Book Chapter

A Review of the Updated Pharmacophore for the Alpha 5 GABA(A) Benzodiazepine Receptor Model

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Abstract

An updated model of the GABA(A) benzodiazepine receptor pharmacophore of the $\alpha 5$ -BzR/GABA(A) subtype has been constructed prompted by the synthesis of subtype selective ligands in light of the recent developments in both ligand synthesis, behavioral studies, and molecular modeling studies of the binding site itself. A number of BzR/GABA(A) $\alpha 5$ subtype selective compounds were synthesized, notably $\alpha 5$ -subtype selective inverse agonist PWZ-029 (**1**) which is active in enhancing cognition in both rodents and primates. In addition, a chiral positive allosteric modulator (PAM), SH-053-2'-F-R-CH₃ (**2**), has been shown to reverse the deleterious effects in the MAM-model of schizophrenia as well as alleviate constriction in airway smooth muscle. Presented here is an updated model of the pharmacophore for $\alpha 5\beta 2\gamma 2$ Bz/GABA(A) receptors, including a rendering of PWZ-029 docked within the $\alpha 5$ -binding pocket showing specific interactions of the molecule with the receptor. Differences in the included volume as compared to $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, and $\alpha 3\beta 2\gamma 2$ will be illustrated for clarity. These new models enhance the ability to understand structural characteristics of ligands which act as agonists, antagonists, or inverse agonists at the Bz BS of GABA(A) receptors.

1. Introduction

The gamma-amino butyric acid A (GABA_A) receptor is a heteropentameric chloride ion channel. This channel is generally made up of two α -subunits, two β -subunits, and a single γ -subunit arranged in an $\alpha\beta\alpha\beta\gamma$ fashion. The GABA_A receptors (GABA_AR) are responsible for a myriad of brain functions. Positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) act on the benzodiazepine (BZ) site of the GABA_AR which can change the conformation of the receptor to inhibit or excite the neurons associated with the ion channel. To date, researchers have been unable to get an X-ray crystal structure of a functional Bz/GABA_AR ion

channel. Recently, Miller and Aricescu [1] have reported the crystal structure of a homopentameric GABA_AR containing the $\beta 3$ -subunit at 3 Å resolution. Although this work provides great promise that other heteropentameric GABA_ARs will be crystallized in the near future, molecular modeling and structure-activity-relationships (SARs) still remain key tools to find better subtype-selective binding agents.

2. Subtype Selective Ligands for $\alpha 5$ GABA(A)/Bz Receptors

Interest in BzR/GABA(A) $\alpha 5$ subtypes began years ago when it was realized that $\alpha 5\beta 3\gamma 2$ Bz/GABA(A) subtypes are located

FIGURE 1: Alpha 5 selective compounds [13]. This figure is modified from that reported in [13].

primarily in the hippocampus. More recently this interest has been confirmed by the report of Möhler et al. [2–5] on $\alpha 5$ “knock-in mice.” This group has provided strong evidence that hippocampal extrasynaptic $\alpha 5$ GABA(A) receptors play a critical role in associative learning as mentioned above [6–11].

Earlier we synthesized a series of $\alpha 5$ subtype selective ligands (RY-023, RY-024, RY-079, and RY-080) based on the structure of Ro 15-4513 and reported their binding affinity [6], as well as several ligands by Atack et al. [12]. These ligands are benzodiazepine receptor (BzR) negative modulators *in vivo* and a number of these compounds have been shown to enhance memory and learning [13]. One of these ligands was shown by Bailey et al. [6] to be important in the acquisition of fear conditioning and has provided further evidence for the involvement of hippocampal GABA(A)/BzR in learning and anxiety [13]. This is in agreement with the work of DeLorey et al. [7] in a memory model with a ligand closely related to $\alpha 5$ subtype selective inverse agonists RY-024 and RY-079 including PWZ-029 (1).

In order to enhance the $\alpha 5$ subtype selectivity, the bivalent form of RY-80 (3) was prepared to provide XLi-093 (4) [13]. The binding affinity of XLi-093 *in vitro* was determined on $\alpha 1-6\beta 3\gamma 2$ LTK cells and is illustrated in Figure 1. This bivalent ligand exhibited little or no affinity at $\alpha 1-4,6\beta 3\gamma 2$ BzR/GABA(A) subtypes, but this $\alpha 5$ ligand had a K_i of 15 nM at the $\alpha 5\beta 3\gamma 2$ subtype [14]. Since this receptor binding study indicated bivalent ligand XLi-093 bound almost exclusively to the $\alpha 5$ subtype, the efficacy of this ligand on GABA(A) receptor subtypes expressed in *Xenopus* oocytes was investigated by Sieghart, Furtmueller, Li, and Cook [14, 15]. Analysis of the data indicated that XLi-093 up to a concentration of 1 μ M did not trigger chloride flux in any one of the GABA(A) subtypes tested. At 1 μ M XLi-093 did not modulate GABA induced chloride flux in $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, or $\alpha 3\beta 3\gamma 2$ receptors, but very slightly inhibited chloride flux in $\alpha 5\beta 3\gamma 2$ subtypes. At 1 μ M, XLi-093 barely influenced benzodiazepine (Valium) stimulation of GABA-induced current in $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$,

FIGURE 2: XLi-093 (4) aligned in the included volume of the pharmacophore receptor model for the $\alpha 5\beta 3\gamma 2$ subtype [17, 18] (this figure is modified from the figure in Clayton et al., 2007) [23].

and $\alpha 3\beta 3\gamma 2$ BzR but shifted the diazepam dose response curve to the right in $\alpha 5\beta 3\gamma 2$ receptors in a very significant manner [16]. Importantly, bivalent ligand XLi-093 was able to dose dependently and completely inhibit diazepam-stimulated currents in $\alpha 5\beta 3\gamma 2$ receptors. This was the first subtype selective benzodiazepine receptor site antagonist at $\alpha 5$ receptors. This bivalent ligand XLi-093 provided a lead compound for all of the bivalent ligands in this research [16].

Illustrated in Figure 2 is XLi-093 (4) aligned excellently within the pharmacophore-receptor model of the $\alpha 5\beta 3\gamma 2$ subtype [14, 16–19]. The fit to the pharmacophore-receptor and the binding data indicate that bivalent ligands will bind to BzR subtypes [14, 19]. It is believed that the dimer enters the binding pocket with one monomeric unit docking while the other monomer tethered by a linker extends out of the protein into the extracellular domain. If this is in fact true that the second imidazole unit is protruding into the extracellular domain of the BzR/GABA(A) $\alpha 5$ binding site,

TABLE 1: Full PDSP panel receptor binding reported (Roth [138]) for XLi-093 and XLi-356.

Cook code	5ht1a	5ht1b	5ht1d	5ht1e	5ht2a	5ht2b	5ht2c	5ht3	5ht5a	5ht6	5ht7	α 1A	β 1B	α 2A	α 2B
XLi093	*	Repeat	*	*	*	*	*	*	*	*	*	*	*	*	*
XLi356	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cook code	α 2C	Beta1	Beta2	CB1	CB2	D1	D2	D3	D4	D5	DAT	DOR	H1	H2	H3
XLi093	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
XLi356	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cook code	H4	Imidaz oline	KOR	M1	M2	M3	M4	M5	MDR	MOR	NET	NMDA	SERT	σ 1	σ 2
XLi093	*	*	2,024.00	*	*	*	*	*	*	*	*	*	*	*	*
XLi356	*	*	6,118.00	*	*	*	*	*	*	*	*	*	*	*	*

Data ("secondary binding") are K_i values. K_i values are reported in nanomolar concentration, Case Western Reserve University. "*" indicates "primary missed" (<50% inhibition at 10 μ M). See full data of the PDSP screen in the report of Clayton [22].

it could have a profound effect on the ligand design. This means other homodimers or even heterodimers may bind to BzR/GABA(A)ergic sites.

In this vein, Wenger, Li, and Cook et al. [13, 20, 21] earlier described preliminary data that XLi-093, an α 5 subtype selective antagonist, enhances performance of C57BL/6J mice under a titrating delayed matching to position schedule of cognition, as illustrated in Figure 3 [14, 16–19]. This indicates, however, that this agent does cross the blood brain barrier.

Bivalent ligands have a preferred linker of 3–5 methylene units, between the two pharmacophores (see XLi-093). This was established by NMR experiments run at low temperatures, X-ray crystallography, and molecular modeling of the ligands in question and will be discussed [14, 17, 18].

Based on this data, additional α 5-subtype selective ligands have been prepared (see Figure 4). The basic imidazobenzodiazepine structure has been maintained [7]; however substituents were varied in regions around the scaffold based on molecular modeling [6]. These are now the most α 5 subtype selective ligands ever reported [22]. Moreover, the ability to increase the subtype selectivity can be done by selecting specific substituents on these ligands to new agents with 400–1000-fold α 5-selectivity over the remaining 5 subtypes. *This is an important step forward to understanding the true, unequivocal physiological responses mediated by α 5 subtypes in regard to cognition (amnesia), schizophrenia, anxiety, and convulsions, all of which in some degree are influenced by α 5 subtypes.* Based on the ligands in Figures 4 and 5, affinity has occurred principally at α 5 subtypes. In addition, since XLi-093 bound very tightly only to α 5 BzR subtypes, the bivalent nature and functionality presented here can be incorporated into other dimeric ligands.

As shown previously in Figure 3, α 5-antagonist XLi-093 (4) was shown to enhance cognition. In another study, a reduction of the two acetylenic groups of XLi-093 resulted in ethyl groups [14], providing a new bivalent ligand (XLi-356, 10) which shows α 5-selective binding with very low affinity for α 1 subtypes (Figure 5). Efficacy (oocyte) data shows XLi-356 is an α 5 negative allosteric modulator [7, 13]. DeLorey et al. have recently shown in mice that XLi-356 does potentially reverse scopolamine induced memory deficits [7]. This bivalent α 5 inverse agonist enhanced cognition

FIGURE 3: XLi-093 (4) effects on cognition enhancement by Wenger et al. (data on statistical significance not shown, unpublished results). Effects of 4 on cognition from the mean delay achieved by C57BL/6J mice titrating delayed matching-to-position schedule.

in agreement with work reported from our laboratory on monovalent inverse agonists RY-10 [6] and RY-23 [7].

The dimers XLi-093 (4) and XLi-356 (10) were sent to Case Western Reserve (NIMH supported PDSP program, Roth et al.) for full panel receptor binding and they do not bind to other receptors at levels of concern (Table 1).

Although XLi-093 (4) was found to be an antagonist at the α 5 subtype, XLi-356 (10) was found to be a weak agonist-antagonist. XLi-356 was found to reverse scopolamine induced memory deficits in mice. When XLi-356 was looked at in audio cued fear conditioning, the results show no activity. This suggests that the effect of XLi-356 is selective through α 5 receptors which are abundant in the hippocampus which is highly associated with contextual memory. Audio cued memory instead is amygdala-based and should not be affected by an α 5 subtype selective compound [39–42].

As illustrated in Figure 6, scopolamine (1 mg/kg) reduced freezing (i.e., impairs memory) generally due to coupling the

Ligand	R ₁	α1	α2	α3	α4	α5	α6
1a^b	CH ₂ OCH ₃	>300	>300	>300	ND	38.8	>300
1b^b	CH ₂ OCH ₃	920	ND	ND	ND	30	ND
1c^b	CH ₂ OCH ₃	362.4	180.3	328.2	ND	6.185	ND
5	CH ₂ Cl	>300	>300	>300	ND	28.5	>300
6	CH ₂ OEt	>300	>300	>300	ND	82.7	>300
7	CH ₃	>89	>70	>91	ND	3.7	>301
8	CO ₂ Et	>1000	>1000	>1000	>1000	64	>1000
9	CO ₂ Et	>2000	>2000	>2000	>2000	176	>2000

^aData shown here are the means of two determinations which differed by less than 10%. ND: not determined (presumably similar to α6).

^b**1a-c** are binding datasets of PWZ-029 (**1**) from three separate laboratories. This figure is modified from that illustrated in reference [22] to indicate the α5 subtype selectivity.

FIGURE 4: Binding data of selected imidazobenzodiazepines [22].

Ligand	α1	α2	α3	α4	α5	α6
XLI-356 (10)	1852	4203	8545	ND	101	5000
RY-068 (11)	>500	877	496	ND	37	>1000
RY-062 (12)	>1000	>1000	>500	ND	172	>2000
RY-069 (13)	692	622	506	ND	19	>1000
RY-I-29 (14)	>1000	>1000	>1000	ND	157	>1000

^aData shown here are the means of two determinations which differed by less than 10%. ND: not determined (presumably greater than 1000 nM; similar to α6).

FIGURE 5: Binding data of selected imidazobenzodiazepines substituted with an E-ring as compared to XLI-356 (**10**).

FIGURE 6: Visual and audio cued data for XLi-356 (10). This figure was modified from that in [22].

context (the cage) with a mild shock. XLi-356 (10 mg/kg) attenuated the impairment of memory returning the freezing to the levels on par with subjects dosed with vehicle. In audio cued memory the response was activated by sound, not the context. XLi-356 was not able to reverse this type of memory effect which is amygdala driven. A similar effect was observed for XLi-093 by Harris et al. [43]. XLi-093 is the most selective antagonist for $\alpha 5$ subtypes reported to date [13, 43] and is a very useful $\alpha 5$ antagonist used by many *in vivo* [22, 44, 45].

Molecular modeling combined with this knowledge was used to generate new lead compounds aimed at the development of $\alpha 5$ -subtype selective positive and negative allosteric modulators to study cognition as well as amnesia mediated by the hippocampus. All of these compounds have been prepared based on the structure of current $\alpha 5$ -subtype selective ligands synthesized in Milwaukee [46] (see Figures 4 and 5), as well as the binding affinity (15 nM)/selectivity of bivalent $\alpha 5$ antagonist XLi-093 (4) [13].

In efforts to enhance $\alpha 5$ -selectivity in regards to cognition, Cook, Bailey, and Helmstetter et al. have employed RY-024 to study the hippocampal involvement in the benzodiazepine receptor in learning and anxiety [14, 19]. Supporting this Harris, DeLorey et al. show in mice that $\alpha 5$ NAMs (1) and RY-10 potentially reversed scopolamine-induced memory impairment. These $\alpha 5$ NAMs provide insight as to how GABA_ARs influence contextual memory, an aspect of memory affected in age associated memory impairment and especially in Alzheimer's disease [13, 62–64]. In addition, Savić et al. have used the $\alpha 1$ preferring antagonist, BCCt, in passive avoidance studies, in which midazolam's amnesic effects are shown to be due to interaction of agonist ligands at $\alpha 5$ in addition to $\alpha 1\beta 3\gamma 2$ BzR subtypes [24, 65].

3. PWZ-029: A Negative Allosteric Modulator

PWZ-029 (1) has been studied extensively as an $\alpha 5$ -GABA_AR inverse agonist and in certain experimental models has

TABLE 2: Affinity of PWZ-029 (1); K_i (nM)^a.

Code	MW	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
PWZ-029 (1)	291.73	>300	>300	>300	ND	38.8	>300
PWZ-029 (1)	291.73	920	ND	ND	ND	30	ND
PWZ-029 (1)	291.73	362	180	328	ND	6	ND

^aData from three separate laboratories.

been shown to enhance cognition. The binding data from three separate laboratories (Table 2) have all shown that it exhibits remarkable selectivity for the $\alpha 5$ subunit-containing receptors, all greater than 60-fold compared to the next subunit.

Electrophysiological efficacy testing done by Sieghart et al. in oocytes demonstrated that PWZ-029 (1) acts as a negative allosteric modulator at the $\alpha 5$ -subunit, with a very weak agonist activity at the $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunits (Figure 7). At a pharmacologically relevant concentration of 0.1 μ M, PWZ-029 exhibits moderate negative modulation at the $\alpha 5$ -subunit, while showing little or no effect at the $\alpha 1$, $\alpha 2$, or $\alpha 3$ -subunits.

Milić et al. reported on the effects of PWZ-029 in the widely used novel object recognition test, which differentiates between the exploration time of novel and familiar objects. As shown by significant differences between the exploration times of the novel and familiar object (Figure 8(a)), as well as the respective discrimination indices (Figure 8(b)), all the three tested doses of PWZ-029 (2, 5 and 10 mg/kg) improved object recognition in rats after the 24 h delay period. Additionally, in the procedure with the 1 h delay between training and testing, the lowest of the tested doses of PWZ-029 (2 mg/kg) successfully reversed the deficit in recognition memory induced by 0.3 mg/kg scopolamine (Figure 9) [25].

The results of the described study showed for the first time that inverse agonism at $\alpha 5$ -GABA_A receptors may be efficacious in both improving cognitive performance in

FIGURE 7: Oocyte electrophysiological data of PWZ-029 (1) [24].

FIGURE 8: The effects of PWZ-029 (1) (2, 5 and 10 mg/kg) on (a) time exploring familiar and novel objects and (b) discrimination indices in the novel object recognition test using a 24 h delay (mean + SEM). Significant differences are indicated with asterisks (paired-samples *t*-test, novel versus familiar, **p* < 0.05, ***p* < 0.01, ****p* < 0.001). A significant difference from zero is indicated with hashes (one sample *t*-test, ##*p* < 0.01). The number of animals per each treatment group was 10. SOL = solvent [25].

FIGURE 9: The effects of 0.3 mg/kg scopolamine (SCOP 0.3) and combination of 0.3 mg/kg scopolamine and PWZ-029 (1) (2, 5, and 10 mg/kg) on the rats' performance in the object recognition task after a 1 h delay: (a) time exploring familiar and novel objects and (b) discrimination index. Data are represented as mean + SEM. Significant differences are indicated with asterisks (paired-samples t -test, novel versus familiar, * $p < 0.05$, ** $p < 0.01$). A significant difference from zero is indicated with hashes (one sample t -test, # $p < 0.05$). The number of animals per each treatment group was 12–15. SAL = saline, SOL = solvent [25].

unimpaired subjects and ameliorating cognitive deficits in pharmacologically impaired subjects, as assessed in two protocols of the same animal model [25].

In a recent by Rowlett et al. [26], negative allosteric modulator PWZ-029 was evaluated in female rhesus monkeys ($n = 4$) in an Object Retrieval test with Detours (ORD; Figure 10 for details). 1 was administered via i.v. catheters in ORD trained monkeys and evaluated for cognition enhancement. A successful trial was determined by the ability of the subject to obtain a food reward within a transparent box with a single open side, with varying degrees of difficulty ("easy" or "difficult" or "mixed" as a combination of both) based on food placement within the box. In "mixed" trials using PWZ-029, no significant results were observed when compared to vehicle (Figure 11(a)). "Difficult" trials, however, exhibited an increasing dose-dependent curve for successful trials (Figure 11(b)). These results were attenuated by a coadministration $\alpha 5$ -antagonist XLI-093 (Figure 11(c)). PWZ-029 was also shown to dose-dependently reverse the cholinergic deficits that were induced by scopolamine (Figure 11(d)) [26].

These findings suggest that PWZ-029 can enhance performance on the ORD task, only under conditions in which baseline performance is attenuated. The effects of PWZ-029 were antagonized in a surmountable fashion by the selective $\alpha 5$ -GABA_A ligand, XLI-093, consistent with PWZ-029's effects being mediating via the $\alpha 5$ -GABA_A receptor. The results are consistent with the view that $\alpha 5$ GABA_A receptors may represent a viable target for discovery of cognitive enhancing agents.

In addition, we have new data showing that modulation of $\alpha 5$ -GABA_ARs by PWZ-029 rescues Hip-dependent memory in an AD rat model [PMID: 23634826] as evidenced by a significant decrease in the latency to reach the hidden platform (memory probe trials) on spatial water maze task (Figure 12). Roche has employed a similar strategy at $\alpha 5$ subtypes and recently has a drug in the clinic to treat symptoms of dementia in Down syndrome patients. It is well known many Down syndrome patients develop Alzheimer's disease or a dementia with a very similar etiology. This is aimed at treating early onset Alzheimer's patients.

4. PWZ-029 Docking within $\alpha 5\gamma 2$ GABA_A Receptor Subunit Homology Model

These studies with PWZ-029 led to the molecular model rendering of the compound docked within the $\alpha 5\gamma 2$ BzR subtype (Figures 13–16). The model figures have the following features:

The docking of PWZ-029 within the GABA_A/BzR shows the molecule bound and interacting with specific amino acids. The A and B rings of the benzodiazepine framework undergo a π -stacking interaction with HIS 105, indicated by the magenta coloring. At the other end of the molecule the methoxy lone pair and imidazole nitrogen lone pair act as a hydrogen bond acceptors with THR 210 and TYR 213, respectively. These interactions are shown by the aqua-blue descriptors.

Methods

ORD task examples of different types of trials

Note: open side indicated with orange lines

Mixed trials			Difficult trials		
Type of trial	Position of opening	Position of food	Type of trial	Position of opening	Position of food
Easy	Left	Outside	Difficult	Left	Deep
Easy	Right	Outside	Difficult	Right	Deep
Easy	Front	Outside	Difficult	Left	Deep
Easy	Left	Outside	Difficult	Right	Deep
Easy	Front	Outside	Difficult	Left	Deep
Easy	Left	Inside	Difficult	Left	Deep
Difficult	Right	Deep	Difficult	Right	Deep
Easy	Left	Inside	Difficult	Left	Deep
Easy	Right	Inside	Difficult	Right	Deep
Easy	Left	Inside	Difficult	Left	Deep
Difficult	Right	Deep	Difficult	Left	Deep
Difficult	Left	Deep	Difficult	Right	Deep
Difficult	Right	Deep	Difficult	Left	Deep
Easy	Left	Inside	Difficult	Right	Deep
Easy	Right	Inside	Difficult	Left	Deep

Subjects:

Rhesus monkeys (*Macaca mulatta*) $N = 5$

Implanted with chronic i.v. catheters

Procedure:

Object retrieval with detours (ORD)

15 trials per session

Easy, difficult, and mixed trials (see diagram)

Tests:

(i) PWZ-029 · mixed trials

(ii) PWZ-029 · difficult trials

(iii) PWZ-029 + XLI-093 · difficult trials

(iv) Scopolamine · mixed trials

(v) PWZ-029 + scopolamine · mixed trials

Measurements: % correct trials—trial in which food is obtained with the first reach

Errors: barrier reaches (hand in contact with closed side of box) and incomplete trials (food grasped but dropped)

FIGURE 10: ORD methods and procedure [26].

