylaluminum[46] in xylene at 80 ^oC in 75% yield, as shown in Scheme 6. The amidodimethylaluminum was prepared freshly by adding trimethylaluminum to a solution of ammonia-saturated dichloromethane.[45]



2 (SH-053-2'F-*S*-CH_3)

10 (MP-III-018.A)

Scheme 6. Synthesis of the C(3)-nitrile **10**.

The characterization of the lead compound **2** was being carried out simultaneously with the synthesis and assessment of the novel analogs. Due to the ability of **2** to potentiate the α5 containing $GABA_AR$ as well as the α2 and α3 subtypes, while inactive at the α1 containing $GABA_AR$, which mediates sedation and other adverse effects, ligand **2** was evaluated in behavioral models in direct comparison with its enantiomer **1**. For cataleptic behavior in the schizophrenia studies,[47] the antipsychotic, haloperidol elicited a significant cataleptic response. In contrast, both C(4)-methyl substituted ligands **1** and **2** did not show any signs of catalepsy in comparison to the vehicle, as illustrated in Figure 3. Moreover, in the prepulse inhibition (PPI) assay, which was used as a classical animal model of schizophrenia, the *S*-isomer **2** significantly reversed the PPI deficit induced by phencyclidine hydrochloride (PCP), an *N*-methyl-D-aspartate (NMDA) antagonist.[48] Ligand **1** antagonized the deficit compared to vehicle but failed to reach a significant effect at the same dosage, as depicted in Figure 4. However, PPI results with the *R*-isomer **1** were trending toward significance but would have required a larger

number of animals. Additionally, in the 6 Hz electroshock (3 seconds duration) assay, both ligands were evaluated for their ability to protect against epileptic seizures. As shown in Figure 5, ligand **2** was able to protect mice from the electroshock-induced seizures for all time points (1/4, 1/2, 1, 2, and 4 hours), which was predicted due to the activation of α2 and α3 subtypes (Figure 5A) as well as α5 subtypes. However, the *R*-enantiomer (α5) **1** was not effective at any time at a relatively higher dose in the 6 Hz paradigm. For the rotarod (n=8) assay, neither **1** nor **2** produced significant ataxia at 100 mg/kg i.p. in mice (Figure 5B). This is in complete agreement with the poor efficacy at α1β3γ2 Bz/GABAergic subtypes previously reported.[30]

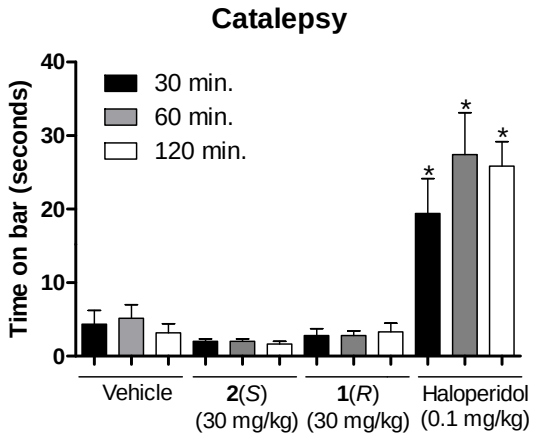


Figure 3. Impact of **1** or **2** treatment (30 mg/kg, ip) on cataleptic behavior. Haloperidol, but not **1** nor **2** produced a significant cataleptic response at 30, 60, and 120 minutes post-injection. The data are expressed as the mean (+SEM) time (sec) subjects spent with both front paws on an elevated bar. *p<0.001, the difference from controls receiving vehicle alone (see Supplementary material for details).

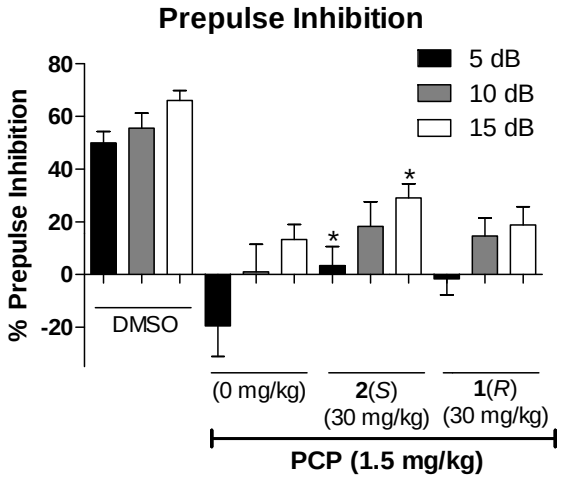


Figure 4. Impact of **1** or **2** treatment (30 mg/kg, IP) on prepulse inhibition. Ligand **2**, but not **1** reversed the PPI deficits induced by PCP when given one hour before testing. The data are expressed as the mean (+SEM) percent prepulse inhibition of the startle response to an auditory stimulus when preceded by a nonstartle-eliciting auditory cue at 5, 10, and 15 decibels. Disruption of prepulse inhibition was obtained in rats by an acute injection of phencyclidine (PCP·HCl, 1.5 mg/kg, SC) 10 minutes prior to testing. *p<0.05, difference from controls receiving phencyclidine with 0 mg/kg drug pretreatment (see Supplementary material for details).

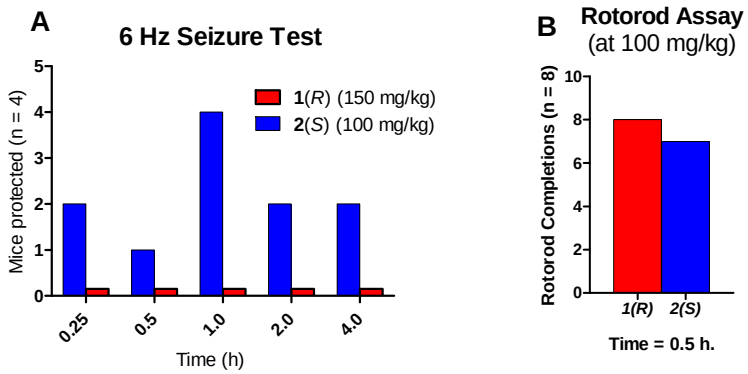


Figure 5. Impact of **1** or **2** treatment on anticonvulsant effects. A) Mice were dosed i.p. with either **1** (150 mg/kg) or **2** (100 mg/kg) and a 6 Hz shock by a corneal electrode was administered at various time points, and the animal observed for convulsions. Ligand **2** was able to protect some subjects while **1** was not able to protect mice from electroshock-induced seizures. B) In the rotarod assay, mice were dosed i.p. with **1** or **2** (100 mg/kg) 30 minutes prior to testing on a rotating rod (6 rpm). Mice that fell 3 times during a 1-minute trial were deemed toxic (ataxic or sedated). (see Supplementary material for details)

The parent compound **2** and its analogs were assayed for metabolic stability *in vitro* with HLM and mouse liver microsomes (MLM) to identify the most stable analogs and the possible trend of steric effects in respect to stability. Moreover, it was important to evaluate stability in both species with respect to applied animal models and future human treatment with such a diverse array of functional groups at C(3). The results of this study are shown in Table 2.

The lead compound **2**, as mentioned before, was found to be rapidly metabolized on HLM with less than 5% remaining after 20 minutes, while **2** exhibited good stability for MLM with 78% remaining after 1 hour. Among the ester analogs, **3, 4a, 4b, 5a-d, 5f** and **5g** an increasing trend in metabolic stability in HLM was observed as the size of the alkyl chain increased. However, esters bearing a linear 2-3 carbon chain (**4a, 5a, 5b, 5f,** and **5g**), regardless of the functional group at the C(8) position (-bromo, -cyclopropyl or -ethynyl), were metabolized very rapidly with less than 5% remaining after 10, 20, and 30 minutes in HLM. A similar trend was observed for MLM, however, the C(8)-bromo and -cyclopropyl replacement of the ethynyl group decreased the metabolic stability (see **2** vs **3**, **4b**; **4a** vs **5a**). It is important to point out that the introduction of an ester function with a longer carbon side-chain or a terminal CF_3 group reduced the metabolic stability significantly, as shown in Table 1. Both ligands **4b** and **5f** could not be detected after 20 minutes in HLM or MLM, respectively. Most of the esters are only slightly more stable in MLM, as compared to HLM, which suggests that longer lipophilic linear alkyl chains in the ester functional group should be avoided in the ligand design. The carboxylic acid **6** was slightly more stable compared to the esters. The stability of thioethyl ester **7** for HLM was improved slightly compared to ethyl ester **2**, which suggests that a sulfur atom in place of an oxygen atom would provide greater metabolic stability. Electronegativity might play a role here. The nitrile **10** was metabolized at a rapid rate with only 9% and 16% of **10** remaining after two hours in HLM and MLM, respectively. As expected, the amide analogs remarkably enhanced the metabolic stability in both HLM and MLM. The trend regarding steric effects on the rate of metabolism of the amides was opposite to that of the esters; bulkier substituents were less metabolically stable. Presumably, bulkier esters are more lipophilic and react faster with P450 enzymes in the liver microsomal extract. The ethyl amide **8b** exhibited the best stability with more than 92% of **8b** remaining after 2 hours, as well as the longest half-life for both types of liver

microsomes among all the amides. The isopropyl amide **8d** was the only ligand, whose metabolism was a little inconsistent, as a result, the isopropyl ester **5c** and amide **8d** shared a similar metabolic pattern. As expected, the replacement of the ester moiety with the oxadiazole bioisosteres **9a-9c** significantly improved the metabolic stability over a 2-hour period of incubation in both HLM and MLM. It is notable that the isopropyl oxadiazole **9c** exhibited the longest half-life (2584 minutes) as compared to all the analogs investigated in the SH-053-2'F-*S*-CH_3 (**2**) series. In brief, it is clear that the amides and substituted oxadiazole functionality at the C(3) position provide the desired ligands with longer half-life values, as compared to other functional groups. Further evaluation of these analogs involved a study of potential cytotoxicity in both HEPG2 (liver) and HEK293 (kidney) cell lines, as summarized in Table 3.

Table 2. *In vitro* microsomal stability of SH-053-2'F-*S*-CH_3 (**2**) analogs

Entry	Structure at C(3) with C(8)-ethynyl		Microsomal stability (HLM) after 2 hr		Microsomal stability (MLM) after 2 hr	
			Half-life (min)	% remaining	Half-life (min)	% remaining
2	ester	$CO_2CH_2CH_3$	< 5[b]	< 5.0[b]	155 ± 9[d]	78.0 ± 0.2[d]
3		$CO_2CH_2CH_3$[e]	< 5[b]	< 5.0[b]	46 ± 3	16.0 ± 0.4
4a		CO_2CH_3[f]	10 ± 1[d]	2.0 ± 0.3[d]	116 ± 13	41.0 ± 0.6
4b		$CO_2CH_2CH_3$[f]	< 5[b]	< 5.0[b]	< 5[b]	< 5.0[b]
5a		CO_2CH_3	119 ± 21[d]	43.0 ± 0.4[d]	216 ± 28	62.0 ± 0.5
5b		$CO_2CH_2CH_2CH_3$	< 5[c]	< 5.0[c]	24 ± 0[d]	12.0 ± 0.2[d]
5c		$CO_2CH(CH_3)_2$	57 ± 2[d]	47.0 ± 0.2[d]	227 ± 16[d]	83.0 ± 0.1[d]
5d		$CO_2C(CH_3)_3$	514 ± 115[d]	89.0 ± 0.2[d]	196 ± 20[d]	78.0 ± 0.2[d]
5f		$CO_2CH_2CF_3$	< 5[a]	< 5.0[a]	< 5[b]	< 5.0[b]
5g		CO_2-cyclopropyl	< 5[a]	< 5.0[a]	19 ± 2[d]	11.0 ± 0.2[d]
6	acid	COOH	358 ± 56	74.0 ± 0.4	139 ± 11[d]	54.0 ± 0.3[d]
7	thioester	$COSCH_2CH_3$	101 ± 19	28.0 ± 0.3	46 ± 6	9.0 ± 0.5
8a	amide	$CONHCH_3$	**1780 ± 39**	**92.0 ± 0.3**	**845 ± 5**	**86.0 ± 0.2**
8b		$CONHCH_2CH_3$	**1978 ± 50**	**93.0 ± 0.3**	**1961 ± 46**	**92.0 ± 0.3**
8c		CONH-cyclopropyl	**1625 ± 803**[d]	**89.0 ± 0.4**[d]	**320 ± 34**[d]	**72.0 ± 0.3**[d]
8d		$CONHCH(CH_3)_2$	107 ± 10	38.0 ± 0.5	28 ± 0	75.0 ± 0.3
8e		$CONHC(CH_3)_3$	205 ± 14	63.0 ± 0.3	142 ± 9	52.0 ± 0.3
8f		$CON(CH_3)_2$	59 ± 5[d]	41.0 ± 0.9[d]	24 ± 2[d]	19.0 ± 0.9[d]
9a	1,2,4-oxadiazole	3-CH_3	**866 ± 213**	**86.0 ± 0.3**	**443 ± 66**	**81.0 ± 0.4**
9b		3-CH_2CH_3	563 ± 123	80.0 ± 0.4	213 ± 17	63.0 ± 0.3
9c		3-$CH(CH_3)_2$	**2584 ± 1920**	**91.0 ± 0.3**	**500 ± 109**	**79.0 ± 0.5**
10	nitrile	CN	41 ± 5	9.0 ± 0.3	58 ± 6	17.0 ± 0.6

[a] Compound was not detected after 10 minutes. [b] Compound was not detected after 20 minutes. [c] Compound was not detected after 30 minutes. [d] Compound was assessed at 1-hour incubation time. [e] C(8)-cyclopropyl. [f] C(8)-bromo. (see Supplementary material for details)

Table 3. *In vitro* cytotoxicity of SH-053-2'F-*S*-CH_3 (**2**) analogs

Entry		Structure at C(3) with C(8)-ethynyl	HEK293 LD_{50} (μM)[a]	HEPG2 LD_{50} (μM)[a]
2	ester	$CO_2CH_2CH_3$	28.0 ± 4.1	**73.5 ± 14.4**
3		$CO_2CH_2CH_3$[b]	**58.0 ± 6.0**	**87.0 ± 6.6**
4a		CO_2CH_3[c]	**98.5 ± 7.9**	**136.8 ± 6.3**
4b		$CO_2CH_2CH_3$[c]	**187.0 ± 49.7**	**>400**
5a		CO_2CH_3	**68.2 ± 4.3**	**136.6 ± 6.3**
5b		$CO_2CH_2CH_2CH_3$	**53.2 ± 4.4**	**56.3 ± 4.0**
5c		$CO_2CH(CH_3)_2$	48.9 ± 4.4	39.9 ± 2.8
5d		$CO_2C(CH_3)_3$	12.3 ± 1.6	21.4 ± 1.9
5e		$CO_2C(CH_3)_2CH_2CH_3$	17.2 ± 1.6	17.1 ± 1.4
5f		$CO_2CH_2CF_3$	**138.1 ± 31.8**	**>400**
5g		CO_2-cyclopropyl	35.8 ± 3.3	**85.1 ± 3.8**
6	acid	COOH	**>400**	**>400**
7	thioester	$COSCH_2CH_3$	20.5± 4.7	30.2 ± 9.7
8a	amide	$CONHCH_3$	**>200**	**>200**
8b		$CONHCH_2CH_3$	**95.5 ± 12.6**	**65.4 ± 7.3**
8c		CONH-cyclopropyl	**63.8 ± 3.0**	**93.5 ± 5.7**
8d		$CONHCH(CH_3)_2$	**80.2 ± 11.1**	**56.9 ± 4.8**
8e		$CONHC(CH_3)_3$	33.6 ± 3.0	43.4 ± 4.0
8f		$CON(CH_3)_2$	**>400**	**>400**
9a	1,2,4-oxadiazole	3-CH_3	**69.3 ± 2.3**	**>180**
9b		3-CH_2CH_3	46.9 ± 6.6	35.1 ± 3.7
9c		3-$CH(CH_3)_2$	32.5 ± 7.6	21.7 ± 1.8
10	nitrile	CN	**81.6 ± 6.7**	**57.1 ± 5.6**

[a] Compounds were incubated at different concentrations with the specific cells for 48 hours, followed by detection of cell viability using a Cell-Titer Glo (Promega). The results were normalized using DMSO (negative control) and 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (150 mM in DMSO final concentration, positive control). Data were acquired by three independent experiments carried out in quadruplet. [b] C(8)-cyclopropyl. [c] C(8)-bromo (see Supplementary material for details)

Examination of the cell viability assay indicated that ligands with a C-3 carboxylic acid substituent of *S*-isomer analog **6** and dimethyl amide **8f** were non-toxic even at 400 μM (LD_{50} > 400 μM) in the presence of HEK293 and HEPG2 cells. Compounds C(8)-bromo ethyl ester **4b** and trifluoroethyl ester **5f** were non-toxic at doses higher than 400 μM (LD_{50} > 400 μM) for the HEPG2 cells, and exhibited an LD_{50} of 187 μM and 138 μM in HEK293 cells, respectively. Methyl amide **8a** showed no toxicity at concentrations below 200 μM for both cell lines. Esters **3, 4a, 5a, 5b,** amides **8b**-**8d**, methyl oxadiazole **9a** and nitrile **10**, were safe at lower concentrations with LD_{50} values of >50 μM for both cell lines. Usually, these ligands potentiate the $GABA_A$Rs at nanomolar concentrations, consequently far below the measured LD_{50} values of 50 μM. The compounds with moderate cytotoxicity (30 μM <LD_{50} <50 μM) were the isopropyl ester **5c**, cyclopropyl ester **5g**, *t*-butyl amide **8e** and ethyl oxadiazole **9b**. Ligands that exhibited pronounced cytotoxicity (LD_{50} <30 μM) for HEK293 kidney cells were ethyl ester **2**, *t*-butyl ester **5d**, 2-methyl butyl ester **5e**, thioethyl ester **7**, and isopropyl oxadiazole