5. Subtype Selective Agonists for $\alpha 5$ GABA_A/Bz Receptors

Möhler has proposed that $\alpha 5$ selective inverse agonists or $\alpha 5$ selective agonists might enhance cognition [5, 13, 16–18, 86]. This is because of the extrasynaptic pyramidal nature of $\alpha 5\beta 3\gamma 2$ subtypes, located almost exclusively in the hippocampus. Because of this, a new “potential agonist” which binds solely to $\alpha 5\beta 3\gamma 2$ subtypes was designed by computer modeling (see Figure 17). This ligand (DM-I-81, **9**) has an agonist framework and binds only to $\alpha 5\beta 3\gamma 2$ subtypes [13, 17, 18, 86]. The binding potency at $\alpha 5$ subtypes is 176 nM. Although the 8-pendant phenyl of DM-I-81 was lipophilic and bound to the L_2 pocket, additional work on the 8-position of this scaffold has been abandoned and generally left as an acetylene or halide function, with a few exceptions. The steric bulk of the 8-phenyl moiety was felt detrimental to activity and potency which may have led to the weak binding affinity.

6. Alpha 5 Positive Allosteric Modulators in Schizophrenia

In addition to inverse agonists, a number of other $\alpha 5$ -GABA_AR positive allosteric modulators (PAMs) have been

synthesized. These compounds, such as SH-053-2'-F-R-CH₃ (**2**), have been shown to decrease the firing rate of synapses controlling cognition and can be used to treat schizophrenia.

The following is reported by Gill, Cook, and Grace et al. [27–38].

There are a number of novel benzodiazepine-positive allosteric modulators (PAMs), selective for the $\alpha 5$ subunit of the GABA_A receptor, including SH-053-2'-F-R-CH₃ (**2**), which has been tested for its ability to effect the output of the HPC (hippocampal) in methylazoxymethanol-(MAM-) treated animals, which can lead to hyperactivity in the dopamine system [27–38]. In addition, the effect of this compounds (**2**) response to amphetamine in MAM-animals on the hyperactive locomotor activity was examined. Schizophrenic-like symptoms can be induced into rats when treated prenatally with DNA-methylating agent, methylazoxymethanol, on gestational day (GD) 17. These neurochemical outcomes and changes in behavior mimic those found in schizophrenic patients. Systemic treatment with (**2**) resulted in a reduced number of spontaneously active DA (dopamine) neurons in the VTA (ventral tegmental area) of MAM animals (Figure 18) to levels seen in animals treated with vehicle (i.e., saline). To confirm the location of action, **2** was also directly infused into the ventral HPC (Figure 19)

FIGURE 11: Cognitive-enhancing effects of PWZ-029 in the rhesus monkey Object Retrieval with Detours (ORD) task ($n = 5$ monkeys). (a) Effects of PWZ-029 on ORD tests consist of both easy and difficult trials; (b) PWZ-029 enhanced performance on the ORD task when tested with difficult trials only; (c) enhancement of ORD performance by 0.018 mg/kg of PWZ-029 was attenuated by the $\alpha 5$ GABA_A-preferring antagonist XLI-093 and this antagonism was surmountable by increasing the PWZ-029 dose; (d) PWZ-029 reversed performance impaired by 0.01 mg/kg of scopolamine [26]. * $p < 0.05$ versus vehicle, ** $p < 0.05$ versus Scopolamine.

and was shown to have the same effect. Moreover, HPC neurons in both SAL and MAM animals showed diminished cortical-evoked responses following $\alpha 5$ -GABA_A RPA treatment. This study is important for it supports a treatment of schizophrenia that targets abnormal HPC output, which in turn normalized dopaminergic neuronal activity [27–38]. This is a novel approach to treat schizophrenia.

The pathophysiology of schizophrenia has identified hippocampal (HPC) dysfunction as a major mediator as reported by many including Anthony Grace [27–38]. This included morphological changes, reduced HPC volume, and GAD67 expression [27, 28] that have been reported after death in the brains of patients with schizophrenia. Both HPC activation and morphology changes have been identified that can precede psychotic symptoms or correlate with severity of cognitive deficits [29–33]. This has been shown in a cognitive test during baseline and activation.

Many animal models of schizophrenia were essential to behavioral pathology and have delivered new knowledge about the network disturbances that contribute to CNS disorder. This study shows that the offspring of MAM-treated

animals showed both structural and behavioral abnormalities. These were consistent with those observed in patients with schizophrenia. The animals had reduced limbic cortical and HPC volumes with increased cell packing density and showed increased sensitivity to psychostimulants [34–36]. In addition, the startle response in prepulse inhibition was reduced in MAM-treated animals and deficits in latent inhibition were observed [35]. Furthermore, a pathological rise in spontaneous dopamine (DA) activity by the ventral tegmental area (VTA) was observed that can be attributed to aberrant activation within the ventral HPC [36]. It was suggested that reductions in parvalbumin- (PV-) stained interneurons might be the reason for the hyperactivation of the HPC and disruption of normal oscillatory activity in the HPC and cortex of MAM animals [38, 61]. At least this is the prevailing hypothesis at the moment put forth by many investigators (see references cited in [27–38]).

Selective $\alpha 5$ -GABA_A R positive allosteric modulator (2) was successful in reversing the pathological increase in tonic DA transmission in methylazoxymethanol rats by targeting abnormal hippocampal activity. In addition, the $\alpha 5$ -PAM was

FIGURE 12: PWZ-029 rescues spatial memory deficits in AD model as evidenced by a decrease in the latency to reach the hidden platform (probe test) in the water maze relative to vehicle (VEH, * $p < 0.05$).

FIGURE 13: PWZ-029 docked within $\alpha 5\gamma 2$ BzR binding site (BS).

FIGURE 14: PWZ-029 docked with amino acid residues.

FIGURE 15: PWZ-029 docked with A.A. residue interactions.

FIGURE 16: PWZ-029 docked with interactions. (1) HIS 105 π -stacking interaction with centroid of PWZ-029. (2) TYR 213 phenol OH hydrogen bonding to imidazole nitrogen lone pair. (3) THR 210 OH and lone pair on methoxy of PWZ-029. (4) $\alpha 5$ ribbon being green. (5) $\gamma 2$ ribbon being yellow. (6) Hydrogen bonding being aqua blue. (7) π -stacking being magenta.

able to reduce the behavioral sensitivity to psychostimulants observed in MAM rats (Figures 20 and 21). This suggests that novel $\alpha 5$ -partial allosteric modulators should be effective in alleviating dopamine-mediated psychosis. However, if this drug can also restore rhythmicity within HPC-efferent structure, it may also affect other aspects of this disease state such as cognitive disabilities and negative symptoms. This study, using the MAM-model to induce symptoms of schizophrenia, shows that the use of $\alpha 5$ -GABA_AR targeting compounds could be an effective treatment in schizophrenic patients. The selective targeting solely of $\alpha 5\beta 3\gamma 2$ subunits, as opposed to unselective BZDs such as diazepam, could provide relief from the psychotic symptoms without producing adverse effects such as sedation [27–38].

As reported by Gill, Grace et al. [36, 38, 47–61].

Often initial antipsychotic drug treatments (APD) for schizophrenia are ineffective, requiring a brief washout period prior to secondary treatment. The impact of withdrawal from initial APD on the dopamine (DA) system is unknown. Furthermore, an identical response to APD therapy between normal and pathological systems should not be assumed. In another study by Gill, Grace et al., $\alpha 5$ positive allosteric modulator SH-053-2 F-R-CH₃ (2) was used in the MAM neurodevelopmental model of schizophrenia which was used to study impact of withdrawal from repeated haloperidol (HAL) on the dopamine system [36, 38, 47–61].

The following studies were designed to provide insight as to why a new drug to treat schizophrenia may be effective in Phase II clinical trials but fail in Phase III because of the large number of patients required for the study. Many of these patients in Phase III studies have altered neuronal pathways in the CNS because of long-term treatment with antipsychotics (sometimes 10–20 years) [36, 38, 47–61].

Importantly, spontaneous dopamine activity reduction was observed in saline rats withdrawn from haloperidol with an enhanced locomotor response to amphetamine, indicating the development of dopamine supersensitivity. In addition, PAM treatment, as well as ventral HPC inactivation, removed the depolarization block of DA neurons in withdrawn HAL treated SAL rats. In contrast, methylazoxymethanol rats withdrawn from HAL displayed a reduction in spontaneous dopamine activity and enhanced locomotor response that

DM-I-81 aligned in the included volume of the pharmacophore/receptor model for the $\alpha1\beta3\gamma2$ (blue) and $\alpha5\gamma3\beta2$ (red) subtypes

Binding data	$\alpha1$	$\alpha2$	$\alpha3$	$\alpha4$	$\alpha5$	$\alpha6$
DM-I-81 (9)	>2000	>2000	>2000	>2000	176	>2000

FIGURE 17: The $\alpha5$ selective agonist DM-I-81 (9), bound within the $\alpha1$ and $\alpha5$ subtypes. Binding data shown as K_i (nM).

was unresponsive to PAM treatment with SH-053-2'-F-R-CH₃ or ventral HPC inactivation [36, 38, 47–61].

Prior HAL treatment withdrawal can restrict the efficacy of subsequent pharmacotherapy in the MAM model of schizophrenia. This is an extremely important result indicating that testing a new drug for schizophrenia in humans treated for years with both typical and atypical antipsychotics may result in a false negative with regard to treatment. Studies that support this hypothesis follow here [36, 38, 47–61].

Novel therapeutics for the treatment of schizophrenia that exhibit initial promise in preclinical trials often fail to demonstrate sufficient efficacy in subsequent clinical trials. In addition, relapse or noncompliance from initial treatments is common, necessitating secondary antipsychotic intervention [47, 48]. Studies have shown that between 49 and 74% of schizophrenia patients discontinue the use of antipsychotic drug (APD) treatments within 18 months due to adverse side-effects [48, 49]. Current pharmacotherapies for schizophrenia target the pathological increase in dopamine system activity, as mentioned above. Common clinical practice for secondary antipsychotic application involves a brief withdrawal period from the initial APD. Unfortunately, the success of even secondary treatments is far from being optimal with the rehospitalization of patients being a common occurrence. The impact of repeated antipsychotic treatment and subsequent withdrawal on the dopamine system has not been adequately assessed [36, 38, 47–61].

As indicated above, schizophrenia is a complex chronic psychiatric illness characterized by frequent relapses despite ongoing treatment. The search for more effective pharmacotherapies for the treatment of schizophrenia continues unabated. It is not uncommon for novel pharmaceuticals to demonstrate promise in preclinical trials but fail to show an adequate response in subsequent clinical trials. Indeed, evaluating the benefits of one APD versus another is complicated by clinical trials beset with high attrition rates and poor

efficacy in satisfactorily reducing rehospitalization [47, 49–52].

Previous work from the Gill, Grace et al.'s laboratory [36, 38, 47–61] with the MAM model of schizophrenia has identified a potential novel therapeutic, a $\alpha5$ GABAAR PAM. The dopamine system pathology in the MAM model is likely the result of excessive output from the ventral HPC [36]. The $\alpha5$ GABAAR PAM was identified as a potential therapeutic due to the relatively selective expression of $\alpha5$ GABAAR in the ventral HPC and its potential for reducing HPC activity [53–60]. When either administered systemically or directly infused into the ventral HPC, the $\alpha5$ GABAAR PAM (SH-053-2'-F-R-CH₃) was effective in reducing the dopamine system activation in MAM rats [38]. Anthony Grace, Gill et al. showed $\alpha5$ GABAAR PAM treatment was also effective in reducing the enhanced behavioral response to amphetamine in MAM rats, as stated above. Data from the present study sought to delineate whether the $\alpha5$ GABAAR PAM (SH-053-2'-F-R-CH₃) would remain effective in MAM rats withdrawn from prior neuroleptic treatment, a common occurrence in the patient population. In both SAL and MAM rats, there was a reduction in the spontaneous activity of dopamine neurons in the VTA after 7 days withdrawal from repeated HAL treatment. However, MAM rats continued to exhibit a greater activation of the dopamine system in comparison to SAL rats. Treatment with the $\alpha5$ GABAAR PAM was no longer effective in reducing the activity of dopamine neurons in the VTA in withdrawn HAL treated MAM rats. In contrast, $\alpha5$ GABAAR PAM treatment in the withdrawn HAL treated SAL rats instead increased the spontaneous activity of dopamine in the VTA (Figures 22–25) [36, 38, 47–61].

Similar to the effects seen following $\alpha5$ GABAAR PAM treatment, ventral HPC inactivation in withdrawn HAL treated SAL rats restored normal dopamine system activity by increasing the number of spontaneously active dopamine neurons. The disparate effect of withdrawal from HAL on

FIGURE 18: Treatment with SH-053-2'F-R-CH₃ (0.1 mg/kg, i.v.; patterned bars) normalizes the aberrant increase in the number of spontaneously firing dopamine neurons (expressed as cells/track) in methylazoxymethanol acetate- (MAM-) treated animals (a). There was no effect of SH-053-2'F-R-CH₃ treatment in control animals (open bars, (a)–(c)) or on firing rate and burst activity in MAM animals (dark bars; (b)–(c)) (* $P < 0.05$, two-way ANOVA, Holm-Sidak *post hoc*; $N = 5$ –7 rats/group) [27–38].

the dopamine system between SAL and MAM rats provides a vital clue for the inconsistencies between preclinical trials for novel therapeutics that utilize normal subjects and subsequent clinical trials in a patient population [36, 38, 47–61].

The data suggests underlying dopamine system pathology alters the impact of withdrawal from prior repeated HAL in the MAM model of schizophrenia. In addition, subsequent novel APD treatment loses efficacy following withdrawal from repeated HAL in MAM animals. This certainly has relevance to Phase III clinical trials of new drugs to treat schizophrenia [36, 38, 47–61].

7. GABA_A $\alpha 5$ Positive Allosteric Modulators Relax Airway Smooth Muscle

Emala, Gallos, et al. [66–75] have found that novel $\alpha 5$ -subtype selective GABA_A positive allosteric modulators relax

airway smooth muscle from rodents and humans. The clinical need for new classes of bronchodilators for the treatment of bronchoconstrictive diseases such as asthma remains a major medical issue. Few novel therapeutics have been approved for targeting airway smooth muscle (ASM) relaxation or lung inflammation in the last 40 years [66]. In fact, several asthma-related deaths are attributed, in part, to long-acting β -agonists (LABA) [67]. Adherence to inhaled corticosteroids, the first line of treatment for airway inflammation in asthma, is very poor [68, 69]. Therapies that break our dependence on β -agonism for ASM relaxation would be a novel and substantial advancement.

These ASM studies were undertaken due to a pressing clinical need for novel bronchodilators in the treatment of asthma and other bronchoconstrictive diseases such as COPA. There are only three drug classes currently in clinical

FIGURE 19: Hippocampal (HPC) infusion of SH-053-2'F-R-CH₃ (1 μM/side; patterned bars) normalizes the aberrant increase in the number of spontaneously firing dopamine neurons (expressed as cells/track) in methylazoxymethanol acetate- (MAM-) treated animals (a). There was no effect of SH-053-2'F-R-CH₃ treatment in control animals (open bars, (a)–(c)) or on firing rate in MAM animals (dark bars; (b)). Hippocampal (HPC) infusion of SH-053-2'F-R-CH₃ significantly reduced the percentage of spikes occurring in bursts of dopamine (DA) neurons in MAM and control animals (c) (* $P < 0.05$, two-way ANOVA, Holm-Sidak *post hoc*; $N = 7$ rats/group) [27–38].

use as acute bronchodilators in the United States (methylxanthines, anticholinergics, and β -adrenoceptor agonists) [70]. Thus, a novel therapeutic approach that would employ cellular signaling pathways distinct from those used by these existing therapies involves modulating airway smooth muscle (ASM) chloride conductance via GABA_A receptors to achieve relaxation of precontracted ASM [71, 72]. However, widespread activation of all GABA_A receptors may lead to undesirable side effects (sedation, hypnosis, mucus formation, etc.). Thus, a strategy that selectively targets a subset of GABA_A channels, those containing α subunits found to be expressed in airway smooth muscle, may be a first step in limiting side effects. Since human airway smooth muscle contains only $\alpha 4$ or $\alpha 5$ subunits [72], ligands with selectivity for these subunits are an attractive therapeutic option. Concern regarding nonselective GABA_A receptor activation

is not limited to the airway or other peripheral tissues. GABA_A receptor ligands are classically known for their central nervous system effects of anxiolysis, sedation, hypnosis, amnesia, anticonvulsion, and muscle relaxant effects. Such indiscriminate activation of GABA_A receptors in the CNS is exemplified by the side effects of classical benzodiazepines (such as diazepam) which were the underpinning for the motivation of a search for benzodiazepine (BZD) ligands that discriminate among the α subunits of GABA_A receptors [73–75].

A novel approach to identify novel benzodiazepine derivatives to selectively target GABA_A channels containing specific α subunits was developed by Cook et al. in the 1980s that employed a pharmacophore receptor model based on the binding affinity of rigid ligands to BDZ/GABA_A receptor sites (as reviewed in 2007 [23]). From this series

FIGURE 20: Extracellular recording traces illustrate the reduction in evoked responses in the ventral hippocampal (HPC) to entorhinal cortex stimulation in both MAM- and saline-treated animals (a, b). Treatment with SH-053-2'-F-R-CH₃ (0.1 mg/kg, i.v.) decreases the evoked excitatory response (dashed lines) of ventral HPC neurons to entorhinal cortex stimulation in both MAM- and saline-treated animals (c) (**p* < 0.05 for saline and ⁺*p* < 0.05, two-way repeated measures ANOVA, Holm-Sidak *post hoc*) [27–38].

of receptor models for $\alpha_{1-6}\beta\gamma_2$ subtypes a robust model for α_5 subtype selective ligands emerged, the result of which included the synthesis of a novel $\alpha_5\beta_3\gamma_2$ partial agonist modulator, SH-053-2'-F-R-CH₃ (2). The discovery of this and related ligands selective for α_5 BDZ/GABA_A-ergic receptors and the realization that only α_4 and α_5 subunits are expressed in GABA_A channels on human airway smooth muscle yielded an ideal opportunity for targeting these α_5 -subunit containing GABA_A channels for bronchorelaxation [66–75].

The GABA_A α_5 subunit protein was first localized to the ASM layer of human trachea while costaining for the smooth muscle specific protein α actin (Figure 26). The first panel of Figure 26 shows GABA_A α_5 protein stained with fluorescent green and blue fluorescent nuclear staining (DAPI). The second panel is the same human tracheal smooth muscle section simultaneously stained with a protein specific for

smooth muscle, α actin, and the third panel is a merge of the first two panels showing costaining of smooth muscle with GABA_A α_5 and α actin proteins. The fourth panel is a control omitting primary antibodies but including nuclear DAPI staining [66–75].

After demonstrating the protein expression of GABA_A receptors containing the α_5 subunit, functional studies of isolated airway smooth muscle were performed in tracheal airway smooth muscle from two species. Human airway smooth muscle suspended in an organ bath was precontracted with a concentration of acetylcholine that was the EC₅₀ concentration of acetylcholine for each individual airway smooth muscle preparation. The induced contraction was then relaxed with a β -agonist (isoproterenol) in the absence or presence of the GABA_A α_5 ligand SH-053-2'-F-R-CH₃ (2). Figure 27(a) shows that the amount of relaxation

FIGURE 21: Treatment with SH-053-2'F-R-CH₃ (10 mg/kg, i.p.) reduced the aberrant increased locomotor response to D-amphetamine (0.5 mg/kg i.p.) observed in MAM rats (a). MAM animals demonstrated a significantly larger peak locomotor response than both saline-treated animals and MAM animals pretreated with the alpha-5 PAM (b) (there was a significant difference between MAM-vehicle and all other groups, * $p < 0.05$, two-way repeated measures ANOVA, Holm-Sidak *post hoc*) [27–38].

induced by 10 nM isoproterenol was significantly increased if 50 μ M SH-053-2'F-R-CH₃ (2) was also present in the buffer superfusing the airway smooth muscle strip. Studies were also performed in airway smooth muscle from another species, guinea pig, that measured direct relaxation of a different contractile agonist, substance P. As shown in Figure 27(b), the amount of remaining contractile force 30 minutes after a substance P-induced contraction was significantly reduced in airway smooth muscle tracheal rings treated with SH-053-2'F-R-CH₃ (2) [66–75].

Following these studies in intact airway smooth muscle, cell based studies were initiated in cultured human airway smooth muscle cells to directly measure plasma membrane chloride currents and the effects of these currents on intracellular calcium concentrations. SH-053-2'F-R-CH₃ (2) induced a Cl⁻ current *in vitro* using conventional whole cell patch clamp techniques [66–75]. These electrophysiology studies were then followed by studies to determine the effect of these plasma membrane chloride currents on intracellular calcium concentrations following treatment of human airway smooth muscle cells with a ligand whose receptor couples through a Gq protein pathway, a classic signaling pathway that mediates airway smooth muscle contraction.