9c. These data revealed that increasing the size of the substituent of the oxadiazole moiety may increase cytotoxicity; most of the esters are considered to be cytotoxic if given at very high doses (much higher than any therapeutic doses), unless a different group at C(8) was introduced (see **3**, **4a**, and **4b**). The trifluoroethyl ester was fairly cytotoxic perhaps because the trifluoroethanol group is a much better leaving group. *In vitro* toxicity can be used as a measure to estimate *in vivo* toxicity, however, variations can occur. For instance, lead compound **2** exhibited a fairly low LD_{50} value, however it was not toxic in a number of pharmacological assays in the mice, rats and primates.[23-25, 30-32] The cytotoxicity assays indicated which analogs were least cytotoxic but were not potent enough (nM) to rule out further pharmacological studies in models of schizophrenia or depression.

To assess the possibility of undesired CNS side effects (α1-mediated), a locomotor coordination study of each analog was conducted by placing mice on a rotating rod for a maximum of 3 minutes after oral administration by gavage at a dose of 40 mg/kg (Figure 6). The mice were also observed for loss of righting reflex, an indication of undesired CNS effects. Most of the treated mice exhibited no sedation or ataxia, nor loss of righting reflection vs the control diazepam. Most of the compounds exhibited sensorimotor steadiness at all three time-points, which indicated no sedative/ataxic effects. However, some amides did exbihit some sensorimotor deficits. Methyl amide **8a** and ethyl amide **8b** effected severe motor impairment, while the cyclopropyl amide **8c** and dimethyl amide **8f** exhibited only minor impairment. The bulkier isopropyl amide **8d** and *t*-butyl amide **8e** did not show any motor impairment. These results suggested that these particular amides, which produced sedation/ataxia, are sedating presumably because of some efficacy at the α1-$GABA_AR$ subtype. In regard to the pharmacological effects of the amide side-chain, it is obvious that a smaller alkyl chain promoted a more sedating effect, which should not result from metabolism nor cytotoxicity since these two assays indicated that the amides with smaller substituents were highly stable and non-toxic.

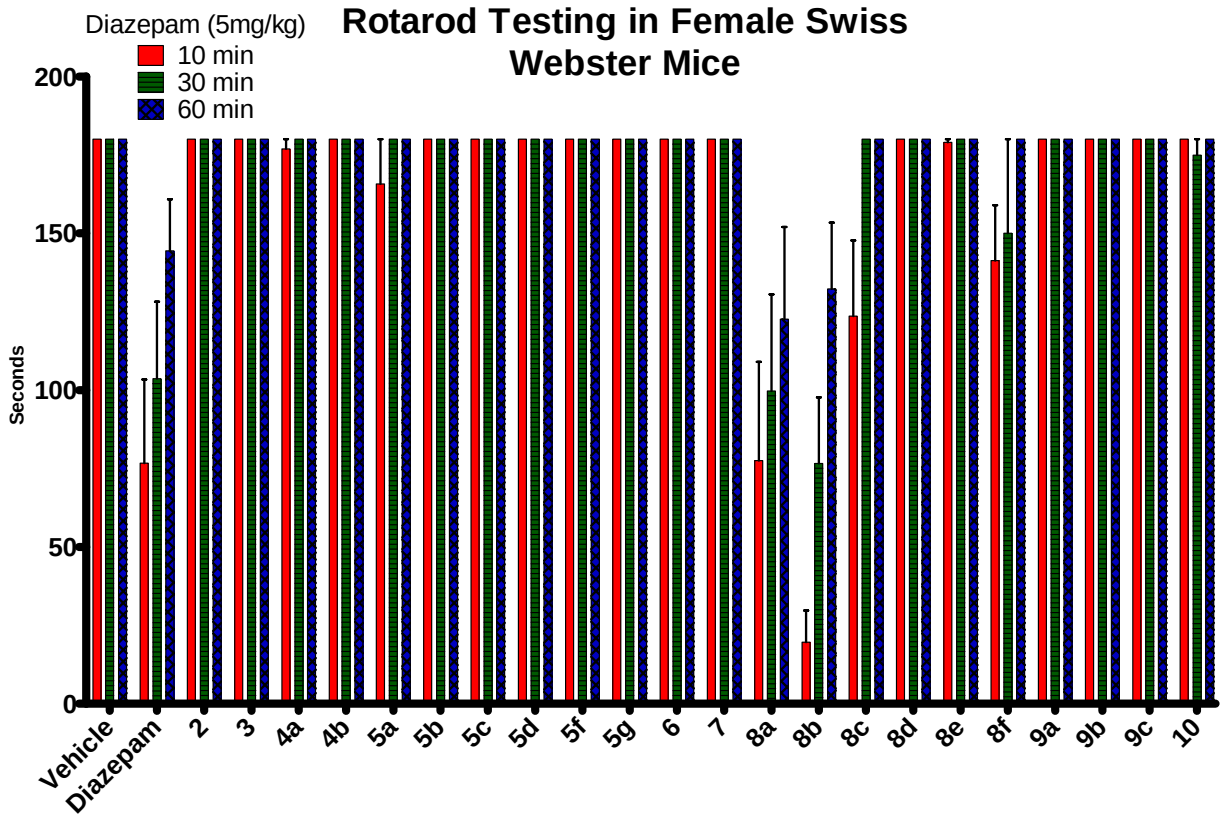


Figure 6. Effect of compounds on sensorimotor coordination. (see Supplementary materials for details)

After examination of all results for the series of C(4)-*S*-CH_3 substituted enantiomers described above, the best two compounds, which exhibited excellent stability, minimal or no toxicity and did not exhibit motor impairments, were cyclopropyl amide GL-I-55 (**8c**) and methyl oxadiazole GL-I-65 (**9a**). Moreover, it was suggested that the bioisosteres GL-I-66 (**9b**) and GL-I-81 (**9c**), which were very metabolically stable and exhibited no sensorimotor impairment, will need to be administrated at low concentrations in behavioral studies to avoid adverse chronic side effects. Amides **8a** and **8b** exerted excellent stability and no cytotoxicity, however, these two ligands were sedating at 40 mg/kg p.o. Esters (**2-5g**) including the lead compound **2** exhibited either cytotoxicity or were metabolically less stable in the presence of HLM and MLM. Thioester **7**, isopropyl amide **8e** and nitrile **10** were not metabolically stable enough to encourage further studies. Carboxylic acid **6** exhibited excellent properties in all three assays, however, the acid functionality will prevent the penetration of the blood brain barrier which was demonstrated for the *R* enantiomer.[27] This property is not suitable for the treatment of schizophrenia and depression. Cognitive deficits developed in these two disease states are still poorly managed with current drugs. Although the data is only preliminary, *S*-CH_3 ligand **9a** demonstrated procognitive effects (C+) in the Y-maze paradigm. The *S*-CH_3 ligand **8f** was active in the antidepressant assay (forced swim test) and procognitive (A++ and C++) assay.[50] However, **8f** was metabolized fairly rapidly in HLM as shown in Table 2. To date, these two ligands along with **8c** in this *S*-enantiomeric series seemed to be the best candidates for additional preclinical behavioral testing, while in the *R*-CH_3 series (α5 subtype selective series) a number of ligands have promising pharmacological effects for the treatment of schizophrenia and depression.[23-25,31,35,50]

Conclusions

It is clear that ligand design to target specific $GABA_AR$ subtypes without adverse effects is very difficult, in part, due to the absence of a crystal structure of the $\alpha_{1\text{-}6}\beta_3\gamma_2$ ion channel, as well as the diversity of the receptor subunit expression in different regions of the brain. Although a crystal structure has been reported for a pentameric GABA ion channel (5 beta subunits form the ion pore), which is an important result going forward, nevertheless, the lack of a benzodiazepine binding site in this structure renders it ineffective for ligand design.[49] In the present research, a number of ligands have been successfully designed and synthesized with the C(4) *S*-CH_3 stereochemistry. This series of esters, amides, and bioisosteres of the lead compound **2** are devoid of the liable ester functionality at C(3) to enhance metabolic stability. The synthesis of these analogs, importantly, was inexpensive and straightforward with improved yields over the previous routes.[43] More importantly, these reactions can be easily scaled up to gram quantities. The novel ligands **8c** and **9a** are metabolically stable in the presence of human and mouse liver microsomes and exhibit excellent cytotoxicity (LD_{50}) values. Furthermore, they do not induce sedative effects, which is a major problem of classical benzodiazepine drugs. Taken together, this research of the *S*-CH_3 enantiomers has provided several novel ligands that possess good safety and metabolic profiles for further *in vivo* behavioral testing in animal models in both schizophrenia and depression.[23-25, 31-32] The *in vivo* activity of **8c** and **9a** in models of schizophrenia and depression is ongoing and preliminary studies look very promising.[50]

Experimental Section

General. Unless specified, all reactions were performed in oven-dried round-bottom flasks or a screw-cap test tube or heavy walled pressure vessel under an argon atmosphere, if required. All organic solvents were anhydrous or purified when necessary by standard methods and purchased from commercial suppliers, as well as all the chemicals. Reactions were monitored by TLC on plates from Dynamic Adsorbents, Inc. under a UV light. The purification of some analogs was completed by flash chromatography on silica gel (230-400 mesh, Dynamic Adsorbents). The melting points were determined on a Stuart melting point SMP3 apparatus manufactured by Barloworld Scientific US Ltd. The optical rotation values were recorded on a Jasco DIP-370 Digital Polarimeter. The ^{1}H and ^{13}C NMR spectra were obtained on Bruker Spectrospin 300 MHz instrument, in $CDCl_3$, or DMSO. Chemical shifts were reported in δ (ppm) relative to TMS as an internal standard. Multiplicities are presented as follows: singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q), multiplet (m). The purity of all analogs was determined to be > 95%, by HPLC-MS performed on a Shimadzu LCMS-2020 and HRMS was performed on a Shimadzu LCMS-IT-TOF at the Milwaukee Institute for Drug Discovery in the Shimadzu Laboratory for Advanced and Applied Analytical Chemistry.

(*S*)-Ethyl-8-cyclopropyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-78, 3). To a solution of the bromo ethyl ester **4b** (5.0 g, 11.3 mmol) in toluene (100 mL) and water (14.5 mL), cyclopropylboronic acid (4.9 g, 57.0 mmol), potassium phosphate (10.3 g, 48.6 mmol) and bis(triphenylphosphine)palladium(II) diacetate (0.85 g, 1.13 mmol) were added under argon. A reflux condenser was attached and the mixture was degassed under vacuum with argon; this process was repeated four times. The mixture was stirred and heated to 100 °C. After 12 h the reaction was completed on analysis by TLC (silica gel) and it was then cooled to rt. Water (10 mL) was added and the mixture was extracted with EtOAc (3 × 15 mL), after which the filtrate was washed with brine (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The black residue which resulted was purified by a wash column (silica gel, EtOAc/hexane 7:3) to afford the desired 8-cyclopropyl-imidazobenzodiazepine **3** as a white solid (3.74 g, 82%): mp 106-108 °C; $[\alpha]_D^{25}$ +65.34 (*c* 0.81, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.54 (t, *J* 7.0 Hz, 1H), 7.48 – 7.32 (m, 2H), 7.19 (t, *J* 7.6 Hz, 2H), 7.08 – 6.85 (m, 2H), 6.63 (q, *J* 6.9 Hz, 1H), 4.34 (dd, *J* 14.4, 7.1 Hz, 2H), 2.11 (d, *J* 7.0 Hz, 1H), 1.91 – 1.72 (m, *J* 4.0 Hz, 1H), 1.37 (t, *J* 7.1 Hz, 3H), 1.23 (d, *J* 7.0 Hz, 2H), 0.95 (d, *J* 8.1 Hz, 2H), 0.59 (dd, *J* 10.1, 4.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.2 (s), 163.1 (s), 160.1 (d, J_{C-F} 250.1 Hz), 143.9 (s), 141.6 (s), 134.8 (s), 132.1 (s), 131.7 (s), 131.5 (s), 131.2 (d, J_{C-F} 1.3 Hz), 129.2 (d, J_{C-F} 12.8 Hz), 129.1 (d, J_{C-F} 9.4 Hz), 128.4 (s), 127.9 (s), 124.3 (d, J_{C-F} 3.3 Hz), 121.9 (s), 116.0 (d, J_{C-F} 21.6 Hz), 60.6 (s), 50.0 (s), 15.0 (s), 14.6 (s), 14.4 (s), 9.9 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{23}FN_3O_2$ 404.1769; found 404.1766.

(*S*)-Methyl-8-bromo-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (MP-III-059, 4a). The ethyl ester SH-053-2'F-*S*-CH_3 **2** (2.0 g, 4.52 mmol) was dissolved in methanol (120 mL). Sodium methoxide (0.98 g, 18.1 mmol) was added in one portion and the solution was heated to reflux. The reaction mixture was monitored on analysis by TLC (silica gel, EtOAc/hexane 4:1) until the starting material had been consumed. This took about 30 min. The reaction mixture was cooled to rt and then quenched with a saturated aq solution of $NaHCO_3$ (20 mL). Water (50 mL) was then added to the solution and the methanol was removed under reduced pressure. The product was extracted with EtOAc (3 x 100 mL) and the organic layers were combined, washed with brine (50 mL) and dried (Na_2SO_4). The solution was concentrated under reduced pressure. The solid, which resulted, was purified by flash column chromatography (silica gel, EtOAc/hexane 4:1) which provided pure methyl ester **4a** as an off-white solid (1.68 g, 87% yield): mp 194-195 °C; $[\alpha]_D^{25}$ –4.55 (*c* 0.22, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.70 (d, *J* 8.5 Hz, 1H), 7.57 (t, *J* 7.0 Hz,

1H), 7.51 – 7.36 (m, 3H), 7.23 (t, *J* 7.5 Hz, 1H), 7.02 (t, *J* 9.3 Hz, 1H), 6.67 (q, *J* 7.2 Hz, 1H), 3.89 (s, 3H), 2.13 (d, *J* 7.3 Hz, 1H), 1.26 (d, *J* 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.3 (s), 162.7 (s), 160.1 (d, J_{C-F} 251.3 Hz), 141.6 (s), 134.9 (s), 133.6 (s), 133.0 (s), 132.2 (s), 132.1 (s) 131.2 (d, J_{C-F} 2.0 Hz), 131.1 (s), 129.2 (s), 128.4 (d, J_{C-F} 12.5 Hz), 124.6 (d, J_{C-F} 3.4 Hz), 123.7 (s), 121.0 (s), 116.2 (d, J_{C-F} 21.4 Hz), 51.9 (s), 50.0 (s), 14.9 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{16}BrFN_3O_2$ 428.0404; found 428.0402.

(*S*)-Methyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (MP-III-021, 5a). The ethyl ester SH-053-2'F-*S*-CH_3 **2** (150 mg, 0.387 mmol) was dissolved in MeOH (20 mL). Sodium methoxide (84 mg, 1.55 mmol) was added in one portion and the solution was heated to reflux. The reaction mixture was monitored by analysis by TLC (silica gel, EtOAc/hexane 4:1) until the starting material had been consumed. This took about 30 min. The reaction mixture was cooled to rt and then quenched with a saturated aq solution of $NaHCO_3$ (4 mL). Water (10 mL) was then added to the solution and the methanol was removed under reduced pressure. The methyl ester **5a** was then extracted with EtOAc (3 x 40 mL). The organic layers were combined, washed with brine (10 mL) and dried (Na_2SO_4). The solution was concentrated under reduced pressure. The solid, which resulted, was purified by flash column chromatography (silica gel, EtOAc/hexane 4:1) which provided pure methyl ester **5a** as an off-white solid (117 mg, 81% yield): mp 119-120 °C; $[\alpha]_D^{25}$ +6.00 (*c* 2.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.71 (d, *J* 8.3 Hz, 1H), 7.59 (t, *J* 9.5 Hz, 2H), 7.49-7.40 (m, 2H), 7.29-7.21 (m, 1H), 7.04 (t, *J* 9.3 Hz, 1H), 6.69 (q, *J* 7.2 Hz, 1H), 3.92 (s, 3H), 3.16 (s, 1H), 2.16 (d, *J* 7.3 Hz, 1H), 1.29 (d, *J* 7.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.7 (s), 161.2 (s), 161.2 (d, J_{C-F} 251.1 Hz), 141.5 (s), 135.5 (s), 135.0 (s), 134.5 (s), 134.1 (s), 132.3 (d, J_{C-F} 7.1 Hz), 131.3 (s), 129.3 (s), 129.2 (s), 128.6 (d, J_{C-F} 9.4 Hz), 124.6 (d, J_{C-F} 3.2 Hz), 122.3 (s), 121.8 (s), 116.4 (d, J_{C-F} 21.2 Hz), 81.3 (s), 79.9 (s), 51.9 (s), 49.9 (s), 14.9 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{17}FN_3O_2$ 374.1299; found 374.1307.