SH-053-2'F-R-CH₃ (2) attenuated an increase in intracellular calcium concentrations induced by a classic Gq-coupled ligand, bradykinin (Figure 28(a)) [66–75]. The attenuation by SH-053-2'F-R-CH₃ (2) was significantly blocked by the GABA_A antagonist gabazine (Figure 28(b)) indicating that SH-053-2'F-R-CH₃ (2) was modulating GABA_A receptors for these effects on cellular calcium [66–75].

The major findings of these studies are that human airway smooth muscle expresses $\alpha 5$ subunit containing GABA_A receptors that can be pharmacologically targeted

by a selective agonist. The GABA_A $\alpha 5$ subunit selective ligand SH-053-2'F-R-CH₃ (2) relaxed intact guinea pig airway smooth muscle contracted with substance P and augmented β -agonist-mediated relaxation of intact human airway smooth muscle. The mechanism for these effects was likely mediated by plasma membrane chloride currents that contributed to an attenuation of contractile-mediated increases in intracellular calcium, a critical event in the initiation and maintenance of airway smooth muscle contraction [66–75].

8. Recent Discovery of Alpha 5 Included Volume Differences: L₄ Pocket as Compared to Other Bz/GABAergic Subtypes

The findings in both the MAM-model of schizophrenia and the relaxation of airway smooth muscle have led to the study of SH-053-2'F-R-CH₃ and related compounds bound within the $\alpha 5$ -GABA_A/BzR (Figure 29). The SH-053-R-CH₃ (15) and SH-053-S-CH₃ (16) isomers have been previously described [23]. These compounds along with SH-053-2'F-R-CH₃ and SH-053-2'F-S-CH₃ have been tested for binding affinity and show selectivity for the $\alpha 5$ -subunit (Table 3).

From examination of Figure 30 and Tables 3 and 4, it is clear the (R)-isomers bound to the $\alpha 5$ subtype while the (S)-isomers were selective for $\alpha 2/\alpha 3/\alpha 5$ subtypes.

From this data, these compounds were then used in examining the $\alpha 5$ -binding pocket, most specifically the fluoroserries. In regard to molecular modeling, depicted in Figure 30 is the included volume and ligand occupation of the SH-053-2'F-S-CH₃ (17) and SH-053-2'F-R-CH₃ (2) enantiomers in the $\alpha 5$ subtype as well as the $\alpha 2$ subtype. It

FIGURE 22: Repeated haloperidol treatment caused a reduction in the number of spontaneously active dopamine neurons in both SAL and MAM rats injected with vehicle compared to untreated control animals. Treatment with SH-053-2'F-R-CH₃ (0.1 mg/kg, i.v.) reversed the haloperidol-induced reduction in cells/track in SAL, but not MAM, rats (a). Repeated haloperidol treatment had no effect on the firing rate of dopamine neurons recorded in SAL or MAM rats treated with vehicle. However, SH-053-2'F-R-CH₃ caused an increase in firing rate of dopamine neurons in repeatedly haloperidol-treated SAL rats (b). Repeated haloperidol treatment, as well as SH-053-2'F-R-CH₃ injection, had no impact on the percentage of spikes occurring in bursts for dopamine neurons recorded in SAL and MAM rats (c) [36, 38, 47–61]. * $p < 0.05$.

is clear a new pocket (L_4) has been located in the $\alpha 5$ subtype permitting **2** as well as **17** to bind to the $\alpha 5$ subtype. Examination of both ligands in the $\alpha 2$ subtype clearly illustrates the analogous region in the $\alpha 2$ subtype is not present and thus does not accommodate **2** for the pendant phenyl which lies outside the included volume in the space allocated for the receptor protein itself [23].

9. BzR GABA(A) Subtypes

In terms of potency, examination of the values in Table 4 [87], it is clear the R-isomer (**2**) shows more selectivity towards the $\alpha 5$ -subunit, while the S-isomer (**17**) is potent at the $\alpha 2/3/5$ subunits. It is important, as postulated earlier [23], that the major difference in GABA(A)/Bz receptors subtypes stems

FIGURE 23: Repeated haloperidol treatment caused a reduction in the number of spontaneously active dopamine neurons in both SAL and MAM rats microinfused with vehicle in the ventral HPC compared to untreated control animals. Infusion of TTX in the ventral HPC reversed the haloperidol-induced reduction in cells/track in SAL, but not MAM, rats (a). Repeated haloperidol treatment had no effect on the firing rate of dopamine neurons recorded in SAL or MAM rats infused with vehicle or TTX in the ventral HPC (b). Repeated haloperidol treatment had no effect on the percentage of spikes occurring in bursts for dopamine neurons recorded in SAL or MAM rats infused with vehicle or TTX in the ventral HPC (c) [36, 38, 47–61]. * $p < 0.05$.

from differences in asymmetry in the lipophilic pockets L_1 , L_2 , L_3 , L_4 , and L_{Di} in the pharmacophore/receptor model and indicates even better functional selectivity is possible with asymmetric BzR ligands.

The synthetic switching of chirality at the C-4 position of imidazobenzodiazepines to induce subtype selectivity was

successful. Moreover, increase of the potency of imidazobenzodiazepines can be achieved by substitution of the 2'-position hydrogen atom with an electron rich atom (fluorine) on the pendant phenyl ring in agreement with Haefely et al. [88], Fryer [89, 90], and our own work [22, 91]. The biological data on the two enantiomeric pairs of benzodiazepine ligands

FIGURE 24: Administration of apomorphine (80 mg/kg i.v.) increased the number of spontaneously active dopamine neurons in SAL rats withdrawn from repeated HAL, while having no effect on the number of active dopamine neurons in MAM rats withdrawn from repeated HAL [36, 38, 47–61]. * $p < 0.05$.

TABLE 3: Binding affinity at $\alpha\beta\gamma 2$ GABA_A receptor subtypes (values are reported in nM).

Compound ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
SH-053-R-CH ₃ , (15)	2026	2377	1183	>5000	949.1	>5000
SH-053-S-CH ₃ , (16)	1666	1263	1249	>5000	206.4	>5000
SH-053-2'F-R-CH ₃ , (2)	759.1	948.2	768.8	>5000	95.17	>5000
SH-053-2'F-S-CH ₃ , (17)	350	141	1237	>5000	19.2	>5000

^aData shown here are the means of two determinations which differed by less than 10%.

TABLE 4: Oocyte electrophysiological data of benzodiazepines^a [87].

Compound	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$
SH-053-2'F-R-CH ₃ (2)	111/154	124/185	125/220	183/387
SH-053-2'F-S-CH ₃ (17)	116/164	170/348	138/301	218/389

^aEfficacy at $\alpha\beta\gamma 2$ GABA_A receptor subtypes as % of control current at 100 nM and 1 μ M concentrations. Data presented as percent over baseline (100) at concentrations of 100 nM/1 μ M.

confirm the ataxic activity of BZ site agonists is mediated by $\alpha 1\beta 2/3\gamma 2$ subtypes, as reported in [23, 91–93]. The antianxiety activity in primates of the S isomers was preserved with no sedation. In only one study in rodents was any sedation observed; the confounding sedation was observed in both the S isomer (functionally selective for $\alpha 2$, $\alpha 3$, and $\alpha 5$ receptor subtypes) and R isomer (essentially selective for $\alpha 5$ subtype) and may involve at least, in part, agonist activity at $\alpha 5$ BzR subtypes. There are some $\alpha 5$ BzR located in the spinal cord which might be the source of the decrease in locomotion with SH-053-2'F-R-CH₃ and SH-053-2'F-S-CH₃; however,

this is possibly some type of stereotypical behavior. Hence in agreement with many laboratories including our own [23, 92, 93] the best potential nonsedative, nonamnesic, antianxiety agents stem from ligands with agonist efficacy at $\alpha 2$ subtypes essentially silent at $\alpha 1$ and $\alpha 5$ subtypes (to avoid sedation) [91]. It must be pointed out again; however, in primates Fischer et al. [87] observed a potent anxiolytic effect with no sedation with the 2'F-S-CH₃ (17) isomer, while the 2'F-R-CH₃ (2) isomer exhibited only a very weak anxiolytic effect.

Numerous groups have done modeling and SAR studies on different classes of compounds which have resulted in a few different pharmacophore models based on the benzodiazepine binding site (BS) of the GABA_A receptor [94]. These models are employed to gain insight in the interactions between the BS and the ligand. These have been put forth by Loew [7, 95, 96], Crippen [97, 98], Coddington [76, 77, 99–101], Fryer [89, 90, 94], Gilli and Borea [102–105], Tebib et al. [106], and Gardner [107], as well as from Professors Sieghart, Cromer, and our own laboratory [21, 39, 40, 76, 78–82, 108–118].

The Milwaukee-based pharmacophore/receptor model is a comprehensive building of the BzR using radioligand binding data and receptor mapping techniques based on 12 classes of compounds [20, 23, 39, 40, 42, 111, 119–122]. This model (Figure 31) [79] has brought together previous models which have used data from the activity of antagonists, positive allosteric modulators, and negative allosteric modulators and included the new models for the “diazepam-insensitive” (DI) sites [123]. Four basic anchor points, H_1 , H_2 , A_2 , and L_1 , were assigned, and 4 additional lipophilic regions were defined as L_2 , L_3 , L_{Di} , and the new L_4 (see captions in Figure 31 for details); regions S_1 , S_2 , and S_3 represent negative areas of steric repulsion. As previously reported, the synthesis of both partial agonists and partial inverse agonists has been achieved by using parts of this model [99, 100, 104, 105, 119, 124–127].

The cloning, expression, and anatomical localization of multiple GABA(A) subunits have facilitated both the identification and design of subtype selective ligands. With the availability of binding data from different recombinant receptor subtypes, affinities of ligands from many different structural classes of compounds have been evaluated.

Illustrated in Figure 31 is the [3,4-*c*]quinolin-3-one CGS-9896 (18) (dotted line), a diazadiindole (19) (thin line), and diazepam (20) (thick line) fitted initially to the inclusive pharmacophore model for the BzR. Sites H_1 (Y210) and H_2 (H102) represent hydrogen bond donor sites on the receptor protein complex while A_2 (T142) represents a hydrogen bond acceptor site necessary for potent inverse activity *in vivo*. L_1 , L_2 , L_3 , L_4 , and L_{Di} are four lipophilic regions in the binding pharmacophore. Descriptors S_1 , S_2 , and S_3 are regions of negative steric repulsion.

Based on SAR data obtained for these ligands at 6 recombinant BzR subtypes [128–132], an effort has been undertaken to establish different pharmacophore/receptor models for BzR subtypes. The alignment of the twelve different structural classes of benzodiazepine receptor ligands was earlier based on the least squares fitting of at least three points. The coordinates of the four anchor points (A_2 , H_1 , H_2 , and L_1) employed in the alignment are outlined in Figure 32.

FIGURE 25: Repeated haloperidol treatment causes an enhancement in the locomotor response to D-amphetamine (0.5 mg/kg, i.p.) in SAL animals that is reduced by pretreatment with SH-053-2'F-R-CH₃ (10 mg/kg, i.p.) (a). MAM rats treated repeatedly with haloperidol exhibit a locomotor response following D-amphetamine similar to untreated MAM rats. However, repeated haloperidol treatment blocks the effect of SH-053-2'F-R-CH₃ pretreatment in decreasing the locomotor response in MAM rats (b). Untreated MAM rats demonstrated a significantly larger peak locomotor response than untreated SAL rats. In addition, SH-053-2'F-R-CH₃ pretreatment significantly reduced the peak locomotor response in untreated MAM rats, while having no effect in repeatedly haloperidol-treated MAM rats. In contrast, repeated haloperidol treatment enhanced the peak locomotor response to amphetamine in SAL rats that was reduced by SH-053-2'F-R-CH₃ pretreatment (c) [36, 38, 47–61]. * $p < 0.05$.

Herein are described the results from ligand-mapping experiments at recombinant BzR subtypes of 1,4-benzodiazepines, imidazobenzodiazepines, β -carbolines, diindoles, pyrazoloquinolinones, and others [126]. Some of the differences and similarities among these subtypes can be gleaned from this study and serve as a guide for future drug design.

10. $\alpha 1$ Updates

10.1. Beta-Carbolines. A series of 3,6-disubstituted β -carbolines was prepared and evaluated for their *in vitro* affinity

at $\alpha x\beta 3\gamma 2$ GABA(A)/BzRr subtypes by radioligand binding assays in search of $\alpha 1\beta 3\gamma 2$ subtype selective compounds (Figure 33). A potential therapeutic application of such antagonist analogs is to treat alcohol abuse [133, 134]. Analogues of β CCt (21) were synthesized *via* a carbonyldiimidazole-mediated method by Yin et al. [85] and the related 6-substituted β -carboline-3-carboxylates including WYS8 (27) were synthesized from 6-iodo β CCt (29). Bivalent ligands (42 and 43) were also synthesized to increase the scope of the structure-activity relationships (SAR) to larger ligands. An

FIGURE 26: Protein expression of the GABA_A $\alpha 5$ subunit in intact human trachea-bronchial airway smooth muscle. Representative images of human tracheal airway smooth muscle sections using confocal microscopy are depicted following single, double, and triple immunofluorescence labeling. The antibodies employed were directed against the GABA_A $\alpha 5$ subunit (green), α -smooth muscle actin (SMA; red), and/or the nucleus via DAPI counterstain (blue). Panels illustrate the following staining parameters from left to right: (1st) costaining of DAPI and GABA_A $\alpha 5$ subunit; (2nd) α -SMA staining alone; (3rd) triple-staining of GABA_A $\alpha 5$, α -SMA, and DAPI; (4th) DAPI nucleus counterstain, with primary antibodies omitted as negative control. Modified from [66–75].

FIGURE 27: SH-053-2'-F-R-CH₃ (2) mediated activation of $\alpha 5$ subunit containing GABA_A channels induces relaxation of precontracted airway smooth muscle. (a) SH-053-2'-F-R-CH₃ (2) (SH-053) potentiates β -agonist-mediated relaxation of human airway smooth muscle. Cotreatment of human airway smooth muscle strips with SH-053-2'-F-R-CH₃ (2) (50 μ M) significantly enhances isoproterenol (10 nM) mediated relaxation of an acetylcholine EC₅₀ contraction compared to isoproterenol alone ($N = 8$ /group, \$\$ = $p < 0.01$). Modified from [66–75]. (b) SH-053-2'-F-R-CH₃ (2) activation of $\alpha 5$ containing GABA_A receptors induces direct relaxation of substance P-induced airway smooth muscle contraction. Compiled results demonstrating enhanced spontaneous relaxation (expressed as % remaining force at 30 minutes following a 1 μ M substance P mediated contraction) following treatment with SH-053-2'-F-R-CH₃ (2) compared to treatment with vehicle control ($n = 4$ -5/group, \$\$ = $p < 0.01$) [66–75].

initial SAR on the first analogs demonstrated that compounds with larger side-groups at C6 were well tolerated as they projected into the L_{Di} domain (see 42 and 43) [85]. Moreover, substituents located at C3 exhibited a conserved stereo interaction in lipophilic pocket L_1 , while N2 likely participated in hydrogen bonding with H_1 . Three novel β -carboline ligands (21, 23, and 27) permitted a comparison of the pharmacological properties with a range of classical benzodiazepine

receptor antagonists (flumazenil, ZK93426) from several structural groups and indicated these β -carbolines were “near GABA neutral antagonists.” Based on the SAR, the most potent (*in vitro*) $\alpha 1$ selective ligand was the 6-substituted acetylenyl β CCt (WYS8, 27). In a previous study both 21 and 23 were able to reduce the rate at which rats self-administered alcohol in alcohol preferring and HAD rats but had little or no effect on sucrose self-administration [85].

FIGURE 28: SH-053-2'-F-R-CH₃ (**2**) mediated activation of $\alpha 5$ containing GABA_A receptors attenuates bradykinin-induced elevations in cytosolic Ca^{2+} in human airway smooth muscle cells. (a) Representative Fluo-4 Ca^{2+} fluorescence (RFU) tracing illustrating pretreatment with SH-053-2'-F-R-CH₃ (**2**) (SH-053) (10 μM) reduces cytosolic Ca^{2+} response to bradykinin (1 μM). This effect is reversed in the presence of gabazine (200 μM , GABA_A receptor antagonist). Modified from [66–75]. (b) Compiled results illustrating SH-053-2'-F-R-CH₃ (**2**) pretreatment of GABA_A $\alpha 5$ receptors on human airway smooth muscle cells attenuates bradykinin-induced elevations in intracellular Ca^{2+} compared to levels achieved following pretreatment with vehicle control (\$\$ = $p < 0.01$). While gabazine-mediated blockade of GABA_A channels does not significantly affect bradykinin-induced intracellular calcium increase compared to vehicle control, gabazine treatment did reverse SH-053-2'-F-R-CH₃ (**2**) ability to attenuate bradykinin-induced elevations in intracellular calcium thereby illustrating a GABA_A channel specific effect (n.s. = not significant). Modified from [66–75].

FIGURE 29: Structures of enantiomers with 2'-H (**15**, **16**) and 2'-F (**2**, **17**).

3-PBC (**23**) was also active in baboons [134]. This data has been used in updating the pharmacophore model in the $\alpha 1$ -subtype.

11. The Updated Included Volume Models

Illustrated in Figure 34 is the included volume of the updated pharmacophore receptor model of the $\alpha 1\beta 3\gamma 2$ subtype of Clayton [22]. The current model for the $\alpha 1\beta 3\gamma 2$ subtype has several new features. The cyclopropyl group of CD-214 extended 2 Å past the A_2 descriptor slightly increasing its volume. The trimethylsilyl group of QH-II-82 and WYS7 illustrates how well bulky groups are tolerated near the

entrance of the binding pocket. Despite not being as potent, dimers of beta carbolines, WYS2 and WYS6, bound to $\alpha 1$ subtypes at 30 nM and 120 nM, respectively. Their ability to bind, albeit weakly, supports the location of the binding site entrance from the extracellular domain. The included volume of the $\alpha 1\beta 3\gamma 2$ subtype was previously 1085.7 cubic angstroms. The volume has now been measured as 1219.2 cubic angstroms. Volume measurements should be used carefully as the binding site is not enclosed and the theoretical opening near L_{DI} is not clearly demarcated. Dimers were excluded from the included volume exercise because although they bound to the receptor, they represented compounds which were felt to extend outside the receptor binding pocket when docked to the protein. Where appropriate,

SH-053-2'F-S-CH₃ 17 fits the pharmacophore in the included volume of the alpha 2 subtype

The left image of figure rotated 90°. It can be clearly seen that 17 fits within the included volume

SH-053-2'F-R-CH₃ 2 does not fit the pharmacophore in the included volume of the alpha 2 subtype

The left image of figure rotated 90°. It can be clearly seen that the conformation of 2 is such that the pendant 6-phenyl sticks outside the included volume

SH-053-2'F-S-CH₃ 17 fits the pharmacophore in the included volume of the alpha 5 subtype

The left image of figure rotated 90°. It can be clearly seen that 17 fits within the included volume

SH-053-2'F-R-CH₃ 2 fits the pharmacophore in the included volume of the alpha 5 subtype

The left image of figure rotated 90°. It can be clearly seen that 2 fits within the included volume

FIGURE 30: Included volume and ligand occupation of the SH-053-2'F-S-CH₃ 17 and SH-053-2'F-R-CH₃ 2 enantiomers in the α 5 and γ 2 pharmacophore/receptor models. This figure was modified and reproduced from that reported by Clayton et al. in [22, 23].

FIGURE 31: The two-dimensional representation of the Milwaukee-based unified pharmacophore with 3 amino acids in the binding site based on the rigid ligand template [23, 39, 42, 76–80]. This figure has been modified from that reported for PAMs, NAMs, and antagonists in [22, 23].

FIGURE 32: The schematic representation of the descriptors for the initial inclusive BzR pharmacophore based on the rigid ligands (diindoles) [79, 81–84]. This figure has been modified from that reported in [79].

their monomers were included in the included volume analysis. Ligands considered for the included volume in Table 5 exhibited potent binding at $\alpha 1$ subtypes ($K_i \leq 20 \text{ nM}$) but were not necessarily subtype selective. The binding data for ligands at α_{2-6} -subtypes follow (Tables 6–10; structures located in Clayton [22] and Supporting Information, Appendix III in Supplementary Material available online at <http://dx.doi.org/10.1155/2015/430248>).

12. The $\alpha 1\beta 3\gamma 2$ Receptor Subtype

The focus of this research was aimed at diazepam sensitive receptors; additional features to the $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ receptors were not identified (see Table 5, Figures 34 and 35). The major new feature identified for the $\alpha 5\beta 3\gamma 2$ receptor was a new L_4 pocket. This new lipophilic pocket was identified with SH-053-R-CH₃ (**15**) and SH-053-S-CH₃ (**16**) chiral enantiomers as well as the 2'F analogs [74, 135, 136].

ZK-93423

Ligands	R ₆	R ₃	α1	α2	α3	α4	α5	α6
21(βCCt)	H	CO ₂ tBu	0.72	15	18.9	1000	111	>5,000
22(βCCE)	H	CO ₂ Et	1.2	4.9	5.7	ND	26.8	2,700
23(3-PBC)	H	OnPr	5.3	52.3	68.8	1000	591	>1,000
24(WYB14)	TMS—≡	CO ₂ tBu	6.8	30	36	2000	108	1000
25(WY-B-25)	TMS—≡	CO ₂ CH ₂ CF ₃	17	59	88	200	1444	>3000
26(WY-B-99-1)	TMS—≡	CO ₂ Et	4.4	4.5	5.58	2000	47	2000
27(WYS8)	H—≡	CO ₂ tBu	0.972	111	102	2000	1473	1980
28(WY-B-26-2)	H—≡	CO ₂ CH ₂ CF ₃	4.5	44.6	42.7	2000	124	2000
29(iodo-βCCt)	I	CO ₂ tBu	14.4	44.9	123	>4000	65.3	>4000
30(WY-B-20)	I	CO ₂ CH ₂ CF ₃	12	39	47	2000	122	3000
31(iodo-βCCE)	I	CO ₂ Et	4.8	31	34	1000	286	1000
32(WY-B-08)	I	CO ₂ CH ₂ (CF ₃) ₂	78	301	131	3000	681	3000
33(WYS13)		CO ₂ tBu	2.4	13	27.5	NA	163	5000
34(WYB27-1)		CO ₂ CH ₂ CF ₃	26	143	117	3000	127	2000
35(WYS12)		CO ₂ tBu	37	166	314	NA	2861	5000
36(WYB27-2)		CO ₂ CH ₂ CF ₃	9.2	13	72	2000	449	2000
37(WYS15)		CO ₂ tBu	3.63	2.02	44.3	NA	76.5	5000
38(WYB29-2)		CO ₂ CH ₂ CF ₃	25	137	125	2000	299	2000
39(CMA57)	F	COC ₃ H ₇	3.7	27	40	NA	254	>2500
40(CM-A-82a)	C(CH ₃) ₃	CO ₂ tBu	2.78	8.93	24.5	1000	7.49	1000
41(CM-A-87)	F	CO ₂ tBu	1.62	4.54	14.7	1000	4.61	1000
42(WY-S-2)	Bcct—≡—Bcct		30	124	100	>300	>300	>4000
43(WY-S-6)	Bcct—≡—≡—Bcct		120	1059	3942	5000	5000	5000

^a Affinity of compounds at GABA(A)/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam or [³H] Ro-15-4513 binding to HEK cell membranes expressing human receptors of composition α1β3γ2, α2β3γ2, α3β3γ2, α4β3γ2, α5β3γ2, and α6β3γ2 [85]. Data represent the average of at least three determinations with a SEM of ±5%.

FIGURE 33: ^a Affinities (K_i = nM) of 3,6-disubstituted β-carbolines at αxβ3γ2 ($x = 1-3, 5, 6$) receptor subtypes [85]. The structures versus code numbers of all ligands in the tables of this review can be found in the Ph.D. thesis of Terry Clayton (Ph.D. thesis, University of Wisconsin-Milwaukee, Milwaukee, WI, December, 2011) [22] and in the Supporting Information.

TABLE 5: These ligands bound with potent affinity for $\alpha 1$; ligands bound with K_i values <20 nM at this subtype.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
WY-TSC-4 (WYS8)	0.007	0.99	1.63		51.04	
SH-TSC-2 (BCCT)	0.03	0.0419	0.035		69.32	
QH-II-090 (CGS-8216)	0.05	0.08	0.12		0.25	17
XLI-286	0.051	0.064	0.118		0.684	
QH-II-077	0.06	0.08	0.05		0.12	4
QH-II-092	0.07	0.03	0.04	ND	0.17	ND
JYI-57	0.076	0.076	0.131	ND	0.036	ND
QH-II-085	0.08	0.06	0.02	ND	0.08	ND
XHE-II-024	0.09	0.18	0.32	14	0.24	11
PWZ-007A	0.11	0.1	0.09	ND	0.2	10
CGS8216	0.13	ND	ND	ND	ND	46
SPH-121	0.14	1.19	1.72	ND	4	479
QH-II-075	0.18	0.21	0.25	ND	1.3	40
PZII-028	0.2	ND	0.2	ND	0.32	1.9
CGS9895	0.21	ND	ND	ND	ND	9.3
PWZ-0071	0.23	0.17	0.12	ND	0.44	17.31
XHE-III-24	0.25	ND	8	222	10	328
JYI-42	0.257	0.146	0.278	ND	0.256	ND
CGS9896	0.28	ND	ND	ND	ND	181
JYI-64 (C17H12N4FBr)	0.305	1.111	0.62	ND	0.87	5000
PZII-029	0.34	ND	0.79	ND	0.52	10
BRETAZENIL	0.35	0.64	0.2	ND	0.5	12.7
FG8205	0.4	2.08	1.16	ND	1.54	227
YT-5	0.421	0.6034	36.06	ND	1.695	ND
6-PBC	0.49	1.21	2.2	ND	2.39	1343
QH-146	0.49	ND	0.76	ND	7.7	10000
DM-II-90 (C17H12N4BrCl)	0.505	1	0.63	ND	0.37	5000
SPH-165	0.63	2.79	4.85	ND	10.4	1150
BCCt	0.72	15	18.9	ND	110.8	5000
SH-I-048A	0.774	0.1723	0.383	ND	0.11	ND
alprazolam	0.8	0.59	1.43	ND	1.54	10000
Ro15-1788	0.8	0.9	1.05	ND	0.6	148
WYS10 C14H9F3N2O2	0.88	36	25.6	ND	548.7	15.3
WY-B-15	0.92	0.83	0.58	2080	4.42	646
WY-A-99-2 (WYS8)	0.972	111	102	2000	208	1980
XHE-III-06a	1	2	1	5	1.8	37
Xli366 C22H21N3O2	1	ND	ND	ND	ND	ND
JYI-59 (C22H13N3O2F4)	1.08	2.6	11.82	ND	11.5	5000
WYSC1 C16H16N2O2	1.094	5.44	12.3	ND	69.8	21.2
MLT-I-70	1.1	1.2	1.1	ND	40.3	1000
SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
BCCE	1.2	4.9	5.7	ND	26.8	2700
XHE-III-04	1.2	2	1.1	219	0.4	500
XLi350 C17H11ClN2O	1.224	1.188	ND	ND	2.9	ND
XHE-III-49	1.3	5.5	4.2	38.7	11.3	85.1
PWZ-009A1	1.34	1.31	1.26	ND	0.84	2.03
DM-239	1.5	ND	0.53	ND	0.14	6.89
XLi351 C21H21ClN2OSi	1.507	0.967	ND	ND	1.985	ND
XLi352 C18H13ClN2O	1.56	0.991	ND	ND	1.957	ND

TABLE 5: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
TG-4-39	1.6	34	24	5.6	1.4	23
TG-II-82	1.6	2.9	2.8	ND	1	1000
CM-A87	1.62	4.54	14.73	1000	4.61	1000
QH-II-082	1.7	1.8	1.6	ND	6.1	100
JYI-49 (C20H12N3O2F4Br)	1.87	2.38	ND	ND	6.7	3390
LJD-III-15E	1.93	14	19	ND	70.8	1000
SPH-38	2	5.4	10.8	ND	18.5	3000
XHE-I-093	2	7.1	8.9	1107	20	1162
MSA-IV-35	2.1	16	21	ND	995	3000
JYI-19 (C23H23N3O3S)	2.176	205	ND	ND	34	12.7
FLUNITRAZEPAM	2.2	2.5	4.5	ND	2.1	2000
YCT-5	2.2	11.46	16.3	ND	200	10000
TJH-IV-51	2.39	17.4	14.5	ND	316	10000
WYS13 C20H18N2O3	2.442	13	27.5	ND	163	5000
YT-III-25	2.531	5.786	5.691	ND	0.095	ND
XHE-III-14	2.6		10	13	2	7
WYS9 C16H15IN2O2	2.72	22.2	23.1	ND	562	122
JYI-47	2.759	2.282	0.511	ND	0.427	ND
CM-A82a	2.78	8.93	24.51	1000	7.49	1000
TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9
XLi268 C17H13BrN4	2.8145	0.6862	ND	ND	0.6243	ND
JYI-54 (C24H15N3O3F4)	2.89	172	6.7	ND	57	1890
MMB-II-74	3	24.5	41.7	500	125.7	1000
MMB-III-016	3	1.97	2	1074	0.26	211
MMB-III-16	3	1.97	2	1074	0.26	211
QH-II-080b	3	3.7	4.7	ND	24	1000
YCT-7A	3	23.8	30.5	ND	240	10000
JYI-32 (C20H15N3O2BrF)	3.07	4.96	ND	ND	2.92	52.24
Ro15-4513	3.3	2.6	2.5	ND	0.26	3.8
XHE-II-017	3.3	10	7	258	17	294
XLi-JY-DMH ANX3	3.3	0.58	1.9	ND	4.4	5000
MLT-II-18	3.4	11.7	11	ND	225	10000
TJH-V-88	3.41		30	ND	140.9	10000
XLI-2TC	3.442	1.673	44.08	ND	1.121	
WYS15 C22H20N2O2	3.63	2.02	44.3	ND	76.5	5000
CM-A57	3.7	27	40	ND	254	1000
XHE-II-006b	3.7	15	12	1897	144	1000
JYI-60	3.73	1.635	4.3	ND	1.7	5000
RY-008	3.75	7.2	4.14	ND	1.11	44.3
MLT-II-18	3.9	12.2	24.4	ND	210	10000
OMB-18	3.9	1.2	3.4	1733	0.8	5
WY-B-09-1	3.99	8	32	1000	461	2000
SHU-1-19	4	12	7	48	14	84
ZK 93423	4.1	4.2	6	ND	4.5	1000
WY-B-23-2 (WYS11)	4.2	37.7	39	2000	176	69.4
WY-B-23-2 (WYS11)	4.2	37.7	73	ND	176	69.4
WY-B-99-1	4.4	4.5	5.58	2000	47	2000
WY-B-26-2	4.45	44.57	42.66	2000	124	2000
XHE-II-006a	4.7	4.4	20	1876	89	3531

TABLE 5: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
CM-B01	4.8	31	34	1000	286	1000
PWZ-085	4.86	13	8.5	ND	0.55	40
MLT-II-16	5.05	10.41	18.4	ND	260	10000
3 PBC	5.3	52.3	68.8	ND	591	1000
MA-3-PROPOXYL	5.3	52.3	68.8	ND	591	1000
TJH-IV-43	5.42	30.19	48.9	ND	475	10000
DMCM	5.69	8.29	4	ND	1.04	134
DM-139	5.8	ND	169	ND	9.25	325
XHE-II-073A (R ENRICHED)	5.9	11	10	15	1.18	140
MSR-I-032	6.2	18.7	4	ND	3.3	74.9
JYI-70 (C19H13N4F)	6.3	2.1	ND	ND	0.56	5000
XLi343 C20H19ClN2OSi	6.375	17.71	ND	ND	150.5	ND
3 EBC	6.43	25.1	28.2	ND	826	1000
DM-146	6.44	ND	148	ND	4.23	247
DM-215	6.74	ND	7.42	ND	0.293	8.28
ZG69A	6.8	16.3	9.2	ND	0.85	54.6
ZG-69a (Ro15-1310)	6.8	16.3	9.2	ND	0.85	54.6
WY-B-14 (WYS7)	6.84	30	36	2000	108	1000
YT-II	6.932	0.8712	3.518	ND	5.119	ND
SVO-8-67	7	41	26	15	2.3	191
MLT-II-34	7.04	15.95	22.3	ND	158	1000
SPH-195	7.2	168.5	283.5	ND	271	10000
XHE-I-065	7.2	17	18	500	57	500
ZG-234	7.25	22.14	9.84	ND	0.3	5.25
SH-I-04	7.3	6.136	5.1	ND	7.664	ND
XHE-I-038	7.3	5	34	ND	132	1000
XHE-III-13	7.3	ND	7.1	880	1.6	311
WY-B-25	7.6	40	66	2000	263	2000
CM-A49 (R)	7.7	32.5	43	ND	69	1000
SVO-8-14	8	25	8	6.9	0.9	14
TG-4-29	8.3	10.2	6.9	ND	0.4	7.61
XHE-II-002	8.3	18	13	3.9	1.5	11
WY-B-14 (WYS7)	8.5	165	245	ND	1786	5000
XHE-II-011	9	60	39	3233	90	1000
WY-B-27-2	9.19	111	72	2000	449	2000
QH-II-063	9.4	9.3	31	ND	7.7	3000
JC184 C13H9BrN2OS	9.606	10.5	ND	ND	6.709	ND
ZG-208	9.7	11.2	10.9	ND	0.38	4.6
RY-I-31	10	45	19	ND	6	1000
WY-B-23-1	10	33	43	1000	189	2000
RY-098	10.1	22.2	16.5	ND	1.68	100
H2148 C18H15N3	10.98	5000	ND	ND	256	5000
SVO-8-20	11	40	28	19	8.6	138
XHE-II-073B (S-ENRICHED)	11	17	12	33	2.1	269
SH-I-085	11.08	4.866	13.75	ND	0.24	ND
PWZ-096	11.1	36	16.9	ND	1.07	51.5
ZG-168	11.2	10.7	9.2	ND	0.47	9.4
CM-A77	11.51	51.9	105.16	1000	42.62	1000
WY-B-20	12	39	47	2000	122	3000
ABECARNIL	12.4	15.3	7.5	ND	6	1000

TABLE 5: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
SH-I-89S	12.78	8.562	8.145	ND	3.23	ND
ZG-213	12.8	49.8	30.2	ND	3.5	22.5
EDC-I-071	12.9	83.1	ND	ND	314	5000
MMB-III-14	13	13	6.9	333	1.1	333
DM-173	13.1	ND	38.1	ND	0.78	118
XLI-348	13.56	11.17	1.578	ND	82.05	ND
EDC-I-093	13.6	423	ND	ND	2912	5000
diazepam	14	20	15	ND	11	ND
XLi223 C22H20BrN3O2	14	8.7	18	1000	10	2000
WYSC2 C15H11F3N2O2	14.14	113	170	ND	518	61.2
SH-I-030	14.42	11.04	19.09	ND	1.89	ND
CM-A100	14.49	44.91	123.8	1000	65.31	1000
RY-033	14.8	56	25.3	ND	1.72	22.9
HJ-I-037	15.07	8.127	28.29	ND	0.818	ND
YT-6	15.31	87.8	60.49	ND	1.039	ND
EDC-II-044	15.4	ND	293	ND	323	1000
CM-A58	16	120	184	ND	1000	1012
QH-II-067a	16	31	52	ND	199	3000
CD-214	16.4	48.2	42.5	ND	9.8	168
JYI-06 (C23H23N3O4)	16.5	5.48	5000	ND	12.6	5000
CM-A50 (S)	17	59	88	ND	144	1000
RY-061	17	13	6.7	ND	0.3	31
ZG-224	17.1	33.7	50	ND	2.5	30.7
ZG-63A	17.3	21.6	29.1	ND	0.65	4
DM-II-30 (C20H13N3O2BrF3)	17.6	13.4	28.51	ND	7.8	5000
CM-A64	18	60	116	ND	216	1000
RY-071	19	56	91	ND	7.2	266
WZ-113	19.2	13.2	13.4	ND	11.5	300
YT-III-23	19.83	23.65	19.87	ND	1.105	ND
CM-E09b	20	22	19	55	0.45	69
MMB-II-90	20	24	5.7	9	0.25	36

^aAffinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam or [³H] Ro15-4513 binding to HEK cell membranes expressing human receptors of compositions $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ [139]. Data represent the average of at least three determinations with a SEM of $\pm 5\%$. The structures of these ligands are in the Ph.D. thesis of Clayton (2011) [22] and Supporting Information.

13. The $\alpha 2\beta 3\gamma 2$ Receptor Subtype

See Table 6 and Figures 36 and 37.

14. The $\alpha 3\beta 3\gamma 2$ Receptor Subtype

See Table 7 and Figures 38 and 39.

15. The $\alpha 4\beta 3\gamma 2$ Receptor Subtype

See Table 8 and Figures 40 and 41.

16. The $\alpha 5\beta 3\gamma 2$ Receptor Subtype

The multiple volume contours displayed in Figures 34–47 were created using the mvolume function (multiple volume contour function) in Sybyl and compounds with binding affinity at the receptor less than or equal to 20 nM. To create the overlays, first, the display (dsp) and contour (cnt) files were created for the $\alpha 5\beta 3\gamma 2$ receptor subtype and the $\alpha 1\beta 3\gamma 2$ receptor subtype by overlaying the compounds for each of these receptors (see Table 9 and Figures 42–45). Using the mvolume function, a logical expression was entered to create the surfaces making up the union as well as the included volume for each receptor subtype itself. It is clear from the

TABLE 6: Ligands with potent affinity for $\alpha 2$; ligands bound with K_i values <20 nM at this subtype. The structures of these ligands are in the Ph.D. thesis of Clayton (2011) [22].

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
QH-II-092	0.07	0.03	0.04	ND	0.17	ND
SH-TSC-2 (BCCT)	0.03	0.0419	0.035	ND	69.32	ND
QH-II-085	0.08	0.06	0.02	ND	0.08	ND
XLI-286	0.051	0.064	0.118	ND	0.684	ND
JYI-57	0.076	0.076	0.131	ND	0.036	ND
QH-II-090 (CGS-8216)	0.05	0.08	0.12	ND	0.25	17
QH-II-077	0.06	0.08	0.05	ND	0.12	4
PWZ-007A	0.11	0.1	0.09	ND	0.2	10
JYI-42	0.257	0.146	0.278	ND	0.256	ND
PWZ-0071	0.23	0.17	0.12	ND	0.44	1731
SH-I-048A	0.774	0.1723	0.383	ND	0.11	ND
XHE-II-024	0.09	0.18	0.32	14	0.24	11
QH-II-075	0.18	0.21	0.25	ND	1.3	40
XLi-JY-DMH ANX3	3.3	0.58	1.9	ND	4.4	5000
alprazolam	0.8	0.59	1.43	ND	1.54	10000
YT-5	0.421	0.6034	36.06	ND	1.695	ND
BRETAZENIL	0.35	0.64	0.2	ND	0.5	12.7
XLi268 Cl7H13BrN4	2.8145	0.6862	ND	ND	0.6243	ND
WY-B-15	0.92	0.83	0.58	2080	4.42	646
YT-II	6.932	0.8712	3.518	ND	5.119	
Ro15-1788	0.8	0.9	1.05	ND	0.6	148
XLi351 C21H21ClN2OSi	1.507	0.967	ND	ND	1.985	ND
WY-TSC-4 (WYS8)	0.007	0.99	1.63	ND	51.04	ND
XLi352 Cl8H13ClN2O	1.56	0.991	ND	ND	1.957	ND
DM-II-90 (Cl7H12N4BrCl)	0.505	1	0.63	ND	0.37	5000
JYI-64 (Cl7H12N4FBr)	0.305	1.111	0.62	ND	0.87	5000
XLi350 Cl7H11ClN2O	1.224	1.188	ND	ND	2.9	ND
SPH-121	0.14	1.19	1.72	ND	4	479
MLT-I-70	1.1	1.2	1.1	ND	40.3	1000
OMB-18	3.9	1.2	3.4	1733	0.8	5
6-PBC	0.49	1.21	2.2	ND	2.39	1343
YT-III-271	32.54	1.26	2.35	ND	103	ND
PWZ-009A1	1.34	1.31	1.26	ND	0.84	2.03
DM-II-72 (Cl15H10N20BrCl)	5000	1.37	ND	ND	2.02	5000
JYI-60 (Cl7H11N2OF)	3.73	1.635	4.3	ND	1.7	5000
XLI-2TC	3.442	1.673	44.08	ND	1.121	ND
QH-II-082	1.7	1.8	1.6	ND	6.1	100
TC-YT-II-76	101.1	1.897	5.816	ND	11.99	ND
MMB-III-016	3	1.97	2	1074	0.26	211
MMB-III-16	3	1.97	2	1074	0.26	211
XHE-III-06a	1	2	1	5	1.8	37
XHE-III-04	1.2	2	1.1	219	0.4	500
WYS15 C22H20N2O2	3.63	2.02	44.3	ND	76.5	5000
FG8205	0.4	2.08	1.16	ND	1.54	227
JYI-70 (Cl19H13N4F)	6.3	2.1	ND	ND	0.56	5000
JYI-47	2.759	2.282	0.511	ND	0.427	ND
JYI-49 (C20H12N3O2F4Br)	1.87	2.38	ND	ND	6.7	3390
FLUNITRAZEPAM	2.2	2.5	4.5	ND	2.1	2000
JYI-59 (C22H13N3O2F4)	1.08	2.6	11.82	ND	11.5	5000

TABLE 6: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
RoI5-45I3	3.3	2.6	2.5	ND	0.26	3.8
SPH-165	0.63	2.79	4.85	ND	10.4	1150
YT-II-76	95.34	2.797	0.056	ND	0.04	ND
TG-II-82	1.6	2.9	2.8	ND	1	1000
QH-II-080b	3	3.7	4.7	ND	24	1000
TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9
PS-I-34B C20H17N4BrO	ND	4.198	3.928	ND	ND	ND
ZK 93423	4.1	4.2	6	ND	4.5	1000
XHE-II-006a	4.7	4.4	20	1876	89	3531
WY-B-99-1	4.4	4.5	5.58	2000	47	2000
CM-A87	1.62	4.54	14.73	1000	4.61	1000
OMB-19	22	4.6	20	3333	3.5	40
SH-I-085	11.08	4.866	13.75	ND	0.24	ND
BCCE	1.2	4.9	5.7	ND	26.8	2700
JYI-32 (C20H15N3O2BrF)	3.07	4.96	ND	ND	2.92	52.24
XHE-I-038	7.3	5	34	ND	132	1000
SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
SPH-38	2	5.4	10.8	ND	18.5	3000
WYSC1 C16H16N2O2	1.094	5.44	12.3	ND	69.8	21.2
JYI-06 (C23H23N3O4)	16.5	5.48	5000	ND	12.6	5000
XHE-III-49	1.3	5.5	4.2	38.7	11.3	85.1
YT-III-25	2.531	5.786	5.691	ND	0.095	ND
SH-I-04	7.3	6.136	5.1	ND	7.664	ND
XHE-I-093	2	7.1	8.9	1107	20	1162
RY-008	3.75	7.2	4.14	ND	1.11	44.3
DMH-D-053 (C43H30N6O4)	236	7.4	272	5000	194.2	5000
WY-B-09-1	3.99	8	32	1000	461	2000
HJ-I-037	15.07	8.127	28.29	ND	0.818	ND
DMCM	5.69	8.29	4	ND	1.04	134
SH-I-89S	12.78	8.562	8.145	ND	3.23	ND
XLi223 C22H20BrN3O2	14	8.7	18	1000	10	2000
CM-A82a	2.78	8.93	24.51	1000	7.49	1000
QH-II-063	9.4	9.3	31	ND	7.7	3000
	9.4	9.3	31	ND	7.7	3000
XHE-II-017	3.3	10	7	258	17	294
TG-4-29	8.3	10.2	6.9	ND	0.4	7.61
MLT-II-16	5.05	10.41	18.4	ND	260	10000
JC184 C13H9BrN2OS	9.606	10.5	ND	ND	6.709	ND
ZG-168	11.2	10.7	9.2	ND	0.47	9.4
XHE-II-073A (R ENRICHED)	5.9	11	10	15	1.18	140
XLI-8TC	21.52	11.01	2.155	ND	4.059	ND
SH-I-030	14.42	11.04	19.09	ND	1.89	ND
XLI-348	13.56	11.17	1.578	ND	82.05	ND
ZG-208	9.7	11.2	10.9	ND	0.38	4.6
YT-TC-3	141.4	11.43	118.1	ND	29.22	ND
YCT-5	2.2	11.46	16.3	ND	200	10000
MLT-II-18	3.4	11.7	11	ND	225	10000
XHE-II-O53-ACID	50.35	11.8	44	ND	5.9	5000
SHU-1-19	4	12	7	48	14	84
RY-067	21	12	10	ND	0.37	42