General procedure for the synthesis of esters (5b-5e). The ethyl ester SH-053-2'F-*S*-CH_3 **2** (200 mg, 0.52 mmol) was stirred in dry THF (20 mL) in an oven-dried flask under an argon atmosphere at 40 °C. Simultaneously, the corresponding anhydrous alcohol R^2-OH (5 mL) was stirred in a separate oven-dried flask under argon at 40 °C. Small pieces of freshly cut Li rod (excess) were quickly added to the dry alcohol solution and the suspension was stirred for 30 min under argon. The ethyl ester solution was then added to the alcohol/lithium mixture and the reaction was monitored on analysis by TLC (silica gel) until the starting material had been consumed. This took 30-45 min. The reaction mixture was then quenched with a saturated aq solution of $NaHCO_3$ aq (15 mL) and the product was extracted with EtOAc (3 x 20 mL). The organic layers were combined, washed with brine (20 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure. The solid, which resulted, was purified by flash column chromatography to afford the pure corresponding esters **5b-5e**.

(*S*)-Propyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-32, 5b). The ester **5b** was prepared from **2** following the general procedure for esters with dry 1-propanol. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 7:3) to yield the pure propyl ester **5b** as a white powder (0.17 g, 82%): mp 204-205 °C; $\mathbf{[\alpha]_D^{25}}$ +5.26 (*c* 0.19, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.71 (d, *J* 7.8 Hz, 1H), 7.59 (dd, *J* 15.9, 7.7 Hz, 2H), 7.46 (dd, *J* 14.0, 6.5 Hz, 2H), 7.31 – 7.22 (m, 1H), 7.05 (t, *J* 9.2, 1H), 6.71 (q, *J* 6.6 Hz, 1H), 4.42 – 4.22 (m, 2H), 3.16 (s, 1H), 1.94 – 1.76 (m, 2H), 1.29 (d, *J* 6.8 Hz, 3H), 1.03 (t, *J* 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.2 (s), 163.0 (s), 160.1 (d, J_{C-F} 250.9 Hz), 141.6 (s), 135.2 (s), 134.9 (s), 134.5 (s), 133.9 (s), 132.0 (d, J_{C-F} 8.3 Hz), 131.2 (s), 129.6 (s), 129.6 (s), 128.7 (d, J_{C-F} 11.6 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.2 (s), 121.6 (s), 116.2 (d, J_{C-F} 21.4 Hz), 81.4 (s), 79.7 (s), 66.4 (s), 50.1 (s), 22.1 (s), 14.9 (s), 10.5 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{21}FN_3O_2$ 402.1612; found 402.1615.

(*S*)-Isopropyl 8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-31, 5c). The isopropyl ester **5c** was prepared from **2** following the general procedure for esters with dry isopropanol. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 3:7) to yield the pure isopropyl ester **5c** as a white powder (0.20 g, 97%): mp 231-232 °C; **$[\alpha]_D^{25}$** +14.89 (*c* 0.94, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.69 (d, *J* 8.2 Hz, 1H), 7.58 (dd, *J* 16.6, 7.7 Hz, 2H), 7.44 (dd, *J* 14.2, 6.6 Hz, 2H), 7.25 (t, *J* 7.5 Hz, 1H), 7.04 (t, *J* 9.2 Hz 1H), 6.70 (q, *J* 7.0 Hz, 1H), 5.36 – 5.20 (m, 1H), 3.15 (s, 1H), 1.40 (dd, *J* 9.4, 6.4 Hz, 6H), 1.28 (d, *J* 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.2 (s), 162.5 (s), 160.1 (d, J_{C-F} 251.9 Hz), 141.6 (s), 135.2 (s), 134.8 (s), 134.6 (s), 133.9 (s), 131.9 (d, J_{C-F} 8.7 Hz), 131.2 (s), 129.9 (s), 129.6 (s), 128.7 (d, J_{C-F} 6.0 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.2 (s), 121.6 (s), 116.2 (d, J_{C-F} 21.5 Hz), 81.4 (s), 79.7 (s), 68.3 (s), 50.1 (s), 22.0 (s), 14.9 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{21}FN_3O_2$ 402.1612; found 402.1610.

(*S*)-*tert*-Butyl 8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-30, 5d). The *t*-butyl ester **5d** was prepared from **2** following the general procedure for esters with dry *t*-butanol. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 3:7) to yield the pure *t*-butyl ester **5d** as a white powder (0.21 g, 96%): mp 214-215 °C; **$[\alpha]_D^{25}$** + 15.29 (*c* 0.85, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.69 (d, *J* 7.3 Hz, 1H), 7.65 – 7.51 (m, 2H), 7.45 (dd, *J* 15.0, 6.9 Hz, 2H), 7.28 – 7.21 (m, 1H), 7.05 (t, *J* 9.3 Hz, 1H), 6.67 (q, *J* 6.9 Hz, 1H), 3.15 (s, 1H), 1.63 (s, 9H), 1.28 (d, *J* 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.2 (s), 162.2 (s), 160.1 (d, J_{C-F} 250.7 Hz), 141.2 (s), 135.1 (s), 134.7 (s), 134.6 (s), 133.9 (s), 131.9 (d, J_{C-F} 8.2 Hz), 131.2 (s), 130.9 (s), 129.6 (s), 128.7 (d, J_{C-F} 13.1 Hz), 124.5 (d, J_{C-F} 3.5 Hz), 122.2 (s), 121.5 (s), 116.2 (d, J_{C-F} 21.4 Hz), 81.5 (s), 79.6 (s), 50.2 (s), 28.3 (s), 22.0 (s), 14.9 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{23}FN_3O_2$ 416.1769; found 416.1768.

(*S*)-*tert*-Pentyl 8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-33, 5e). The 2-methylbutyl ester **5e** was prepared from **2** following the general procedure for esters with dry 2-methylbutan-2-ol. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 1:1) to yield the pure 2-methylbutyl ester **5e** as a white powder (0.17 g, 77%): mp 94-96 °C; $[\alpha]_D^{25}$ +34.15 (*c* 0.41, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.69 (d, *J* 8.1 Hz, 1H), 7.66 – 7.50 (m, 2H), 7.45 (dd, *J* 16.3, 8.6 Hz, 2H), 7.29 – 7.19 (m, 1H), 7.05 (t, *J* 9.2 Hz, 1H), 6.66 (q, *J* 7.2 Hz, 1H), 3.15 (s, 1H), 1.98 (q, *J* 7.4 Hz, 2H), 1.59 (s, 6H), 1.28 (d, *J* 3.6 Hz, 3H), 0.99 (t, *J* 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.2 (s), 162.2 (s), 160.1 (d, J_{C-F} 250.2 Hz), 141.1 (s), 135.2 (s), 134.7 (s), 133.9 (s), 132.0 (s), 131.9 (s), 131.2 (d, J_{C-F} 1.6 Hz), 130.8 (s), 129.6 (s), 128.7 (d, J_{C-F} 13.2 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.2 (s), 121.5 (s), 116.2 (d, J_{C-F} 21.5 Hz), 84.2 (s), 81.5 (s), 79.6 (s), 50.2 (s), 33.5 (s), 25.9 (s), 25.7 (s), 14.9 (s), 8.5 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{25}FN_3O_2$ 430.1925; found 430.1928.

(*S*)-8-Ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylic acid (SH-053-2'F-*S*-CH_3-acid, 6). The ethyl ester SH-053-2'F-*S*-CH_3 **2** (5.0 g, 12.91 mmol) was dissolved in EtOH (200 mL), after which sodium hydroxide pellets (4.1 g, 103.25 mmol) were added to the solution. This reaction mixture was heated to 55 ˚C for 0.5 h and the EtOH was removed under reduced pressure. The remaining aq solution was stirred at 0 ˚C for 10 min and then aq HCl (1 M) was added dropwise to the solution until the pH was 5 (pH paper). A pale white precipitate which formed, was left in the solution for 10 min and was then collected by filtration, washed with cold water and the aq layer also allowed to stand at rt for 10 h to yield additional acid **6**. The combined solids were dried under vacuum for 7 h to provide pure acid **6** as a white powder (4.6 g, 99%): mp 217-218 °C; $[\alpha]_D^{25}$ -14.58 (*c* 0.48, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.36 (s, 1H), 7.92 (d, *J* 7.9 Hz, 1H), 7.79 (d, *J* 7.2 Hz, 1H), 7.55 (dt, *J* 7.8, 6.5 Hz, 2H), 7.32 (t, *J* 7.4 Hz, 1H), 7.21 (t, *J* 9.3 Hz, 2H), 6.61 (d, *J* 7.6 Hz, 1H), 4.36 (s, 1H), 1.13 (d, *J* 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.6 (s), 162.9 (s), 159.9 (d, J_{C-F} 242.7 Hz), 141.1 (s), 136.7 (s), 135.6 (s), 134.7 (d, J_{C-F} 2.7 Hz), 133.2 (s), 133.1 (s), 132.6 (d, J_{C-F} 6.1 Hz), 131.9 (s), 129.4 (d, J_{C-F} 2.3 Hz), 128.9 (d, J_{C-F} 8.2 Hz), 125.1 (d, *J* 3.0 Hz), 124.0 (s), 121.2 (s), 116.4 (d, J_{C-F} 20.3 Hz), 83.4 (s),

82.0 (s), 49.8 (s), 15.0 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{15}FN_3O_2$ 360.1143; found 360.1142.