TABLE 6: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
DM-III-01 (C18H12N3O2Br)	5000	12	ND	ND	4.73	5000
MLT-II-18	3.9	12.2	24.4	ND	210	10000
SH-053-2'F	21.99	12.34	34.9	ND	0.671	ND
WYS13 C20H18N2O3	2.442	13	27.5	ND	163	5000
PWZ-085	4.86	13	8.5	ND	0.55	40
MMB-III-14	13	13	6.9	333	1.1	333
RY-061	17	13	6.7	ND	0.3	31
WZ-113	19.2	13.2	13.4	ND	11.5	300
YT-II-83	32.74	13.22	24.1	ND	3.548	ND
DM-II-30 (C20H13N3O2BrF3)	17.6	13.4	28.51	ND	7.8	5000
LJD-III-15E	1.93	14	19	ND	70.8	1000
YT-III-272	295.9	14.98	10.77	ND	103.3	ND
BCCt	0.72	15	18.9	ND	110.8	5000
XHE-II-006b	3.7	15	12	1897	144	1000
ABECARNIL	12.4	15.3	7.5	ND	6	1000
MLT-II-34	7.04	15.95	22.3	ND	158	1000
MSA-IV-35	2.1	16	21	ND	995	3000
JYI-04 (C21H23N3O3)	28.3	16	ND	ND	0.51	1.57
PS-1-35 C23H22N5OBr	ND	16.03	24.41	ND	ND	ND
ZG69A	6.8	16.3	9.2	ND	0.85	54.6
ZG-69a (Ro15-1310)	6.8	16.3	9.2	ND	0.85	54.6
YT-III-42	382.9	16.83	44.04	ND	9.77	ND
XHE-I-065	7.2	17	18	500	57	500
XHE-II-073B (S-ENRICHED)	11	17	12	33	2.1	269
TJH-IV-51	2.39	17.4	14.5	ND	316	10000
SH-I-047	1710	17.52	1222	ND	1519	ND
XLi343 C20H19ClN2OSi	6.375	17.71	ND	ND	150.5	ND
XHE-II-002	8.3	18	13	3.9	1.5	11
YT-III-38	1461	18.21	14.63	ND	3999	
JYI-72 (C22H21N4SiF)	48.5	18.5	ND	ND	11.5	5000
MSR-I-032	6.2	18.7	4	ND	3.3	74.9
JC208 C15H10N2OS	22.42	18.89	ND	ND	5.039	ND
diazepam	14	20	15	ND	11	ND

^a Affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam or [³H] Ro15-4513 binding to HEK cell membranes expressing human receptors of compositions $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ [22, 139]. Data represent the average of at least three determinations with a SEM of $\pm 5\%$. ND: not determined.

included volume overlay that the L_2 pocket is deeper for the $\alpha 5$ subtype, as determined previously [13, 21–23, 110, 119]. The new L_4 pocket can be distinguished as the new yellow region of the $\alpha 5\beta 3\gamma 2$ subtype which is due to recently designed R-isomers by Huang [135], Poe and Li.

17. The $\alpha 6\beta 3\gamma 2$ Receptor Subtype

See Table 10 and Figures 46 and 47.

18. Updates to the Previous Model

In addition to the newly discovered L_4 pocket, the updated library of binding affinity led to two specific updates in the previous model (Figure 48).

19. QSAR

A nontraditional quantitative structure activity relationship (QSAR) approach was executed to observe steric and electrostatic preferences for each receptor subtype. A subset of the compounds used in each subtype pharmacophore/receptor model were chosen with a good cross section of scaffold variety. The compounds used in the COMFA maps are the imidazobenzodiazepines published previously [110, 137] and additionally alternative scaffolds which bound with < 20 nM at the respective subtype [22].

The interest here was in creation of steric and electrostatic maps of the comparative molecular field analyses (COMFA) created from molecular spreadsheets. A variety of compounds selective for each subtype were selected and placed into a dataset used to build the CoMFA models. Activities (K_i

TABLE 7: Ligands with potent affinity for $\alpha 3$; ligands bound with K_i values <20 nM at this subtype. The structures of these ligands are in the Ph.D. thesis of Clayton (2011) [22].

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
QH-II-085	0.08	0.06	0.02	ND	0.08	ND
SH-TSC-2 (BCCT)	0.03	0.0419	0.035	ND	69.32	ND
QH-II-092	0.07	0.03	0.04	ND	0.17	ND
QH-II-077	0.06	0.08	0.05	ND	0.12	4
YT-II-76	95.34	2.797	0.056	ND	0.04	ND
PWZ-007A	0.11	0.1	0.09	ND	0.2	10
XLI-286	0.051	0.064	0.118	ND	0.684	ND
QH-II-090 (CGS-8216)	0.05	0.08	0.12	ND	0.25	17
PWZ-0071	0.23	0.17	0.12	ND	0.44	17.31
JYI-57	0.076	0.076	0.131	ND	0.036	ND
BRETAZENIL	0.35	0.64	0.2	ND	0.5	12.7
PZII-028	0.2	ND	0.2	ND	0.32	1.9
QH-II-075	0.18	0.21	0.25	ND	1.3	40
JYI-42	0.257	0.146	0.278	ND	0.256	ND
XHE-II-024	0.09	0.18	0.32	14	0.24	11
SH-I-048A	0.774	0.1723	0.383	ND	0.11	ND
JYI-55	41.39	ND	0.504	ND	24.75	ND
JYI-47	2.759	2.282	0.511	ND	0.427	ND
DM-239	1.5	ND	0.53	ND	0.14	6.89
WY-B-15	0.92	0.83	0.58	2080	4.42	646
JYI-64 (C17H12N4FBr)	0.305	1.111	0.62	ND	0.87	5000
DM-II-90 (C17H12N4BrCl)	0.505	1	0.63	ND	0.37	5000
QH-146	0.49	ND	0.76	ND	7.7	10000
PZII-029	0.34	ND	0.79	ND	0.52	10
WYS19 C26H32N2O4Si	ND	ND	0.89	ND	ND	ND
XHE-III-06a	1	2	1	5	1.8	37
Ro15-1788	0.8	0.9	1.05	ND	0.6	148
MLT-I-70	1.1	1.2	1.1	ND	40.3	1000
XHE-III-04	1.2	2	1.1	219	0.4	500
FG8205	0.4	2.08	1.16	ND	1.54	227
PWZ-009A1	1.34	1.31	1.26	ND	0.84	2.03
alprazolam	0.8	0.59	1.43	ND	1.54	10000
XLI-348	13.56	11.17	1.578	ND	82.05	ND
QH-II-082	1.7	1.8	1.6	ND	6.1	100
WY-TSC-4 (WYS8)	0.007	0.99	1.63	ND	51.04	ND
SPH-121	0.14	1.19	1.72	ND	4	479
XLi-JY-DMH ANX3	3.3	0.58	1.9	ND	4.4	5000
MMB-III-016	3	1.97	2	1074	0.26	211
MMB-III-16	3	1.97	2	1074	0.26	211
XLI-8TC	21.52	11.01	2.155	ND	4.059	ND
6-PBC	0.49	1.21	2.2	ND	2.39	1343
YT-III-271	32.54	1.26	2.35	ND	103	ND
Ro15-4513	3.3	2.6	2.5	ND	0.26	3.8
TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9
TG-II-82	1.6	2.9	2.8	ND	1	1000
OMB-18	3.9	1.2	3.4	1733	0.8	5
YT-II	6.932	0.8712	3.518	ND	5.119	ND

TABLE 7: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
PS-1-34B C20H17N4BrO	ND	4.198	3.928	ND	ND	ND
DMCM	5.69	8.29	4	ND	1.04	134
MSR-I-032	6.2	18.7	4	ND	3.3	74.9
RY-008	3.75	7.2	4.14	ND	1.11	44.3
XHE-III-49	1.3	5.5	4.2	38.7	11.3	85.1
JYI-60 (C17H11N2OF)	3.73	1.635	4.3	ND	1.7	5000
FLUNITRAZEPAM	2.2	2.5	4.5	ND	2.1	2000
XLI-317	60.24	24.05	4.562	ND	0.295	ND
QH-II-080b	3	3.7	4.7	ND	24	1000
SPH-165	0.63	2.79	4.85	ND	10.4	1150
SH-I-04	7.3	6.136	5.1	ND	7.664	ND
SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
WY-B-99-1	4.4	4.5	5.58	2000	47	2000
YT-III-25	2.531	5.786	5.691	ND	0.095	ND
BCCE	1.2	4.9	5.7	ND	26.8	2700
MMB-II-90	20	24	5.7	9	0.25	36
TC-YT-II-76	101.1	1.897	5.816	ND	11.99	ND
ZK 93423	4.1	4.2	6	ND	4.5	1000
RY-061	17	13	6.7	ND	0.3	31
JYI-54 (C24H15N3O3F4)	2.89	172	6.7	ND	57	1890
TG-4-29	8.3	10.2	6.9	ND	0.4	7.61
MMB-III-14	13	13	6.9	333	1.1	333
XHE-II-017	3.3	10	7	258	17	294
SHU-1-19	4	12	7	48	14	84
XHE-III-13	7.3	ND	7.1	880	1.6	311
DM-215	6.74	ND	7.42	ND	0.293	8.28
ABECARNIL	12.4	15.3	7.5	ND	6	1000
SVO-8-14	8	25	8	6.9	0.9	14
XHE-III-24	0.25	ND	8	222	10	328
SH-I-89S	12.78	8.562	8.145	ND	3.23	ND
PWZ-085	4.86	13	8.5	ND	0.55	40
XHE-I-093	2	7.1	8.9	1107	20	1162
ZG-168	11.2	10.7	9.2	ND	0.47	9.4
ZG69A	6.8	16.3	9.2	ND	0.85	54.6
ZG-69a (Ro15-1310)	6.8	16.3	9.2	ND	0.85	54.6
ZG-234	7.25	22.14	9.84	ND	0.3	5.25
XHE-II-073A (R ENRICHED)	5.9	11	10	15	1.18	140
RY-067	21	12	10	ND	0.37	42
XHE-III-14	2.6	ND	10	13	2	7
YT-III-272	295.9	14.98	10.77	ND	103.3	ND
SPH-38	2	5.4	10.8	ND	18.5	3000
ZG-208	9.7	11.2	10.9	ND	0.38	4.6
MLT-II-18	3.4	11.7	11	ND	225	10000
DM-II-33 (C20H13N3O2BrCl3)	88.6	85	11.6	ND	26.2	5000
JYI-59 (C22H13N3O2F4)	1.08	2.6	11.82	ND	11.5	5000
XHE-II-006b	3.7	15	12	1897	144	1000
XHE-II-073B (S-ENRICHED)	11	17	12	33	2.1	269
CM-B44 (ss)	32	43	12	379	4.3	485

TABLE 7: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
WYSCI C16H16N2O2	1.094	5.44	12.3	ND	69.8	21.2
JYI-48	75.59	90.68	12.78	ND	31.28	ND
XHE-II-002	8.3	18	13	3.9	1.5	11
RY-076	26	27	13	ND	0.7	22
WZ-113	19.2	13.2	13.4	ND	11.5	300
SH-I-085	11.08	4.866	13.75	ND	0.24	ND
CM-E10	23	26	14	215	0.51	96
TJH-IV-51	2.39	17.4	14.5	ND	316	10000
YT-III-38	1461	18.21	14.63	ND	3999	ND
CM-A87	1.62	4.54	14.73	1000	4.61	1000
diazepam	14	20	15	ND	11	ND
RY-053	49	29	15	ND	1	46
YCT-5	2.2	11.46	16.3	ND	200	10000
RY-098	10.1	22.2	16.5	ND	1.68	100
PWZ-096	11.1	36	16.9	ND	1.07	51.5
XLi223 C22H20BrN3O2	14	8.7	18	1000	10	2000
XHE-I-065	7.2	17	18	500	57	500
SH-I-02B	29.82	1315	18	ND	74.05	ND
MLT-II-16	5.05	10.41	18.4	ND	260	10000
RY-024 C19H19N3O3	26.9	26.3	18.7	ND	0.4	5.1
BCCt	0.72	15	18.9	ND	110.8	5000
LJD-III-15E	1.93	14	19	ND	70.8	1000
CM-E09b	20	22	19	55	0.45	69
RY-I-31	10	45	19	ND	6	1000
SH-I-030	14.42	11.04	19.09	ND	1.89	ND
YT-III-23	19.83	23.65	19.87	ND	1.105	ND
XHE-II-006a	4.7	4.4	20	1876	89	3531
OMB-19	22	4.6	20	3333	3.5	40
XHE-III-06b	32	33	20	299	28.6	740

^a Affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam or [³H] Ro15-4513 binding to HEK cell membranes expressing human receptors of compositions $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ [22, 139]. Data represent the average of at least three determinations with a SEM of $\pm 5\%$. ND: not determined.

values) were converted to logarithmic units for this study. A CoMFA descriptor set was created based on the $-\log(K_i)$ of over 70 structures. The goal was to derive an alternative three-dimensional shape of the receptor using biological activity of the most selective compounds. Structures were determined by crystal structure where available or by calculation. Charges were provided based on the Gasteiger-Huckel model. Conformations were kept consistent based on previous studies of low energy conformations [110]. It should be noted that this was not a traditional QSAR study as nonselective compounds were excluded. Therefore, K_i values did not cross 3 log units. This was acceptable since the goal was not to create a predictive QSAR predictive algorithm, rather a map of the receptor based on sterics and electrostatics. Hydrogen acceptor radii were set to 3.0 and the hydrogen donor radii were set to 2.6 based on recommendations from Certara

(Tripos). Analyses were executed using PLS (partial least squares). The details of modeling will be further discussed in the SI.

For each of the following QSAR models (Figures 49–64), green areas represent desirable steric bulk and yellow represents undesirable steric bulk. Positive electrostatic contributions are represented by blue and negative electrostatic contributions are represented by red.

20. The $\alpha 1\beta 3\gamma 2$ Receptor Subtype

See Figures 49–52.

21. The $\alpha 2\beta 3\gamma 2$ Receptor Subtype

See Figures 53–56.

TABLE 8: Ligands with potent affinity for $\alpha 4$; ligands bound with K_i values <20 nM at this subtype. The structures of these ligands are in the Ph.D. thesis of Clayton (2011) [22].

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
CM-D45 C19H21N3O4	90.5	65.5	30.3	0.15	1.65	0.23
CM-D44	34.3	56.3	20.7	0.33	0.57	0.92
XHE-III-74	77	105	38	0.42	2.2	5.8
TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9
SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
XHE-II-002	8.3	18	13	3.9	1.5	11
XHE-III-06a	1	2	1	5	1.8	37
RY-080 C17H15N3O3	28.4	21.4	25.8	5.3	0.49	28.8
TG-4-39	1.6	34	24	5.6	1.4	23
SVO-8-14	8	25	8	6.9	0.9	14
RY-023 C22H27N3O3Si	197	142.6	255	7.8	2.61	58.6
MMB-II-90	20	24	5.7	9	0.25	36
XHE-III-14	2.6		10	13	2	7
XHE-II-024	0.09	0.18	0.32	14	0.24	11
XHE-II-073A (R ENRICHED)	5.9	11	10	15	1.18	140
SVO-8-67	7	41	26	15	2.3	191
CM-B31i (ss)	90	184	78	18	4.9	121
SVO-8-20	11	40	28	19	8.6	138

^a Affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam or [³H] Ro15-4513 binding to HEK cell membranes expressing human receptors of compositions $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ [22, 139]. Data represent the average of at least three determinations with a SEM of $\pm 5\%$. ND: not determined.

22. The $\alpha 3\beta 3\gamma 2$ Receptor Subtype

See Figures 57–60.

23. The $\alpha 5\beta 3\gamma 2$ Receptor Subtype

From the CoMFA maps several observations (Figure 65) can be made. The yellow steric regions near L_3 in the $\alpha 5\beta 3\gamma 2$ map are unique. This illustrated that, in general, benzodiazepines lacking a pendant phenyl are more suited to targeting the $\alpha 5$ subtype. The L_{Di} region of the $\alpha 1$ subtype is most tolerable for compounds with steric interactions in this location while the $\alpha 3$ subtype receptor compounds prefer no steric interaction in this location. Negative electrostatics are most preferred by the L_3 pocket of the $\alpha 2$ and $\alpha 5$ receptors. In general, the $\alpha 1$ subtype receptor prefers molecules without a dipole. It should be noted that none of the analogs are ionic in nature and the charges for this model were provided by the Gasteiger-Huckel model. For this reason more emphasis is placed on the steric relationships which exclude interactions in the pharmacophores. In the future a QSAR study which includes nonbinding benzodiazepines in the data set along with activity data will permit the creation of a predictive algorithm which will be very useful in lead targeting (see Figures 61–65).

24. Conclusion

Benzodiazepines, β -carbolines, and other classes of compounds readily target the GABA_A receptors. The difficulty is finding subtype selective ligands, since there is no crystal structure of the Bz/GABA_A-ergic site itself, just one composed of five beta-subunits which has no Bz site to date. The $\alpha 5$ -BzR/GABA_A subunit has recently been shown to be important in the search to treat numerous cognition-based illnesses including Alzheimer's, schizophrenia, bipolar, and depression, as well as more recently a bronchodilator, potentially important in the treatment of asthma. As an inverse agonist, PWZ-029 was able to counteract the memory-impairing effects of scopolamine, a muscarinic antagonist, in both object recognition tests and object retrieval tests in rodents, and was active in primates, as well as samaritan Alzheimer's rats. The implications of these tests point to a use as a possible treatment for Alzheimer's disease. The docking of PWZ-029 in the $\alpha 5\gamma 2$ GABA_A R-subunit details the interactions between the pharmacophore/receptor model binding site and this important negative allosteric modulator. Furthermore, $\alpha 5$ -BzR/GABA_A positive allosteric modulator, SH-053-2'-F-R-CH₃, was shown to reverse deleterious effects in the MAM-model of schizophrenia. The recent discovery of $\alpha 5$ -GABA_A R in airway smooth muscle by Emala et al. has also lead to the testing of SH-053-2'-F-R-CH₃ as a bronchodilator. This SH-053-2'-F-R-CH₃ was found to be effective in relaxing precontracted airway smooth muscle, as well as attenuating calcium-ion entry through the plasma membrane. In addition, XLi-093 (an $\alpha 5$ receptor antagonist), a potently binding $\alpha 5$ -subtype selective bivalent ligand, has been shown to inhibit the $\alpha 5$ -cognition deficits effected by diazepam and is a very good $\alpha 5$ benzodiazepine receptor site antagonist. It has also been shown to reverse the effects of $\alpha 5$ PAMs and NAMs in both rodent and primate models. These findings led to the exploration of the $\alpha 5$ -binding pocket in the Milwaukee-based pharmacophore.

New features have been introduced to the unified pharmacophore/receptor model based on many substance classes that act at the diazepam sensitive and diazepam insensitive BzR binding sites of GABA_A receptors. The major new feature identified for the $\alpha 5\beta 3\gamma 2$ receptor was a new L_4 pocket which was found by using pendant 6-phenyl benzodiazepines with a R-CH₃ at the prochiral center at C4. Further enhancement of potency was achieved by addition of 2'-F or 2'-N substituent in the pendant phenyl ring at C-6. While these changes have led to enhanced subtype selective ligands, the overall development guided by this pharmacophore model described here has led to new agents with varying, fascinating pharmacological profiles, ranging from use in cognition-based diseases such as Alzheimer's and schizophrenia, to use as a bronchodilator. This research on updating the Milwaukee-based pharmacophore/receptor model can be used in the rational design for improving the selectivity of $\alpha 5$ ligands. As the library of compounds increases, the data which follows can then be further evaluated and can lead to more insight to the identification of the possible roles each individual residue may have with the binding pocket.

TABLE 9: Ligands with potent affinity for $\alpha 5$; ligands bound with K_i values <20 nM at this subtype. The structures of these ligands are in the Ph.D. thesis of Clayton (2011) [22].

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
JYI-57	0.076	0.076	0.131	ND	0.036	ND
YT-II-76	95.34	2.797	0.056	ND	0.04	ND
QH-II-085	0.08	0.06	0.02	ND	0.08	ND
YT-III-25	2.531	5.786	5.691	ND	0.095	ND
SH-I-048A	0.774	0.1723	0.383	ND	0.11	ND
QH-II-077	0.06	0.08	0.05	ND	0.12	4
DM-239	1.5	ND	0.53	ND	0.14	6.89
QH-II-092	0.07	0.03	0.04	ND	0.17	ND
TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9
SH-I-75	1487	989.9	773	ND	0.1825	ND
PWZ-007A	0.11	0.1	0.09	ND	0.2	10
XHE-II-024	0.09	0.18	0.32	14	0.24	11
SH-I-085	11.08	4.866	13.75	ND	0.24	ND
MMB-II-90	20	24	5.7	9	0.25	36
QH-II-090 (CGS-8216)	0.05	0.08	0.12	ND	0.25	17
JYI-42	0.257	0.146	0.278	ND	0.256	ND
MMB-III-016	3	1.97	2	1074	0.26	211
MMB-III-16	3	1.97	2	1074	0.26	211
Ro15-4513	3.3	2.6	2.5	ND	0.26	3.8
DM-215	6.74	ND	7.42	ND	0.293	8.28
XLI-317	60.24	24.05	4.562	ND	0.295	ND
RY-061	17	13	6.7	ND	0.3	31
ZG-234	7.25	22.14	9.84	ND	0.3	5.25
PZII-028	0.2	ND	0.2	ND	0.32	1.9
RY-067	21	12	10	ND	0.37	42
DM-II-90 (C17H12N4BrCl)	0.505	1	0.63	ND	0.37	5000
ZG-208	9.7	11.2	10.9	ND	0.38	4.6
XHE-III-04	1.2	2	1.1	219	0.4	500
TG-4-29	8.3	10.2	6.9	ND	0.4	7.61
RY-024 C19H19N3O3	26.9	26.3	18.7	ND	0.4	5.1
JYI-47	2.759	2.282	0.511	ND	0.427	ND
PWZ-0071	0.23	0.17	0.12	ND	0.44	17.31
CM-E09b	20	22	19	55	0.45	69
ZG-168	11.2	10.7	9.2	ND	0.47	9.4
RY-080 C17H15N3O3	28.4	21.4	25.8	5.3	0.49	28.8
BRETAZENIL	0.35	0.64	0.2	ND	0.5	12.7
CM-E10	23	26	14	215	0.51	96
JYI-04 (C21H23N3O3)	28.3	16	ND	ND	0.51	1.57
PZII-029	0.34	ND	0.79	ND	0.52	10
PWZ-085	4.86	13	8.5	ND	0.55	40
JYI-70 (C19H13N4F)	6.3	2.1	ND	ND	0.56	5000
CM-D44	34.3	56.3	20.7	0.33	0.57	0.92
SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
Ro15-1788	0.8	0.9	1.05	ND	0.6	148
XLi268 C17H13BrN4	2.8145	0.6862	ND	ND	0.6243	ND
ZG-63A	17.3	21.6	29.1	ND	0.65	4
SH-053-2'F	21.99	12.34	34.9	ND	0.671	ND
XLI-286	0.051	0.064	0.118	ND	0.684	ND
SH-I-S66	22.93	30.36	55.26	ND	0.69	ND

TABLE 9: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
RY-076	26	27	13	ND	0.7	22
DM-173	13.1	ND	38.1	ND	0.78	118
OMB-18	3.9	1.2	3.4	1733	0.8	5
HJ-I-037	15.07	8.127	28.29	ND	0.818	ND
PWZ-009A1	1.34	1.31	1.26	ND	0.84	2.03
ZG69A	6.8	16.3	9.2	ND	0.85	54.6
ZG-69a (Ro15-1310)	6.8	16.3	9.2	ND	0.85	54.6
JYI-64 (C17H12N4FBr)	0.305	1.111	0.62	ND	0.87	5000
SVO-8-14	8	25	8	6.9	0.9	14
JYI-03 (C21H21N3O3)	185.4	107	ND	ND	0.954	3.34
TG-II-82	1.6	2.9	2.8	ND	1	1000
RY-053	49	29	15	ND	1	46
YT-6	15.31	87.8	60.49	ND	1.039	ND
DMCM	5.69	8.29	4	ND	1.04	134
PWZ-096	11.1	36	16.9	ND	1.07	51.5
MMB-III-14	13	13	6.9	333	1.1	333
YT-III-23	19.83	23.65	19.87	ND	1.105	ND
RY-008	3.75	7.2	4.14	ND	1.11	44.3
XLI-2TC	3.442	1.673	44.08	ND	1.121	ND
XHE-II-073A (R ENRICHED)	5.9	11	10	15	1.18	140
QH-II-075	0.18	0.21	0.25	ND	1.3	40
RY-054	59	44	27	ND	1.3	126
TG-4-39	1.6	34	24	5.6	1.4	23
XHE-II-002	8.3	18	13	3.9	1.5	11
RY-031 (RY-10)	20.4	27	26.1	ND	1.5	176
FG8205	0.4	2.08	1.16	ND	1.54	227
alprazolam	0.8	0.59	1.43	ND	1.54	1000
XHE-III-13	7.3	ND	7.1	880	1.6	311
CM-D45 C19H21N3O4	90.5	65.5	30.3	0.15	1.65	0.23
RY-098	10.1	22.2	16.5	ND	1.68	100
YT-5	0.421	0.6034	36.06	ND	1.695	ND
JYI-60 (C17H11N2OF)	3.73	1.635	4.3	ND	1.7	5000
RY-033	14.8	56	25.3	ND	1.72	22.9
XHE-III-06a	1	2	1	5	1.8	37
SH-I-030	14.42	11.04	19.09	ND	1.89	ND
XLi352 C18H13ClN2O	1.56	0.991	ND	ND	1.957	ND
XLi351 C21H21ClN2OSi	1.507	0.967	ND	ND	1.985	ND
XHE-III-14	2.6	ND	10	13	2	7
DM-II-72 (C15H10N20BrCl)	5000	1.37	ND	ND	2.02	5000
XHE-II-073B (S-ENRICHED)	11	17	12	33	2.1	269
FLUNITRAZEPAM	2.2	2.5	4.5	ND	2.1	2000
XHE-III-74	77	105	38	0.42	2.2	5.8
SVO-8-67	7	41	26	15	2.3	191
6-PBC	0.49	1.21	2.2	ND	2.39	1343
RY-058	86	40	85	ND	2.4	150
ZG-224	17.1	33.7	50	ND	2.5	31.7
RY-066	83	60	48	ND	2.6	180
RY-023 C22H27N3O3Si	197	142.6	255	7.8	2.61	58.6
XLi350 C17H11ClN2O	1.224	1.188	ND	ND	2.9	ND
JYI-32 (C20H15N3O2BrF)	3.07	4.96	ND	ND	2.92	52.24

TABLE 9: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
SH-I-89S	12.78	8.562	8.145	ND	3.23	ND
MSR-I-032	6.2	18.7	4	ND	3.3	74.9
OMB-19	22	4.6	20	3333	3.5	40
ZG-213	12.8	49.8	30.2	ND	3.5	22.5
YT-II-83	32.74	13.22	24.1	ND	3.548	ND
RY-059	89	70	91	ND	3.7	301
SPH-121	0.14	1.19	1.72	ND	4	479
RY-047	200	124	79	ND	4	340
XLI-8TC	21.52	11.01	2.155	ND	4.059	ND
YT-I-38	945.9	326.8	245.9	ND	4.07	ND
DM-146	6.44	ND	148	ND	4.23	247
CM-B44 (ss)	32	43	12	379	4.3	485
CM-B47	32	63	34	2007	4.4	717
XLi-JY-DMH ANX3	3.3	0.58	1.9	ND	4.4	5000
WY-B-15	0.92	0.83	0.58	2080	4.42	646
ZK 93423	4.1	4.2	6	ND	4.5	1000
JYI-12 (C19H16N3O3F3)	91	39	ND	ND	4.5	6.8
CM-A87	1.62	4.54	14.73	1000	4.61	1000
DM-III-01 (C18H12N3O2Br)	5000	12	ND	ND	4.73	5000
RY-057	73	85	97	ND	4.8	333
JYI-15 (C19H14N3O3F3)	205	812	ND	ND	4.8	22
CM-B31i (ss)	90	184	78	18	4.9	121
RY-079	121.1	141.9	198.4	159	5	113.7
JC208 C15H10N2OS	22.42	18.89	ND	ND	5.039	ND
YT-II	6.932	0.8712	3.518	ND	5.119	ND
XLi270 C19H14N4	36.39	25.81	ND	ND	5.291	ND
XHE-I-051	35	39	42	ND	5.3	979
MMB-II-87	200	333	107	109	5.4	333
XLI-210	231	661	2666	ND	5.4	54.22
XHE-II-O53-ACID	50.35	11.8	44	ND	5.9	5000
ABECARNIL	12.4	15.3	7.5	ND	6	1000
RY-I-31	10	45	19	ND	6	1000
QH-II-082	1.7	1.8	1.6	ND	6.1	100
SH-TSC-1 (PWZ-029)	362.4	180.3	328.2	ND	6.185	ND
XHE-II-065	1000	409	216	37	6.4	175
JYI-49 (C20H12N3O2F4Br)	1.87	2.38	ND	ND	6.7	3390
JC184 C13H9BrN2OS	9.606	10.5	ND	ND	6.709	ND
QH-II-066	76.3	42.1	47.4	2000	6.8	3000
XLI-381	619.9	285.6	3639	ND	7.051	ND
RY-071	19	56	91	ND	7.2	266
RY-I-28	283	318	102	ND	7.2	61
CM-A82a	2.78	8.93	24.51	1000	7.49	1000
YT-III-31	36.39	67.85	129.7	ND	7.59	ND
SH-I-04	7.3	6.136	5.1	ND	7.664	ND
QH-146	0.49	ND	0.76	ND	7.7	1000
QH-II-063	9.4	9.3	31	ND	7.7	3000
JC221 ANX1	106.175	49.405	182	ND	7.7495	362
DM-II-30 (C20H13N3O2BrF3)	17.6	13.4	28.51	ND	7.8	5000
SH-TS-CH ₃	107.2	50.09	20.95	ND	8.068	ND
RY-073	156	88	122	ND	8.5	267

TABLE 9: Continued.

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
SVO-8-20	11	40	28	19	8.6	138
SHU-221-1	66	41	43	3000	9	3000
YT-III-231	51.09	61.46	26.34	ND	9.124	ND
CM-E09a	176	192	122	490	9.2	718
DM-139	5.8	ND	169	ND	9.25	325
YT-III-42	382.9	16.83	44.04	ND	9.77	ND
CD-214	16.4	48.2	42.5	ND	9.8	168
XHE-III-24	0.25	ND	8	222	10	328
XLi223 C22H20BrN3O2	14	8.7	18	1000	10	2000
SPH-165	0.63	2.79	4.85	ND	10.4	1150
JYI-01 (C19H20N3O3Br)	59.2	159	96	ND	10.6	2.88
diazepam	14	20	15	ND	11	ND
XHE-III-49	1.3	5.5	4.2	38.7	11.3	85.1
WZ-113	19.2	13.2	13.4	ND	11.5	300
JYI-59 (C22H13N3O2F4)	1.08	2.6	11.82	ND	11.5	5000
JYI-72 (C22H21N4SiF)	48.5	18.5	ND	ND	11.5	5000
TC-YT-II-76	101.1	1.897	5.816	ND	11.99	ND
JYI-10 (C17H13N3O3F3Br)	5000	368	ND	ND	12.3	23
WZ-069	40	30.5	38.5	ND	12.6	1000
JYI-06 (C23H23N3O4)	16.5	5.48	5000	ND	12.6	5000
RY-072	220	150	184	ND	12.7	361
JYI-14 (C17H14N3O3F3)	32	25	ND	ND	13	565
XHE-II-053	287	45	96	1504	13.8	1000
Xli-347 C34H28N6O7	828.05	690.2	ND	ND	13.87	ND
SHU-1-19	4	12	7	48	14	84
CM-C28 (SR)	176	752	244	290	14	141
CM-E11	333	308	161	394	14	750
XHE-II-012	49	24	31	1042	14	2038
MMB-III-018	117	140	78	3500	14	976
MMB-III-18	117	140	78	3500	14	976
CM-B31c (ss)	118	319	173	37	15	137
CM-B45	230	557	336	265	15	230
XLI-093	1000	1000	858	1550	15	2000
DM-II-20 (C22H14N3O2F3)	54.3	27.14	35.68	ND	15.35	5000
XLi269 C22H22N4Si	221.8	154.2	ND	ND	15.51	ND
SH-O53-S-CH ₃ -2'F	350	141	1237	ND	16	5000
JYI-13 (C21H16N3O4F3)	5000	63.7	ND	ND	16	8.38
CM-B34	472	451	223	114	17	175
XHE-II-017	3.3	10	7	258	17	294
JC222 C16H12N2OS	86.7	45.11	ND	ND	17.63	ND
SPH-38	2	5.4	10.8	ND	18.5	3000
WZ-070	72.7	30.7	53.2	ND	18.6	300
RY-069	692	622	506	ND	19	1000
SH-053-2'F-S-CH ₃	468.2	33.27	291.5	ND	19.2	ND
XHE-I-093	2	7.1	8.9	1107	20	1162

^a Affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam or [³H] Ro15-4513 binding to HEK cell membranes expressing human receptors of compositions $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ [22, 139]. Data represent the average of at least three determinations with a SEM of $\pm 5\%$. ND: not determined.

TABLE 10: Ligands with potent affinity for $\alpha 6$; ligands bound with K_i values <20 nM at this subtype. The structures of these ligands are in the Ph.D. thesis of Clayton (2011) [22].

Cook code ^a	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
CM-D45 C19H21N3O4	90.5	65.5	30.3	0.15	1.65	0.23
CM-D44	34.3	56.3	20.7	0.33	0.57	0.92
JYI-04 (C21H23N3O3)	28.3	16	ND	ND	0.51	1.57
PZII-028	0.2	ND	0.2	ND	0.32	1.9
PWZ-009A1	1.34	1.31	1.26	ND	0.84	2.03
JYI-01 (C19H20N3O3Br)	59.2	159	96	ND	10.6	2.88
JYI-03 (C21H21N3O3)	185.4	107	ND	ND	0.954	3.34
Ro15-4513	3.3	2.6	2.5	ND	0.26	3.8
TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9
JYI-11 (C22H22N3O3F3Si)	5000	5000	ND	ND	648	3.97
QH-II-077	0.06	0.08	0.05	ND	0.12	4
ZG-63A	17.3	21.6	29.1	ND	0.65	4
ZG-208	9.7	11.2	10.9	ND	0.38	4.6
OMB-18	3.9	1.2	3.4	1733	0.8	5
RY-024 C19H19N3O3	26.9	26.3	18.7	ND	0.4	5.1
ZG-234	7.25	22.14	9.84	ND	0.3	5.25
XHE-III-74	77	105	38	0.42	2.2	5.8
JYI-12 (C19H16N3O3F3)	91	39	ND	ND	4.5	6.8
DM-239	1.5	ND	0.53	ND	0.14	6.89
XHE-III-14	2.6	ND	10	13	2	7
TG-4-29	8.3	10.2	6.9	ND	0.4	7.61
DM-215	6.74	ND	7.42	ND	0.293	8.28
JYI-13 (C21H16N3O4F3)	5000	63.7	ND	ND	16	8.38
CGS9895	0.21	ND	ND	ND	ND	9.3
ZG-168	11.2	10.7	9.2	ND	0.47	9.4
PWZ-007A	0.11	0.1	0.09	ND	0.2	10
PZII-029	0.34	ND	0.79	ND	0.52	10
XHE-II-024	0.09	0.18	0.32	14	0.24	11
XHE-II-002	8.3	18	13	3.9	1.5	11
BRETAZENIL	0.35	0.64	0.2	ND	0.5	12.7
JYI-19 (C23H23N3O3S)	2.176	205	ND	ND	34	12.7
SVO-8-14	8	25	8	6.9	0.9	14
SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
WYS10 C14H9F3N2O2	0.88	36	25.6	ND	548.7	15.3
QH-II-090 (CGS-8216)	0.05	0.08	0.12	ND	0.25	17
PWZ-0071	0.23	0.17	0.12		0.44	17.31

^aThe affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam binding to HEK cell membranes expressing human receptors of compositions $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ [139]. Data represent the average of at least three determinations with a SEM of $\pm 5\%$. ND: not determined.

The X-ray structure determination of the $\alpha 5\beta 3\gamma 2$ GABA(A) receptor is eagerly awaited, while that with five $\beta 3$ -subunits has been reported recently (Miller and Aricescu, *Nature* 2014). It is hoped that the proposed orientation may be used by others to gain additional insight into the potential mechanisms underlying binding and modulation at the Bz site, all of which will lead to a better understanding of the

structure and function of GABA(A) receptors, ultimately targeted toward treatment of diseases.

25. Synthesis of Ligands with $\alpha 5$ BzR Subtype Selectivity

Briefly, bromoacetyl bromide was added to 2-aminobenzophenone **44**, followed by treatment with methanol, which had been saturated with ammonia (g) under the cooling of an ice-water bath. The benzodiazepine, **45**, was brominated to provide **46** and then reacted with ethyl isocynoacetate to generate the imidazobenzodiazepine, **47**. A much better one-pot process has now been devised using KtBuO at -30°C [140]. The bromide **48** was subjected to a Stille-type coupling to give DM-I-81 (**9**) [126]. This route (Scheme 1) can be executed on several hundred gram scales.

The benzodiazepine monomers were prepared by the method of Fryer and Gu [89, 141]. The isatoic anhydride was heated with sarcosine in dimethyl sulfoxide to provide amide **49**. Bromination of **49** in a mixture of acetic acid, bromine, and sodium acetate afforded the corresponding monosubstituted bromide **50** in good yield. Deprotonation of **50** with lithium diisopropyl amide (LDA) in THF was followed by treatment with diethyl chlorophosphate to provide the intermediate enol phosphate. The enol phosphate was stirred with a solution of ethyl isocynoacetate and LDA to yield the imidazo congener. Again, a better one-pot procedure has been developed using KtBuO at -30°C in place of LDA at 0°C . A Heck type coupling reaction was employed with the bromide **51** with bis(acetate)bis(triphenylphosphine)palladium(II) to provide the TMS-acetylene **52**. Treatment of **52** with Bu₄NF removed the trimethylsilyl group. Hydrolysis of the ester function of **53** provided the acid **54** in excellent yield and this material was dried scrupulously and subjected to a standard CDI-mediated coupling reaction to furnish bivalent ligand XLi-093 (**4**). The imidazobenzodiazepine diethyl diester XLi-356 (**10**) was obtained from XLi-093 (Scheme 2) in high yield via catalytic hydrogenation (Pd/C, H₂).

26. Synthesis of Bivalents

Inverse agonist **53** was synthesized via the reported procedure. Hydrolysis of the ester function of **53** provided the acid **54** in excellent yield. This material was dried scrupulously and was subjected to a standard CDI-mediated coupling reaction to furnish bivalent ligands **4**, **55**, and **56** in 60% yield (Scheme 3) [13].

The acid **57**, obtained from the ester **47**, which was available from the literature [13], was stirred with CDI in DMF, followed by stirring with the required diol and DBU to provide bromide substituted dimers **58** or **59**, respectively. They were converted into the trimethylsilylacetylenyl **60** or **61**, respectively, under standard conditions (Pd-mediated, Heck-type coupling) [142]. The bisacetylene **62** or **63** (individually) was easily obtained by treatment of the trimethylsilyl ligand **60** or **61** with fluoride anion, as shown in Scheme 4.

FIGURE 34: Overlay of selected compounds for $\alpha 1 \beta 3 \gamma 2$ subtype from Table 5.

FIGURE 38: Overlay of compounds selective for $\alpha 3 \beta 3 \gamma 2$ subtype.

FIGURE 35: Updated $\alpha 1 \beta 3 \gamma 2$ subtype (blue solid) overlaid with the previous model (red wire). Overlap identified where wire and solid overlap.

FIGURE 39: Updated $\alpha 3 \beta 3 \gamma 2$ subtype (blue solid) overlaid with the previous model (red wire). Overlap identified where wire and solid overlap.

FIGURE 36: Overlay of compounds selective for $\alpha 2 \beta 3 \gamma 2$ subtype.

FIGURE 40: Overlay of selected compounds selective for $\alpha 4 \beta 3 \gamma 2$ subtype.

FIGURE 37: Updated $\alpha 2 \beta 3 \gamma 2$ subtype (solid) overlaid with the previous model (red wire). Overlap identified where wire and solid overlap.

FIGURE 41: Updated $\alpha 4 \beta 3 \gamma 2$ subtype (blue solid) overlaid with the previous model (yellow wire). Overlap identified where wire and solid overlap.

FIGURE 42: Overlay of selected compounds selective for $\alpha 5\beta 3\gamma 2$ subtype.

FIGURE 43: Updated $\alpha 5\beta 3\gamma 2$ subtype (blue solid) overlaid with the previous model (yellow wire). Overlap identified where wire and solid overlap.

FIGURE 44: Overlay of the $\alpha 5\beta 3\gamma 2$ receptor (yellow) subtype with the $\alpha 1\beta 3\gamma 2$ receptor (magenta) subtype. Orange surfaces indicate overlapping regions.