General procedure for the synthesis of esters/thioester/amides (5f, 5g, 7, 8b-8f). A mixture of the acid SH-053-2'F-*S*-CH_3-acid **6** (200 mg, 0.56 mmol), thionyl chloride (0.407 mL, 5.6 mmol) and dry DCM (20 mL) was placed in an oven dried round bottom flask under argon. This suspension, which formed, was allowed to reflux at 60 ˚C for 1 h under argon. The absence of the starting material was confirmed on analysis by TLC (silica gel). The organic solvent and excess thionyl chloride were removed under reduced pressure on a rotovapor. This procedure was repeated five times with dry DCM (15 mL). The yellow residue, which remained was dissolved in dry DCM (20 mL) and cooled to 0 ˚C for 10 min under argon. Then the appropriate nucleophilic alcohol/thiol/amine (5.6 mmol), followed by triethylamine (0.78 mL, 5.6 mmol) was added to the reaction mixture at 0˚C and the mixture individually was then allowed to warm to rt and stirred for 1-2 h. After the completion of the reaction by TLC (silica gel), the solvent was removed under reduced pressure. The residue was treated with ice cold water (15 mL) and extracted with DCM (3 x 20 mL). The combined organic layer was washed with brine (20 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by column chromatography to yield the corresponding pure esters, thioesters or amides described below.

(*S*)-2,2,2-Trifluoroethyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-36, 5f). The trifluoroethyl ester **5f** was prepared from carboxylic acid **6** following the general procedure for esters/thioester/amides with dry 2,2,2-trifluoroethanol as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 2:3) to yield the pure trifluoroethyl ester **5f** as a white powder (0.20 g, 82%): mp 226-227 °C; $[\alpha]_D^{25}$ +9.43 (*c* 0.53, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.69 (d, *J* 7.9 Hz, 1H), 7.57 (d, *J* 7.9 Hz, 2H), 7.50 – 7.36 (m, 2H), 7.23 (t, *J* 7.3 Hz, 1H), 7.01 (t, *J* 8.9 Hz, 1H), 6.60 (q, *J* 7.0 Hz, 1H), 4.88 – 4.54 (m, 2H), 3.16 (s, 1H), 1.26 (d, *J* 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.4 (s), 161.1 (s), 160.1 (d, J_{C-F} 250.9 Hz), 143.1 (s), 135.5 (s), 135.3 (s), 134.2 (s), 133.9 (s), 132.1 (d, J_{C-F} 8.1 Hz), 131.2 (s), 129.6 (s), 128.5 (d, J_{C-F} 12.7 Hz), 127.7 (s), 124.5 (d, J_{C-F} 3.2 Hz), 123.0 (q, J_{C-F} 276.5 Hz), 122.3 (s), 122.0 (s), 116.2 (d, J_{C-F} 21.4 Hz), 81.3 (s), 80.0 (s), 60.2 (q, J_{C-F} 36.9 Hz), 50.1 (s), 14.7 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{16}F_4N_3O_2$ 442.1173; found 442.1176.

(*S*)-Cyclopropyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (GL-I-38, 5g). The cyclopropyl ester **5g** was prepared from the carboxylic acid **6** following the general procedure for esters/thioester/amides with dry cyclopropanol as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 3:2) to yield the pure cyclopropyl ester **5g** as a white powder (0.16 g, 71%): mp 230-231 °C; $[\alpha]_D^{25}$ +11.38 (*c* 1.23, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.67 (d, *J* 7.4 Hz, 1H), 7.55 (d, *J* 7.9 Hz, 2H), 7.49 – 7.32 (m, 2H), 7.22 (t, *J* 7.0 Hz, 1H), 7.00 (t, *J* 8.6 Hz, 1H), 6.64 (q, *J* 6.6 Hz, 1H), 4.38 – 4.21 (m, 1H), 3.15 (s, 1H), 1.24 (d, *J* 5.6 Hz, 3H), 0.87 (d, *J* 7.1 Hz, 2H), 0.77 (d, *J* 5.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.8 (s), 163.3 (s), 160.1 (d, J_{C-F} 250.6 Hz), 141.9 (s), 135.2 (s), 134.9 (s), 134.4 (s), 133.9 (s), 132.0 (d, J_{C-F} 9.0 Hz), 131.2 (s), 129.6 (s), 129.0 (s), 128.6 (d, J_{C-F} 11.4 Hz), 124.5 (d, J_{C-F} 3.1 Hz), 122.3 (s), 121.7 (s), 116.2 (d, J_{C-F} 21.5 Hz), 81.4 (s), 79.9 (s), 50.0 (s), 49.3 (s), 14.8 (s), 5.2 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{19}FN_3O_2$ 400.1456; found 400.1453.

(*S*)-S-Ethyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carbothioate (GL-I-77, 7). The thioester **7** was prepared from the carboxylic acid **6** following the general procedure for esters/thioester/amides with dry ethanethiol as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 2:3) to yield the pure thioethyl ester **7** as a white powder (0.18 g, 79%): mp 210-211 °C; $[\alpha]_D^{25}$ +21.43 (*c* 0.98, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.69 (d, *J* 8.1 Hz, 1H), 7.64 – 7.50 (m, 2H), 7.48 – 7.38 (m, 2H), 7.24 (t, *J* 7.3 Hz, 1H), 7.02 (t, *J* 9.1 Hz, 1H), 6.65 (q, *J* 14.0, 6.8 Hz, 1H), 3.16 (s, 1H), 3.09 – 2.89 (m, 2H), 1.33 (t, *J* 7.4 Hz, 3H), 1.26 (d, *J* 6.8 Hz, 3H); ^{13}C NMR (75 MHz,

$CDCl_3$): δ 187.8 (s), 163.2 (s), 160.1 (d, J_{C-F} 250.3 Hz), 138.2 (s), 135.4 (s), 135.2 (s), 134.5 (s), 134.3 (s), 134.0 (s), 132.0 (d, J_{C-F} 8.9 Hz), 131.2 (s), 129.7 (s), 128.6 (d, J_{C-F} 11.7 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.2 (s), 121.8 (s), 116.2 (d, J_{C-F} 21.5 Hz), 81.4 (s), 79.8 (s), 49.9 (s), 22.6 (s), 14.8 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{19}FN_3OS$ 404.1227; found 404.1229.

(*S*)-8-Ethynyl-6-(2-fluorophenyl)-*N*,4-dimethyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (MP-III-023, 8a). The ethyl ester SH-053-2'F-*S*-CH_3 **2** (200 mg, 0.52 mmol) was added to a sealed vessel fitted with a septum at -30 °C and treated with methyl amine (10 mL, 33% wt solution in EtOH). The vessel was sealed with a screw-cap and stirred at 50 °C for 18 h. The solution was then cooled to rt and the methyl amine and ethanol were removed under reduced pressure. The residue, which resulted, was purified by flash column chromatography (silica gel, EtOAc/hexane 3:2) to afford the pure methyl amide **8a** as a pale white powder (0.15g, 79%): mp 136-137 °C; $[\alpha]_D^{25}$ -11.54 (*c* 2.34, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.69 (d, *J* 8.1 Hz, 2H), 7.64 (t, *J* 7.0 Hz, 1H), 7.54 (d, *J* 8.3 Hz, 1H), 7.44 (dd, *J* 14.1, 7.1 Hz, 1H), 7.25 (t, *J* 7.5 Hz, 1H), 7.17 (s, 1H), 7.03 (t, *J* 9.3 Hz, 1H), 6.91 (q, *J* 6.5 Hz, 1H), 3.15 (s, 1H), 2.97 (d, *J* 5.0 Hz, 3H), 1.27 (d, *J* 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4 (s), 162.9 (s), 161.8 (d, J_{C-F} 250.8 Hz), 138.7 (s), 135.3 (s), 134.6 (s), 134.1 (s), 133.5 (s), 132.3 (s), 132.3 (d, J_{C-F} 6.4 Hz), 131.5 (s), 129.5 (s), 128.4 (d, J_{C-F} 10.0 Hz), 124.5 (d, J_{C-F} 3.5 Hz), 122.2 (s), 121.7 (s), 116.3 (d, J_{C-F} 21.5 Hz), 81.4 (s), 79.8 (s), 49.7 (s), 25.7 (s), 15.1 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{18}FN_4O$ 373.1459, found: 373.1462.

(*S*)-N-Ethyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (GL-I-43, 8b). The ethyl amide **8b** was prepared from the carboxylic acid **6** following the general procedure for esters/thioester/amides with dry ethylamine as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 1:1) to yield the pure ethyl amide **8b** as a white powder (0.18 g, 88%): mp 208-209 °C; $[\alpha]_D^{25}$ -13.33 (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.69 (dd, *J* 16.7, 7.9 Hz, 2H), 7.58 (d, *J* 8.2 Hz, 1H), 7.46 (dd, *J* 13.4, 7.2 Hz, 2H), 7.27 (t, *J* 7.4 Hz, 2H), 7.04 (t, *J* 9.3 Hz, 1H), 6.93 (q, *J* 7.2 Hz, 1H), 3.55 – 3.37 (m, 2H), 3.16 (s, 1H), 1.29 (d, *J* 7.2 Hz, 3H), 1.23 (t, *J* 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.8 (s), 162.5 (s), 160.1 (d, J_{C-F} 250.5 Hz), 138.8 (s), 135.0 (s), 134.7 (s), 133.9 (s), 133.4 (s), 131.8 (d, J_{C-F} 4.8 Hz), 131.8 (s), 131.4 (d, J_{C-F} 1.5 Hz), 129.8 (s), 128.8 (d, J_{C-F} 11.9 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.1 (s), 121.4 (s), 116.1 (d, J_{C-F} 21.5 Hz), 81.5 (s), 79.6 (s), 49.9 (s), 33.7 (s), 15.0 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{20}FN_4O$ 387.1614; found 387.1614.

(*S*)-N-Cyclopropyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (GL-I-55, 8c). The cyclopropyl amide **8c** as prepared from the carboxylic acid **6** following the general procedure for esters/thioester/amides with dry cyclopropylamine as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 1:1) to yield the pure cyclopropyl amide **8c** as a white powder (0.19 g, 86%): mp 225-226 °C; $[\alpha]_D^{25}$ -11.77 (*c* 0.17, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.69 (dd, *J* 16.5, 8.1 Hz, 2H), 7.58 (d, *J* 8.3 Hz, 1H), 7.46 (dd, *J* 14.0, 6.8 Hz, 3H), 7.27 (t, *J* 7.3 Hz, 1H), 7.04 (t, *J* 9.3 Hz, 1H), 6.93 (q, *J* 7.2 Hz, 1H), 3.17 (s, 1H), 2.85 (dq, *J* 10.6, 3.6 Hz, 1H), 1.30 (d, *J* 7.3 Hz, 3H), 0.89 – 0.73 (m, 2H), 0.69 – 0.50 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.0 (s), 162.8 (s), 160.1 (d, J_{C-F} 249.8 Hz), 138.9 (s), 135.0 (s), 134.7 (s), 133.9 (s), 133.4 (s), 131.8 (d, J_{C-F} 8.3 Hz), 131.6 (s), 131.4 (s), 129.8 (s), 128.8 (d, J_{C-F} 11.4 Hz), 124.5 (d, J_{C-F} 3.3 Hz), 122.0 (s), 121.4 (s), 116.1 (d, J_{C-F} 21.5 Hz), 81.5 (s), 79.5 (s), 49.9 (s), 22.1 (s), 15.0 (s), 6.6 (s), 6.5 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{20}FN_4O$ 399.1616; found 399.1618

(*S*)-8-Ethynyl-6-(2-fluorophenyl)-N-isopropyl-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (GL-I-57, 8d). The isopropyl amide **8d** was prepared from the carboxylic acid **6** following the general procedure for esters/thioester/amides with dry isopropylamine as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 2:3) to yield the pure isopropyl amide **8d** as a white powder (0.16 g, 74%): mp 238-239 °C; $[\alpha]_D^{25}$ -6.19 (*c* 2.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (s,

1H), 7.62 (dd, *J* 17.0, 7.9 Hz, 2H), 7.51 (d, *J* 8.3 Hz, 1H), 7.46 – 7.33 (m, 2H), 7.21 (t, *J* 7.5 Hz, 1H), 7.02 – 6.94 (m, 2H), 6.88 (q, *J* 6.9 Hz, 1H), 4.21 (dq, *J* 13.4, 6.6 Hz, 1H), 3.14 (s, 1H), 1.22 (dd, *J* 13.5, 7.2 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.8 (s), 161.8 (s), 158.4 (s), 138.8 (s), 135.0 (s), 134.7 (s), 133.9 (s), 133.4 (s), 131.9 (s), 131.7 (s), 131.4 (s), 129.7 (s), 128.8 (d, J_{C-F} 12.7 Hz), 124.4 (d, J_{C-F} 3.3 Hz), 122.1 (s), 121.4 (s), 116.0 (d, J_{C-F} 21.6 Hz), 81.5 (s), 79.6 (s), 49.9 (s), 40.7 (s), 22.9 (s), 22.9 (s), 15.0 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{22}FN_4O$ 401.1772; found 401.1776.

(*S*)-N-(*tert*-Butyl)-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (GL-I-41, 8e). The *t*-butyl amide **8e** was prepared from the carboxylic acid **6** following the general procedure for esters/thioester/amides with dry *t*-butylamine as the nucleophile. The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 1:1) to yield the pure *t*-butyl amide **8e** as a white powder (0.19 g, 83%): mp 136-137 °C; $[\alpha]_D^{25}$ +8.7 (*c* 0.23, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (s, 1H), 7.65 (dd, *J* 15.1, 7.7 Hz, 2H), 7.51 (d, *J* 8.3 Hz, 1H), 7.44 (dd, *J* 10.1, 7.6 Hz, 2H), 7.24 (t, *J* 7.5 Hz, 1H), 7.02 (dd, *J* 15.8, 5.8 Hz, 2H), 6.87 (q, *J* 7.1 Hz, 1H), 3.15 (s, 1H), 1.46 (s, 9H), 1.27 (d, *J* 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.8 (s), 162.1 (s), 160.2 (d, J_{C-F} 250.3 Hz), 138.6 (s), 135.0 (s), 134.8 (s), 133.9 (s), 133.0 (s), 132.6 (s), 131.8 (d, J_{C-F} 7.8 Hz), 131.34 (d, J_{C-F} 2.4 Hz), 129.8 (s), 128.8 (d, J_{C-F} 11.8 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.1 (s), 121.4 (s), 116.1 (d, J_{C-F} 21.5 Hz), 81.6 (s), 79.5 (s), 50.9 (s), 49.9 (s), 29.0 (s), 15.0 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{24}FN_4O$ 415.1926; found 415.1926.

(*S*)-8-Ethynyl-6-(2-fluorophenyl)-*N,N*,4-trimethyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (GL-I-54, 8f) The dimethyl amide **8f** was prepared from **6** following the general procedure with dry dimethylamine as the nucleophile. The crude residue was purified by a column chromatography (silica gel, EtOAc and 1% MeOH) to yield pure dimethyl amide **8f** as a light yellow powder (0.15 g, 71%): mp 142-144 °C; $[\alpha]_D^{25}$ -16.0 (*c* 0.75, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 (s, 1H), 7.73 (d, *J* 8.1 Hz, 1H), 7.63 (t, *J* 7.4 Hz, 1H), 7.52 (d, *J* 8.2 Hz, 1H), 7.48 – 7.41 (m, 2H), 7.26 (t, *J* 7.5 Hz, 1H), 7.04 (t, *J* 9.3 Hz, 1H), 4.33 (q, *J* 6.0 Hz, 1H), 3.16 (s, 1H), 3.14 (s, 3H), 3.01 (s, 3H), 1.94 (d, *J* 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1 (s), 162.0 (s), 160.3 (d, J_{C-F} 252.3 Hz), 135.3 (s), 134.7 (d, J_{C-F} 7.2 Hz), 133.8 (s), 133.7 (s), 132.8 (d, J_{C-F} 12.7 Hz), 132.1 (d, J_{C-F} 9.4 Hz), 131.8 (s), 131.3 (s), 129.3 (s), 127.7 (d, J_{C-F} 10.8 Hz), 124.5 (d, J_{C-F} 3.4 Hz), 122.8 (s), 121.5 (s), 116.2 (d, J_{C-F} 21.7 Hz), 81.6 (s), 79.5 (s), 52.2 (s), 39.1 (s), 35.0 (s), 18.5 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{20}FN_4O$ 387.1616; found 387.1624.