27. Materials, Methods, and Experimental

27.1. Materials and General Instrumentation. Chemicals were purchased from Aldrich Chemical Co. or Tokyo Chemical Industries and were used without further purification except where otherwise noted. Anhydrous THF was distilled from sodium/benzophenone ketyl. TLC analyses were carried out on Merck Kieselgel 60 F₂₅₄, and flash column chromatography was performed on silica gel 60b purchased from

E. M. Laboratories. Melting points were taken on a Thomas-Hoover melting point apparatus or an Electrothermal Model IA8100 digital melting point apparatus and are reported uncorrected. NMR spectra were recorded on a Bruker 300 or 500 MHz multiple-probe spectrometer. Infrared spectra were recorded on a Nicolet DX FTIR BX V5.07 spectrometer or a Mattson Polaris IR-10400 instrument. Low-resolution mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5985B GC-mass spectrometer, while high resolution mass spectral data were taken on a VG autospectrometer (Double Focusing High Resolution GC/Mass Spectrometer, UK). Microanalyses were performed on a CE Elantech EA1110 elemental analyzer. Methods of specific experiments can be found in corresponding cited works.

27.2. Competition Binding Assays. Competition binding assays were performed in a total volume of 0.5 mL of a 50 mM Tris-acetate at 4° degree centigrade for 1 hour using [³H]flunitrazepam as the radioligand. For these binding assays, 20–50 mg of membrane protein harvested with hypotonic buffer (50 mM Tris-acetate pH 7.4 at 4 degree) was incubated with the radiolabel as previously described [139, 143]. Nonspecific binding was defined as radioactivity bound in the presence of 100 μ M diazepam and represented less than 20% of total binding. Membranes were harvested with a Brandel cell harvester followed by three ice-cold washes onto polyethyleneimine-pretreated (0.3%) Whatman GF/C filters. Filters were dried overnight and then soaked in Ecoscint A liquid scintillation cocktail (National Diagnostics; Atlanta, GA). Bound radioactivity was quantified by liquid scintillation counting. Membrane protein concentrations were determined using an assay kit from Bio-Rad (Hercules, CA) with bovine serum albumin as the standard.

27.3. Radioligand Binding Assays (Drs. McKernan and Atack) [12]. In brief, the affinity of compounds for human recombinant GABA(A) receptors was measured by competition binding using 0.5 nM [³H]flunitrazepam. Transfected HEK cells (beta2 gamma2 and desired alpha subtype) were harvested into phosphate-buffered saline, centrifuged at 3,000 g, and stored at –70°C until required. On the day of the assay, pellets were thawed and resuspended in sufficient volume of 50 mM Tris/acetate (pH 7.4 at 4°C) to give a total binding of approximately 1500–2000 dpm. Nonspecific binding was defined in the presence of 100 mM (final concentration) diazepam. Test compounds were dissolved in DMSO at a concentration of 10 mM and diluted in assay buffer to give an appropriate concentration range in the assay, such that the final DMSO concentration in the assay was always less than 1%. Total assay volume was 0.5 mL and assays were carried out in 96-well plates and incubation time started by the addition of 0.1 mL of resuspended cell membranes. Following incubation for 1 hour at 4°C, assays were terminated by filtration through GF/B filters, washed with 10 mL ice cold buffer, dried, and then counted using a liquid scintillation counter. The percentage of inhibition of [³H]flunitrazepam binding, the IC₅₀, and the K_i values were calculated using the Activity Base Software Package (ID Business Solutions,

FIGURE 45: Overlay of the $\alpha 5 \beta 3 \gamma 2$ receptor (yellow) subtype with the $\alpha 1 \beta 3 \gamma 2$ receptor (magenta) subtype (Figure 44 rotated 90°). Orange surfaces indicate overlapping regions.

FIGURE 46: Overlay of selected compounds selective for $\alpha 6 \beta 3 \gamma 2$ subtype.

FIGURE 47: Updated $\alpha 6 \beta 3 \gamma 2$ subtype (blue solid) overlaid with the previous model (yellow wire). Overlap identified where wire and solid overlap.

Guildford, UK) according to the Cheng-Prusoff equation [143]. We have previously reported the synthesis of the following.

1,3-Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxy) propyl diester 4

(XLI-093) (Procedure A), experimental details previously reported [17].

1,5-Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxy) pentyl diester 56 (XLI-210), experimental details previously reported [17].

FIGURE 48: The previous benzodiazepine subtype selective receptor pharmacophore models [23]. (1) The L_2 region in the $\alpha5$ subtype is larger than the $\alpha1$ subtypes. This is a key result. It is the principle difference between $\alpha5$ subtypes compared to $\alpha2$ and $\alpha3$ subtypes, but especially in regard to $\alpha1$ subtypes (L_2 smaller in $\alpha1$). (2) The L_3 region is larger in the $\alpha5$ subtype as compared to the $\alpha1$, $\alpha2$, $\alpha3$, $\alpha4$, and $\alpha6$ BzR sites. R analogs of benzodiazepines with pendant phenyls had increased affinity to $\alpha5$ supporting the larger L_3 pocket in this receptor subtype, while S isomers bound to $\alpha2$, $\alpha3$, and $\alpha5$ subtypes because of different conformational constraints.

FIGURE 49: Steric (left) and electrostatic maps of the $\alpha1\beta3\gamma2$ receptor subtype shown in the transparent mode as seen from the classic perspective.

FIGURE 50: Steric (left) and electrostatic maps of the $\alpha1\beta3\gamma2$ receptor subtype shown in the transparent mode as seen from the classic perspective (Figure 45) rotated 90° .

1,3-Bis(8-ethyl-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxy) propyl diester 10 (Xli-356), experimental previously published [144].

Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxy) dimethyl glycol diester

FIGURE 51: Steric (left) and electrostatic maps of the $\alpha 1\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective.

FIGURE 52: Steric (left) and electrostatic maps of the $\alpha 1\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective rotated 90° .

FIGURE 53: Steric (left) and electrostatic maps of the $\alpha 2\beta 3\gamma 2$ receptor subtype shown in the transparent mode as seen from the classic perspective.

FIGURE 54: Steric (left) and electrostatic maps of the $\alpha 2\beta 3\gamma 2$ receptor subtype shown in the transparent mode as seen from the classic perspective rotated 90° .

ter 55 (Xli-374), experimental details previously reported [17].

8-Bromo-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylic acid 57, experimental details previously reported [17].

FIGURE 55: Steric (left) and electrostatic maps of the $\alpha 2\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective.

FIGURE 56: Steric (left) and electrostatic maps of the $\alpha 2\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective rotated 90° .

FIGURE 57: Steric (left) and electrostatic maps of the $\alpha 3\beta 3\gamma 2$ receptor subtype shown in the transparent mode as seen from the classic perspective.

FIGURE 58: Steric (left) and electrostatic maps of the $\alpha 3\beta 3\gamma 2$ receptor subtype shown in the transparent mode as seen from the classic perspective rotated 90° .

FIGURE 59: Steric (left) and electrostatic maps of the $\alpha 3\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective.

FIGURE 60: Steric (left) and electrostatic maps of the $\alpha 3\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective rotated 90°.

FIGURE 61: Steric (left) and electrostatic maps of the $\alpha 5\beta 3\gamma 2$ receptor subtype shown in the transparent mode as seen from the classic perspective.

FIGURE 62: Steric (left) and electrostatic maps of the $\alpha 5\beta 3\gamma 2$ receptor subtype shown in the transparent mode as seen from the classic perspective rotated 90°.

FIGURE 63: Steric (left) and electrostatic maps of the $\alpha 5\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective.

FIGURE 64: Steric (left) and electrostatic maps of the $\alpha 5\beta 3\gamma 2$ receptor subtype shown in line mode as seen from the classic perspective rotated 90°.

FIGURE 65: Clockwise from the top left, line maps of the $\alpha1\beta3\gamma2$, $\alpha2\beta3\gamma3$, $\alpha3\beta3\gamma2$, and $\alpha5\beta3\gamma2$ CoMFA.

SCHEME 1: Synthesis of 8-substituted imidazobenzodiazepines following chemistry earlier developed by Sternbach, Fryer et al. *Reagents and Conditions*. (a) Bromoacetyl bromide, sodium bicarbonate, and chloroform; (b) ammonia (anhydrous), methanol, and reflux; (c) bromine, sulfuric acid, and acetic acid; (d) sodium hydride, diethyl chlorophosphate, and tetrahydrofuran; (e) sodium hydride, ethyl isocynoacetate, and tetrahydrofuran, -30°C to r.t.; (f) tributyl(phenyl)stannane, $\text{Pd}(\text{PPh}_3)_4$.

1,3-Bis(8-bromo-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxy) propyl diester **59** (DMH-D-070) (Procedure B), experimental details previously reported [17].

1,3-Bis(8-trimethylsilylacetylenyl-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxy) propyl diester **61** (DMH-D-048) (Procedure C), experimental details previously reported [17].

1,3-Bis(8-acetylenyl-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxy) propyl diester **63** (DMH-D-053); experimental details previously reported [17].

Bis(8-bromo-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxy) diethylene glycol diester **58** (DM-III-93), experimental details previously reported [17].

Bis(8-trimethylsilylacetylenyl-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxy) diethylene glycol diester

60 (DM-III-94), experimental details previously reported [17].

Bis(8-acetylenyl-6-phenyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxy) diethylene glycol diester **62** (DM-III-96), experimental details previously reported [17].

Abbreviations

APD:	Antipsychotic drug
ASM:	Airway smooth muscle
BS:	Binding site
BZD, Bz:	Benzodiazepine
BzR:	Benzodiazepine receptor
DA:	Dopamine
DAPI:	4',6-Diamidino-2-phenylindole

SCHEME 3: Synthesis of bivalent analogs of XLi-093 (**4**). *Reagents and Conditions*. (a) 2 M NaOH, EtOH, 70°C; (b) 10% aq HCl; (c) CDI, DMF; (d) diol, DBU.

SCHEME 4: Synthesis of bivalent analogues of DMH-D-053 (**63**). *Reagents and Conditions.* (a) 2 N NaOH, EtOH, and reflux; (b) 10% aq. HCl; (c) CDI, DMF; (d) diol, DBU; (e) trimethylsilylacetylene, Pd(OAc)₂(PPh₃)₂, Et₃N, CH₃CN, and reflux; (f) TBAF*0.5H₂O, THF, -78°C.

GABA: Gamma amino butyric acid
 GABA_A: Gamma amino butyric acid A
 GABA_AR: Gamma amino butyric acid A receptor
 HAL: Haloperidol
 HEK: Human embryonic kidney
 HPC: Hippocampal
 LTK: Leukocyte tyrosine kinase
 MAM: Methylazoxymethanol
 NAM: Negative allosteric modulator
 QSAR: Quantitative structure-activity relationship
 PAM: Positive allosteric modulator
 PV: Parvalbumin
 SAL: Saline
 SH-053: SH-053-2'-F-R-CH₃
 SMA: Smooth muscle actin
 TTX: Tetrodotoxin
 VTA: Ventral tegmental area.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Terry Clayton and Michael M. Poe contributed equally to this work.

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A Review of the Updated Pharmacophore for the Alpha 5 GABA(A) Benzodiazepine Receptor Model

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Supporting Information

With References and Appendices.

Comparative Model of the Benzodiazepine Binding Site

A crystal structure of the heteropentameric GABA(A) receptors has not been reported so far, however the structure determination of the similar acetylcholine binding protein (AChBP)¹ has been of great interest for the GABA(A) receptor researchers. A homopentamer of the GABA channel with the $\beta 3$ subunit has been solved by Miller et al. (*Nature* 2014). The AChBP protein shares less than 20% sequence homology with the extracellular domain of the GABA(A) receptor,² however the overall structural similarity is estimated to be around 60 – 75 %.² Comparative modeling studies have been carried out that used the structure of AChBP to develop models of the extracellular domain of GABA(A)R.^{1, 3, 4} In addition, electron cryomicroscopy (cryo-EM) structures that represent the extracellular and transmembrane portion of the nACh receptor were used the modeling of GABA(A) receptors.² However, the homology of the sequence is not very high, making the prediction of detailed features highly uncertain. Therefore, docking studies with Bz BS ligands⁵ have a more qualitative character and do not explain all differential interactions of GABA(A)R ligands with the existing subdomains. Different experimental structures of the nAChR^{1, 2, 6} demonstrated the structure variation of between members of the same family,^{1, 2, 6} which can help us to extrapolate to which extent GABA(A) receptor subunits differ from each other.

The majority of Bz/GABA(A)R have 1γ , 2α and 2β subunits and each domain has a “plus” and a “minus” side (Figure 1). As a consequence, each subdomain interfaces with the plus and minus sides of neighboring subdomains. The Bz binding site can be found between the $\alpha^+\gamma^-$ subunits and is significantly bigger but similar to the two GABA binding pockets, which are positioned between the $\beta^+\alpha^-$ subunits.⁶⁻⁸ Overall, the coordination of the $\alpha 1\beta 2\gamma 2$ GABA(A) receptor can be described as $\gamma\beta\alpha\beta\alpha$ started counter clockwise from plus to minus (Figure 1).^{4, 5, 9, 10}

Figure 1. Absolute subunit arrangement of the $\alpha 1\beta 2\gamma 2$ GABA(A) receptor when viewed from the synaptic cleft.²⁵

The GABA(A) binding sites are located at the $\beta^+\alpha^-$ subunit interfaces and the modulatory benzodiazepine binding site is located at the $\alpha^+\gamma^-$ subunit interface.

The γ_2 subdomain is important for interacting the benzodiazepines in addition to many other molecules that act via the Bz BS.^{11, 12} Sequence variations that differ between α and γ subdomains are responsible for the subtype selectivity and efficacy of Bz BS ligands.¹¹⁻¹⁴ Three segments have been proposed to generate the Bz BS: 1) The α^+ side, 2) the loops A, B and C, and 3) three segments of the γ^- called loops D, E and F.^{11, 12} X-ray crystallography and EM-structures AChBP¹ and the nAChR¹⁵ confirmed these segments, which create a groove-like pocket between subdomains and that are conserved among the entire superfamily. Trudell and Hagen proposed a near planar cleft BzR binding sites in 1980.^{16, 17}

Figure 2. Alignment and homology model depiction of the so called “loops A-F” and flanking regions of the human sequences of the Bz recognition site in different subunits.^{1,11-15,25}

View of the homology model (Figure 2) with the channel in between units. The membrane can be pictured parallel to the top edge of the structure. Stick representation is used for key residues: Loop A His102, loop B Tyr 160 and loop D Phe77.

After the nAChR structure was available, newer reports of liganded AChBP crystal structures were published.¹⁸ Importantly, these structures showed that the local conformation of the BS is influenced by the ligand. This is particularly the case for the loop C. Thus, this highly mobile structure element responds towards ligand binding and induces smaller changes along its boundary.¹⁸ This phenomena has been observed for other receptors as well and it has been hypothesized that low energy conformers can be separately stabilized by different ligands. Unfortunately, *a priori* prediction of the appropriate conformer/ligand pair is not conclusive at this time. The models can predict changes in binding sites as large as 40% and distances of key residues can vary several Ångströms.

Small changes of the protein conformation can severely change the efficacy of Bz BS ligands.¹⁹ In addition, GABA(A) receptor ligands have different efficacies towards particular GABA(A) receptor subtypes.^{20, 21} The stabilization of the active state can be as small as 1 kcal/mol underlining the fact that proteins are inherently dynamic and can adopt many

conformations.²² Therefore, the prediction of the absolute assignments of specific side chains to specific descriptors for any particular conformation is not possible at present. However, a “suggested” orientation of the pharmacophore in the receptor can be predicted implying conformational flexibility of residues that satisfy pharmacophoric descriptors. The combination of a pharmacophore model and a homology results in a model that assigns large lipophilic areas to specific regions and enables flexible assignment of for instance H-bond bridges or π - π stacking. Water molecules can significantly complicate prediction of binding. Therefore, our group favors the pharmacophore/receptor models for drug design based on the rigid planar ligands ($K_i = 5\text{nM}$ – 20nM) that have introduced by Trudell et al.¹⁷

Relative Orientation of the Pharmacophore within the Comparative Model

The Milwaukee group published a review prior to structure determination of the AChBP that included results of site-directed mutagenesis and gave valuable insights in respect to important side chains that formed the Bz BS and represent pharmacophoric descriptors.²³ The combination of that model and the recent homology models to lymnea AChBP,² aplysia AChBP and the nAChR²⁴ resulted in a novel model that is discussed here. Experimental evidence induces some degree of conformational flexibility of the model that hint towards variable assignments for H-bridge interactions. In addition, specific areas of lipophilic interactions create a binding site geometry flanked by particular amino acid residues (Figure 3).

Figure3. Ribbon illustration of the GABA(A) BzR binding pocket conceived by Clayton et al.²⁵

Combining Homology Model and Experimental Evidence

Docking of diazepam and flumazenil was performed to test our computational ligand docking to homology models as described by the two assignments above. Different models based on different templates were used^{1, 15, 18} and the top 100 ligand poses per model were retained. A query among these poses was conducted based on the following parameters: The ring conformation of diazepam or flumazenil (Figure 4) should be in the active state;²⁶ residues α 1H102/ α 1V203 and α 1Y210/ α 1V212 are new the substituent in the seven position (consistent with the covalent labeling data); the 3'-ester group of flumazenil is near residues γ 2A79 and γ 2T81. The resulting docking poses were consistent with all covalent labeling studies and the steric requirements of 3' substituted imidazobenzodiazepines²⁷ binding.

Figure 4. Structures of BZDs.

All poses were found in the database. Representatively, diazepam is shown in Figure 5.

Figure 5. Diazepam docking pose in $\alpha 1\gamma 2$. The beta subunit is not in the binding pocket.²⁵

In Figure 5, diazepam was shown as space filling structure (turquoise: carbon, white: hydrogen, green: halogen, red: oxygen, and blue: nitrogen). The receptor was presented at ribbon with key amino acids in stick representation. The insert figure represents the empty pocket in the

“upright” position. This orientation is different from the main figure, where the structure has been turned and tilted to bring diazepam into the orientation of the unified pharmacophore model. In this orientation, His102 and Thr207 satisfy H_2 and H_1 , respectively, and His 102, Val 212 and Tyr 210 would represent L_2 a hydrophobic pocket. This orientation of the 7-substituted reactive compounds such as isothiocyanate (see Figure 4, third structure) is in position to react with His102, Tyr 210, Val 212, and Val 203. This orientation was developed in collaboration with Professor Werner Seighart.²⁵

Figure 6. High resolution image of diazepam docking pose in $\alpha 1\gamma 2$ binding pocket.²⁵

The docking pose in Figure 6 and similar predictions satisfy the two definitions for L_2 and A_2 that are discussed above and the following descriptors: H_1 is aligned with the side chains near the top of loop C, the L_{DI} region is close the subdomain interaction (the $\alpha 1$ loop B (Y160) and the $\gamma 2$ region are forming the sheet involving M130, T142 and F77); A_2 is represented by H-bond interaction near $\alpha 1$ Y160, $\gamma 2$ A79, $\gamma 2$ T81, $\gamma 2$ M130 and $\gamma 2$ T142. If these proximity relations that are found in the docking poses are compared with the unified pharmacophore model we can predict the following orientation the residues.

Figure 7. Orthogonal views of the location of residues with respect to pharmacophore in the $\alpha 1\beta 3\gamma 2$ BzR binding pocket.

The Figures 7 and 8 show the orientation of Bz BS residues relative to the pharmacophore model in two views that correspond to a 90° rotation representing the evaluation

of experimental data and the comparative model of the $\alpha 1\beta 2\gamma 2$ GABA(A). The ligand is diazepam. It is possible that more than one side chain could satisfy the same descriptor because inverse agonists stabilize protein conformations that vary from the conformations preferred by agonists,

In addition, there is still some degree of variation in structural details of the poses that represent this orientation. The factors of these structure variations: template and alignment choices, construction of the model and its refinement, and docking algorithms.

With the alignment of XLi-093 and docked Ro15-1788,²⁵ it becomes clear that these compounds are able to orientate towards the extracellular domain instead of solvent accessible space through the subunit boundary. Orientations of the pharmacophore model inside the structural model of the GABA(A) receptor have been proposed in the past.²⁸⁻³⁰

Current refinements of the predicted protein-ligand complexes with the support of the unified pharmacophore model is a promising approach to create 3D structures that are more accurate and applicable for structure-guided drug design. New approaches that combine protein modeling and pharmacophore elucidation are currently investigated.

Figure 8. Unified model of the $\alpha 1\gamma 2$ subtype benzodiazepine receptor with diazepam docked in the binding site.

Construction of the Unified Pharmacophore/Receptor Model

The 150+ BZ ligands^{31,32} that belong to fifteen different scaffolds were used to create a unified pharmacophore/receptor model. The relative affinities, efficacies and functional effects from ligands of the same structural class at the diazepam-sensitive and diazepam-insensitive benzodiazepine receptor binding sites applied. In addition, we took the approximate locations of descriptors (hydrogen bond donor sites, hydrogen bond acceptor sites, lipophilic regions, and regions of steric repulsion) that were based primarily on in vitro binding affinities in account. The compounds from different scaffolds were superimposed in order to satisfy the same descriptors, creating the unified pharmacophore model this included the rigid planar templates (K_i = 5nM-20nM) of Trudell.¹⁷

The model has two hydrogen bond donating groups (H_1 and H_2), one hydrogen bond accepting group (A_2) and one lipophilic site (L_1). There are also lipophilic regions of interaction (L_2 , L_3 , L_4 , and L_{Di}) and regions of negative steric repulsion (S_1 , S_2 and S_3). Important for positive allosteric modulation are occupation of L_2 and/or L_3 , and L_4 as well as interactions at H_1 , H_2 , and L_1 . Inverse agonists only require interactions with the H_1 , L_1 , and A_2 descriptors for good activity *in vivo*.³³⁻³⁷ The L_3 region is lipophilic of nature differentiating diazepam sensitive (DS) and the diazepam insensitive (DI) coordination. Figure 8 depicts the relative locations of the different descriptors and regions and classes of ligands for GABA(A) R are shown in Table 1.