General Procedure for the Preparation of Oxadiazoles (9a-9c). The ethyl ester **2** (200 mg, 0.52 mmol) was dissolved in dry THF (20 mL) at rt under argon. In a separate flask which contained 3Å molecular sieves, the corresponding oxime (2.08 mmol) was dissolved in dry THF (30 mL) under argon and treated with sodium hydride (60% dispersion in mineral oil, 0.57 mmol). The mixture, which resulted, was stirred for 15 min at which point the solution containing the ethyl ester was added. The reaction mixture, which resulted, was stirred at rt for 2 h until the starting material was consumed as indicated on analysis by TLC (silica gel). The reaction mixture was quenched with a saturated aq $NaHCO_3$ solution (50 mL). Water (50 mL) was then added and the product was extracted with EtOAc (3 x 100 mL). The organic layers were combined, washed with brine (30 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure. The solid, which resulted, was purified by flash column chromatography (silica gel) to afford the pure corresponding oxadiazole.

(*S*)-5-(8-Ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)-3-methyl-1,2,4-oxadiazole (GL-I-65, 9a). The methyl oxadiazole **9a** was prepared from **2** following the general procedure for oxadiazoles with the methyl oxime (0.154 g, 2.08 mmol). The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 3:2) to yield pure methyl oxadiazole **9a** as a white powder (0.174 g, 85%): mp 225-226 °C; $[\alpha]_D^{25}$ +32.52 (*c* 2.86, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.74 (d, *J* 8.2 Hz, 1H), 7.63 (d, *J* 8.4 Hz, 2H), 7.48 (dd, *J* 18.1, 11.6 Hz, 2H), 7.27 (t, *J* 7.5 Hz, 1H), 7.06 (t, *J* 9.2 Hz, 1H), 6.75 (q, *J* 7.1

Hz, 1H), 3.18 (s, 1H), 2.47 (s, 3H), 1.35 (d, *J* 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8 (s), 167.4 (s), 163.4 (s), 160.1 (d, J_{C-F} 250.5 Hz), 139.3 (s), 136.3 (s), 135.3 (s), 134.2 (s), 134.1 (s), 132.1 (d, J_{C-F} 8.5 Hz), 131.2 (s), 129.6 (s), 128.6 (d, J_{C-F} 12.3 Hz), 124.8 (s), 124.5 (d, J_{C-F} 3.2 Hz), 122.2 (s), 121.9 (s), 116.2 (d, J_{C-F} 21.4 Hz), 81.3 (s), 80.0 (s), 50.2 (s), 14.9 (s), 11.7 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{17}FN_5O$ 398.1412; found 398.1419.

(*S*)-3-Ethyl-5-(8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)-1,2,4-oxadiazole (GL-I-66, 9b). The ethyl oxadiazole **9b** was prepared from **2** following the general procedure for oxadiazoles with the ethyl oxime (0.183 g, 2.08 mmol). The crude residue was purified by flash column chromatography (silica gel, EtOAc/Hexane 3:2) to yield pure ethyl oxadiazole **9b** as a white powder (0.168 g, 79%): mp 199-200 °C; $[\alpha]_D^{25}$ +50.00 (*c* 0.22, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.75 (d, *J* 8.2 Hz, 1H), 7.63 (d, *J* 8.3 Hz, 2H), 7.54 – 7.40 (m, 2H), 7.27 (t, *J* 7.4 Hz, 1H), 7.06 (t, *J* 9.2 Hz, 1H), 6.75 (q, *J* 14.4, 7.2 Hz, 1H), 3.18 (s, 1H), 2.84 (q, *J* 7.6 Hz, 2H), 1.40 (d, *J* 7.6 Hz, 3H), 1.36 (t, *J* 3.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9 (s), 170.7 (s), 163.4 (s), 160.1 (d, J_{C-F} 251.4 Hz), 139.2 (s), 136.3 (s), 135.3 (s), 134.2 (s), 134.1 (s), 132.0 (d, J_{C-F} 8.7 Hz), 131.2 (s), 129.6 (d, J_{C-F} 6.0 Hz), 128.6 (d, J_{C-F} 11.9 Hz), 125.0 (s), 124.5 (d, J_{C-F} 3.2 Hz), 122.2 (s), 121.9 (s), 116.2 (d, J_{C-F} 21.5 Hz), 81.3 (s), 79.9 (s), 50.2 (s), 19.7 (s), 15.0 (s), 11.5 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{19}FN_5O$ 412.1568; found 412.1569.

(*S*)-5-(8-Ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)-3-isopropyl-1,2,4-oxadiazole (GL-I-81, 9c). The isopropyl oxadiazole **9c** was prepared from **2** following the general procedure for oxadiazoles with the isopropyl oxime (0.212 g, 2.08 mmol). The crude residue was purified by flash column chromatography (silica gel, EtOAc/hexane 3:2) to yield pure isopropyl oxadiazole **9c** as a white powder (0.180 g, 82%): mp 205-206 °C; $[\alpha]_D^{25}$ +22.37 (*c* 0.76, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.66 (d, *J* 8.3 Hz, 1H), 7.60 (d, *J* 8.4 Hz, 1H), 7.53 (t, *J* 7.3 Hz, 1H), 7.44 – 7.30 (m, 2H), 7.17 (t, *J* 7.5 Hz, 1H), 6.96 (t, *J* 9.2 Hz, 1H), 6.67 (q, *J* 7.0 Hz, 1H), 3.14 (s, 1H), 3.09 (q, *J* 7.0 Hz, 1H), 1.32 (d, *J* 7.0 Hz, 6H), 1.28 (d, *J* 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.2 (s), 170.6 (s), 163.4 (s), 160.0 (d, J_{C-F} 251.5 Hz), 139.2 (s), 136.3 (s), 135.4 (s), 134.2 (s), 134.0 (s), 132.0 (d, J_{C-F} 8.3 Hz), 131.1 (s), 129.5 (s), 128.6 (d, J_{C-F} 12.3 Hz), 125.0 (s), 124.5 (d, J_{C-F} 3.1 Hz), 122.3 (s), 121.8 (s), 116.2 (d, J_{C-F} 21.4 Hz), 81.3 (s), 80.0 (s), 50.2 (s), 26.7 (s), 20.6 (s), 20.5 (s), 14.9 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{21}FN_5O$ 426.1725; found 426.1728.

(*S*)-8-Ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carbonitrile (MP-III-018.A, 10). The ethyl ester SH-053-2'F-*S*-CH_3 **2** (0.5 g, 1.40 mmol) was stirred in dry xylene (35 mL) at rt. Using a glass syringe and a metal needle, amidodimethylaluminium (0.67 M, 12.5 mL, 8.42 mmol) was carefully added to the solution of starting material **2**. The reaction was then heated to 80 ˚C in an oil bath and was monitored on analysis by TLC (silica gel) until the starting material had been consumed in 2 h. Once the reaction was complete, the mixture was cooled to rt and then quenched with cold water (15 mL). The product was extracted with EtOAc (5 x 50 mL) and the organic layers were combined, washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure. The solid, which resulted, was purified by flash column chromatography (silica gel, EtOAc/hexane 1:1) which afforded the nitrile **10** as a white solid (0.331 g, 75.4%): mp 131-132 °C; $[\alpha]_D^{25}$ -165.16 (*c* 1.55, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.78 (d, *J* 7.1 Hz, 1H), 7.67 (s, 1H), 7.62 – 7.53 (m, 1H), 7.49 (s, 2H), 7.28 (s, 1H), 7.04 (t, *J* 8.7 Hz, 1H), 4.36 (d, *J* 4.8 Hz, 1H), 3.20 (s, 1H), 2.18 (d, *J* 4.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.6 (s), 161.9 (d, J_{C-F} 253.4 Hz), 143.7 (s), 135.8 (s), 135.7 (s), 134.0 (s), 133.4 (s), 132.9 (d, J_{C-F} 9.7 Hz), 131.4 (s), 129.2 (s), 126.9 (d, J_{C-F} 13.0 Hz), 124.7 (d, J_{C-F} 6.5 Hz), 122.9 (s), 122.6 (s), 116.5 (d, J_{C-F} 19.7 Hz), 114.5 (s), 110.7 (s), 81.1 (s), 80.3 (s), 51.7 (s), 18.1 (s); HRMS (ESI/IT-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{14}FN_4$ 341.1137; found 341.1142.

Preparation of the 0.67 M amidodimethylaluminium solution. The ammonia gas was bubbled into dry DCM (20 mL) at 0 °C until the solution was saturated. This took about 10-15 min. The trimethylaluminum (10 mL,

2.0 M in toluene) was then added to the solution. The solution was stirred at rt for 5 min and transferred directly for use in the reaction above using a glass syringe and metal needle.

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Supplementary Material

Catalepsy, seizure protection in the 6 Hz electroshock, ataxic assessment in the rotorod, microsomal stability, cytotoxitity, and rotorod assays, prepulse inhibition, and the pharmacophore/receptor model can all be found in the online version.

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