Figure 8. The Milwaukee-based unified pharmacophore.²⁵

Table 1. Structures of ligands and their modulation at the $\alpha 1$ subtype.²⁵

diazepam
agonist

flunitrazepam
agonist

midazolam
agonist

triazolam
agonist

Ro15-1788
antagonist

Ro15-4513
inverse agonist

zolpidem
agonist

CL-218,872
partial agonist

β -CCM
inverse agonist

DMCM
inverse agonist

ZK-93423
agonist

6-PBC
partial agonist

β -CCT
antagonist

3-PBC
antagonist

β -CCE
inverse agonist

XLi-093
antagonist at $\alpha 5$

The alignments of several Bz BS ligands within this model are shown in Figure 9.

Ro 15-1788

Ro 15-1788 rotated 90°

diazepam

diazepam rotated 90°

DMCM

DMCM rotated 90°

Figure 9. Alignments of several Bz BS ligands within the pharmacophore model.²⁵

A unified pharmacophore model incorporating many substance classes that act at the DS and DI benzodiazepine binding sites of GABA(A) receptors has been updated to include new substance classes. Compound development guided by this pharmacophore model has led to new agents with interesting pharmacological profiles, particularly enhanced preference for $\alpha 2$ or $\alpha 5$ containing GABA(A) receptor subtypes. Based on the evaluation of experimental data and comparative models of the $\alpha 1\beta 2\gamma 2$ GABA(A) receptor, the location of several residues relative to the descriptors of the pharmacophore/receptor model has been proposed. Although no absolute assignments were made regarding which amino acids satisfy the pharmacophoric descriptors, experimental data strongly indicated definite trends with regard to how ligands of varying pharmacological activity are oriented within the receptor. Because the unified pharmacophore/receptor model accounts for the binding and activity profiles at the six GABA(A) receptor subtypes containing any one of the different alpha subunits, the proposed orientation should also be similar within the different models¹ of the various receptor subtypes. Information to be immediately gained from these proposed orientations can have far reaching benefits, not only for the rational design of selective ligands and the interpretation of ligand docking results, but also for the identification and evaluation of possible roles certain residues may have within the pocket. As structure determination of the GABA(A) receptor is eagerly awaited, it is hoped that these proposed orientations may be used by others to gain additional insight into the potential mechanisms underlying binding and modulation at the Bz site, all of

which will lead to a better understanding of the structure and function of GABA(A) receptors. In the next section the methods used to build the protein model will be explained.

Homology Models of the Benzodiazepine Receptor

The γ -aminobutyric-acid (GABA(A) and GABA_C) pentameric ligand gated ion channels are members of a superfamily of allosteric transmembrane proteins which includes the nicotinic acetylcholine (nAChR), serotonin 5-HT₃, and glycine receptors. Electrophysiological data on GABA(A) is available but attempts at atomic resolution to acquire structural data have so far been unsuccessful. However, in 2001 Smit³⁸ discovered a homologue of the nACh receptor ligand-binding domain from the snail *Lymnaea stagnalis*. Acetylcholine-binding protein (AChBP) is produced in the glial cells of *Lymnaea stagnalis*. In the synaptic cleft, AChBP modulates synaptic transmission. Subunits of the snail glial cells form a stable homopentamer with conserved N terminal domains. Known agonists and antagonists of nAChRs bind to the homologue. For this reason, it is a valuable template for modeling the N-terminus domains of pentameric ligand gated ion channels.

Brejč *et al.* successfully solved the crystal structure of AChBP by X-ray crystallography using weak Pb multiple wavelength anomalous diffraction (MAD) data in two crystal forms.¹ The crystals were grown at room temperature using the hanging-drop vapour diffusion technique. The crystal structure revealed a radially symmetric homopentamer with extracellular dimensions of the nicotinic acetylcholine receptor (nAChR) which correlated with measurements taken using electron microscopy.¹

Figure 10. Orthogonal views of the homopentameric acetylcholine binding protein crystallized from *Lymnaea stagnalis*. The subunits have been assigned different colors for clarity.²

Subunits are homologous and have been colored by chain to distinguish them. In space filling models, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (present during the crystallization process) can be seen bound in the ligand binding site (Figure 10) between each subunit interface.²

The N terminus is located at the “mouth” of the ion channel in the synaptic cleft. A molecule of HEPES was present in the acetylcholine binding pocket. This was verified as the residues implicated with agonist binding in nAChR and AChBP were conserved in the receptor. The residues lie in four regions identified as loops A, B, C, and D. Loops A, B, and C are located on what is referred to as the ‘plus’ face while loops D, E, and F lie on the ‘minus’ face (see Figures 11 and 12). In the heteromeric nAChR, agonist binding takes place between the ‘plus’ face of a subunit and the ‘minus’ face of an α or γ subunit. Site directed mutagenesis and radioligand binding assays have established that residues of the GABA(A) receptor involved in ligand binding align with residues in loops A, B, and C of the α subunit and loops D and E of the γ subunit.^{2, 23, 25}

Figure 11. Single subunit of acetylcholine binding protein crystallized from *Lymnaea stagnalis* (side view).^{2,23}

Figure 12. Single subunit of acetylcholine binding protein crystallized from *Lymnaea stagnalis* (top view).

Since the AChBP is a member of the Cys-loop LGIC superfamily it offers a template for building models of the the ligand binding domains of the Cys-loop superfamily. The AChBP was used to build homology models of the subtypes of the GABA(A) receptors. The ligand binding domains of human GABA(A) receptors ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\beta 2$, and $\gamma 2$) were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB). Originated in 1971 at

Brookhaven National Laboratories, this is the home of the Protein Data Bank. The PDB archive is the repository of information about the 3D structures of large biological molecules such as proteins and nucleic acids. With the protein sequences in hand, a critical first step in building a homology model is to align the raw protein sequence with the protein sequence of a target protein or template structure. Alignments are prepared using multiple sequence alignment software for DNA and proteins.³⁹

After calculating the best match for the selected sequences, they are lined up so identities, similarities, and differences can be seen. Programs which perform this include MAFFT, Muscle, Multalin, ClustalW, and BlastAlign (<http://pbil.univ-lyon1.fr/alignment.html>). Templates can also be found by performing a query of the raw protein sequence against the Protein databank. This is performed using the protein database search program, Gapped Blast.^{39, 40} The report will contain a list of sequences producing significant alignments, PDB code, protein description, a normalized alignment score and an Expect (E) value. In general, a lower E score describes the background noise that exists between sequences. A lower E value correlates to a more significant score. Once a proper alignment of protein sequences was performed, the raw protein sequence was threaded onto the AChBP using Deep View. The crystal structure of the AChBP was downloaded from the Swiss Protein Databank Viewer. Deep View is a Swiss Protein Databank Viewer (<http://ca.expasy.org/spdbv>). Threading is performed after the alignment is complete. Amino acids which have been identified as equivalent between the proteins are “fit”. This is similar, but more precise than the “3 corresponding atoms” technique. Deep View includes a tool, *Magic Fit*,³⁹ which performs the threading procedure for all residues across a raw sequence and a reference protein. Once the preliminary model has been built, a manual inspection can be performed. Considerations during manual inspection include, identifying areas of low homology, and manually aligning gap regions with loops in the reference protein.^{39, 40} Loop regions which correspond to gaps were in the alignment can be modeled by fitting structures from a loop database. In the current model, loop regions were mapped to a loop database by Cromer et al.^{2, 15}

Upon completion of the preliminary model, the following properties can be studied using Deep View and Sybyl²⁵:

1. Surface hydrophobicity
2. Solvent accessibility of N-glycosylation sites after pentamer assembly
3. Acidic, basic, and non polar maps
4. Sybyl X minimizations
5. Steric clashes
6. RMSD of alpha carbons
7. Location of disulfide bonds

In order to improve the prediction of areas of low homology PHD was utilized by Cromer et al.^{2, 39, 40} PHD or more commonly referred to as PredictProtein is a sequence analysis tool and the prediction of protein structure and function. Protein sequences or alignments are submitted to Columbia University; PredictProtein returns multiple sequence alignments, PROSITE sequence motifs, low-complexity regions (SEG), nuclear localisation signals, regions lacking regular structure (NORS) and predictions of secondary structure, solvent accessibility, globular regions, transmembrane helices, coiled-coil regions, structural switch regions, disulfide-

bonds, sub-cellular localization, and functional annotations. Upon request the Rost group will perform fold recognition by prediction-based threading, CHOP domain assignments, predictions of transmembrane strands and inter-residue contacts are also available. PredictProtein is run by Burkhard Rost at Columbia University in New York (www.rostlab.org).⁴⁰ As an alternative, Swiss Model can be used to model subunit loop regions by searching structures from loop databases.

Once subunits were completed, a pentamer was constructed taking into consideration residues determined experimentally to be present in the allosteric and GABA binding site. On the α subunit, this includes α Tyr160, α Tyr210, γ Phe77, and α Ser205.^{23, 25} After manually assembling the subunits and comparing an overlay with AChBP, the structure was energy minimized.^{25, 39}

With protein receptor models for $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ completed a truly *unified* pharmacophore model was assembled for each receptor subtype. Using the Tripos Biopolymer software, the pharmacophore was manually aligned into each protein. With mutagenesis data on residue interaction on key ligands, the pharmacophore was carefully adjusted so as to recreate the protein-ligand interactions discussed previously. The protein models below represent the cumulative results of over 20 years of research. The proteins are oriented with the $\gamma 2$ on the left and the α subunit on the right with loop C reaching over the middle to $\gamma 2$. Diazepam has been docked in each receptor and the pharmacophore is visible.

Protein Models of the $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$ and $\alpha 5\beta 3\gamma 2$ Subtypes

Presented here are the complete unified protein-pharmacophore models. The Milwaukee-based pharmacophore has been inserted into each of the homology models for the receptor subtypes of the benzodiazepine receptor. The models show the similarities we expected. The two oxygens of serine 206 represent the hydrogen bond acceptor A_2 . The hydrogen bond donor, H_1 , is due to the hydroxyl group oxygens of Threonine 231 and Tyrosine 234. Threonine 193 is responsible for the H_2 hydrogen bond donor as its hydroxyl group coordinates with lone pairs of ligands. The imidazole ring of histidine 102 coordinates with phenyl rings of ligands through π - π stacking interactions.

Figure 13. The $\alpha 1\beta 3\gamma 2$ subtype receptor with key residues shown

Figure 14. The benzodiazepine binding site of the $\alpha 1\beta 3\gamma 2$ receptor

Figure 15. The benzodiazepine binding site of the $\alpha 1\beta 3\gamma 2$ receptor rotated 90°

Figure 16. The $\alpha 2\beta 3\gamma 2$ subtype receptor with key residues shown

Figure 17. The benzodiazepine binding site of the $\alpha 2\beta 3\gamma 2$ receptor

Figure 18. The benzodiazepine binding site of the $\alpha 2\beta 3\gamma 2$ receptor rotated 90°

Figure 19. The $\alpha 3\beta 3\gamma 2$ subtype receptor with key residues shown

Figure 20. The benzodiazepine binding site of the $\alpha 3\beta 3\gamma 2$ receptor within the active site

Figure 21. The benzodiazepine binding site of the $\alpha 3 \beta 3 \gamma 2$ receptor rotated 90°

Figure 22. The benzodiazepine binding site of the $\alpha 1 \beta 3 \gamma 2$ receptor with diazepam in the binding pocket.

Figure 23. The benzodiazepine binding site of the $\alpha 1\beta 3\gamma 2$ receptor rotated 90° with diazepam in the binding site

Figure 24. The benzodiazepine binding site of the $\alpha 1\beta 3\gamma 2$ receptor rotated 90° (reverse direction) with diazepam in the binding site

Figure 25. The $\alpha 5\beta 3\gamma 2$ subtype receptor with key residues shown with diazepam in the binding site

Figure 26. The benzodiazepine binding site of the $\alpha 5\beta 3\gamma 2$ receptor with diazepam in the binding site

Figure 27. The benzodiazepine binding site of the $\alpha 5\beta 3\gamma 2$ receptor rotated 90° with diazepam in the binding site

From this unified model which was constructed on binding data from hundreds of compounds, included volume analysis, site directed mutagenesis, manual as well as Sybyl Flexidock and AutoDock algorithms, one can now see how any compound will dock in the benzodiazepine binding site of GABA(A) . Presented below is a compound for further research on drug abuse, WYS8.

Figure 28. Flexidock fit of WYS8

Refinement of WYS8 in the unified pharmacophore receptor was completed using FlexiDock. The protein with the pharmacophore set inside the benzodiazepine receptor is displayed with a ligand docked to the pharmacophore. From the compute function, Flexidock is executed. The pocket needs to be defined for Sybyl if it has not already. Pick atoms which are located near the binding site. All atoms within 4 Angstroms will be automatically selected by Sybyl to assist. For Flexidock to work with rigid proteins the following steps must be taken:

1. Water must be removed
2. Select atoms around the binding pocket
3. Add hydrogens
4. Pick 4Å radius to display around binding pocket
5. Have Sybyl add charges if necessary
6. Set rotatable bonds

From these models, the individual pharmacophores, and the compound database the medicinal chemist has a powerful set of tools to determine future ligands to synthesize in order to deliver subtype selective drugs with reduced side effects. For example, knowing how to dock ligands into the pharmacophore, one can begin taking measurements of ligands and residues and analyzing interactions. In the $\alpha 2\beta 2\gamma 2$ Bz receptor His 102 and the phenyl ring of diazepam have a π stacking distance of 4.1 Angstroms. Next we will discuss how one can properly dock ligands into the binding site.

Docking Studies Using AutoDock 4.2

Docking of ligands into the new binding sites of the homology models was also executed using the suite of *Autodock Tools* developed at Scripps Institute.^{41, 42} The general procedure entails preparing *pdb* file formats of the receptor (protein) as well as the ligand. Once these docking grids and docking parameters are set, the docking run will produce multiple potential docking reports. Prior knowledge from SAR studies and mutagenesis studies is then used to select those for a parallel run (clusters) and a final docking prediction is developed.

Preparing the Ligand for Autodock

The ligand is loaded into Autodock as a *pdb* file. Once in *Autodock*, Gasteiger partial charges are determined along with aromatic carbons, rotatable bonds and torsional degrees of freedom. A new “*pdgqt*” file is created which has this information attached to it. Preparation of the receptor begins with loading it too as a *pdb* file into Autodock. Water molecules must be removed from the protein if present for *Autodock* processing. If any residues are flexible, these need to be identified in the receptor. Once identified, torsion angles can be assigned. Bonds in side chains can be manually or automatically selected as rotatable, unrotatable, or non-rotatable. *Autodock* needs two files when docking ligands in proteins with flexible residues, a flexible PDBQT file and a Rigid PDBQT file. Before docking can begin the receptor is checked for Gasteiger charges. A map is created which identifies possible hydrogen bonding interactions.

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Next, a grid box (Figure 29) is constructed around the general vicinity of the receptor if this is known.

Figure 29. Setting the autogrid in Autodock for the Bz BS protein

With all the appropriate information stored in a parameter file, the docking program will know which map file to use, details about the ligand rules, the potential presence of flexible residues and which docking algorithm to use. Four different docking algorithms are currently available in AutoDock. Monte Carlo simulated annealing (SA) was used. Briefly, the ligand makes random moves in the receptor and the energy of the new position is compared to the old one. Moves in which the energy is lowered are accepted.

Figure 30. WYS8

Docking WYS8

WYS8 was drawn in ChemDraw Ultra 8.0 (Figure 30). Lone pairs were added and the molecule was minimized in ChemDraw 3D using MM2 to a minimum RMS gradient of 0.100. This was converted to a PDB file to be loaded into AutoDock 4. Upon loading into Autodock,

Gasteiger charges were added, 14 non-polar hydrogens were merged, 10 aromatic carbons were identified, 3 rotatable bonds were detected, and TORSDOF was set to 3.

Figure 31. Rotatable bonds are identified along with root atom in ligand WYS8

The root atom with the smallest largest sub-tree (in this case an acetylenic hydrogen) is marked with a green sphere and the root portion (red) is identified. The rotatable bonds are colored green. The receptor was rigid for this analysis.

Figure 32. AutoDock result rendering was performed using Tripos Benchware.

Using Tripos Benchware 3D Explorer 2.6, one of the results was modeled (Figure 32). Key residues were identified and the protein surface five angstroms from the ligand was mapped showing its lipophilic potential. The results using AutoDock and a rigid protein will be much improved when flexible residues are allowed in the binding pocket along with rotatable bonds. The docking in Figure 28 is preferred when considering the orientation in the Milwaukee-based pharmacophore.

Homology models which have the pharmacophore aligned in the binding pocket have been presented for the $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 2\gamma 2$, and $\alpha 5\beta 2\gamma 2$ GABA(A) receptors. The binding site was constructed in light of years of work including structure-activity-relationships (SAR), site directed mutagenesis, and docking studies of monomer and dimers alike. The

$\alpha 4\beta 2\gamma 2$ and $\alpha 6\beta 2\gamma 2$ receptors have not been made as these “diazepam-insensitive” binding sites are not a focus of our research to date. An updated included volume analysis has been presented using ligands from 15 different structural families. The new included volume analyses and the unified pharmacophore protein models will continue to drive the design of novel and selective benzodiazepine receptor ligands. From these new ligands, scientists will increase their understanding of the physiological responses of the specific receptors, the design of pharmaceuticals with reduced side effects, and treatments for schizophrenia, drug addiction and Alzheimer’s disease. The $\alpha 5\beta 2\gamma 2$ receptors are of particular interest because of their concentration in the hippocampus. The discovery of a larger L_3 pocket in the $\alpha 5\beta 2\gamma 2$ receptor versus the other subtypes will provide medicinal chemists with a structural advantage in designing more subtype selective ligands. From the unified pharmacophore protein models presented here, the design of new compounds for the GABA(A) will be more productive than ever.

Future Work

Screening and modeling of more subtype selective ligands will further our current understanding of the benzodiazepine receptor and its function. Continued work on the molecular spreadsheet which will include all the compounds designed in the Milwaukee laboratory as well as other organizations will create a powerful QSAR algorithm for answering the age old question, “What compound should we make next?” This study will need to include all binding and non-binding compounds in order to create a predictive program with a high degree of accuracy (r^2 , q^2) in predicting activity.

Site directed mutagenesis needs to be continued in which receptors of all subtypes are tested to determine how a residues substitution affects each of the subtypes. Such experimentation will shed further light on how ligands are positioned in the binding pocket and what residues they are interacting with. Adding to the new pharmacophore protein alignments which can now be executed, docking runs using *Autodock* in which the residues in the receptor are set to flexible may give further information as to docking preferences of ligands helping to better understand key interactions in each of the different subtypes.

All of these activities have allowed us to develop the current ligands as well as propose next generation ligands. The new pharmacophores will aid in furthering our understanding of GABA and in designing better drugs for the CNS until a complete GABA(A) protein receptor ion channel is captured in high resolution.

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Appendix I. Key Amino Acid Sequences

Data Table 1. Amino acid sequence of the alpha subunit of the acetylcholine receptor in the *lymnaea stagnalis* (Great Pond Snail)

>sp|P58154|ACHP_LYMST Acetylcholine-binding protein OS=Lymnaea stagnalis PE=1 SV=1
MRRNIFCLACLWIVQACLSLDRADILYNIRQTSRPDVIPTQRDRPVAVSVSLKFINILEV
NEITNEVDVVFVWQQTTWSDRTLAWNSSHSPDQVSVPISSLWVPDLAAAYNAISKPEVLTPO
LARVVSDEGEVLYMPSIRQRFSCDVSGVDTESGATCRIKIGSWTHHSREISVDPTTENSDD
SEYFSQYSRFEILDVTQKKNSVTYSCCEAYEDVEVSLNFRKKGRSEIL

Data Table 2. Amino acid sequence of the alpha subunit of the acetylcholine receptor in the *Torpedo californica* (Pacific Electric Ray)

>sp|P02710|ACHA_TORCA Acetylcholine receptor subunit alpha OS=Torpedo californica GN=CHRNA1 PE=1 SV=1
MILCSYWHVGLVLLLFSCCGLVLGSEHETRLVANLLENYNKVIRPVEHHTHFVDITVGLQ
LIQLISVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSSDDVWLPDLVLYNA
DGDFAIVHMTKLLLDYTGKIMWTPPAIFKSYCEHVTHFPDQQNCTMKLGIWTDGDKV
SISPESDRPDLSTFMESGEWVMKDYRGWKHWVYYTCCPDTPYLDITYHFIMQRIPLYFVV
NVIIPCLLSFLTGLVFYLPDTSGEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGK
YMLFTMIFVISSIIITVVVINTHHRSPSTHTMPQWVRKIFIDTIPNVMFFSTMKRASKEK
QENKIFADDIDISDISGKQVTGEVIFQTPLIKNPDKSAIEGVKYIAEHMKSDEESSNAA
EEWKYVAMVIDHILLCVFMLICIGTVSVFAGRLIELSQEG

Data Table 3. Amino acid sequence of the alpha 1 subunit of the rat GABA(A) receptor

>sp|P62813|GBRA1_RAT Gamma-aminobutyric acid receptor subunit alpha-1 OS=Rattus norvegicus GN=Gabra1 PE=1 SV=1
MKKSRLSDYLWAWTLILSTLSGRSYGQPSQDELKDNTTVFTRILDRLLDGYDNRLRPGL
GERVTEVKTDIFVTSFGPVSDHDMETIDVFFRQSWKDERLKFKGPMTVLRNLNLMASKI
WTPDTFFHNGKKSVAHNMTMPNKLRLITEDGTLTYMRLTVRAECPMHLEDFPMDAHACP
LKFGSYAYTRADEVVYEWTRPARSVVVAEDGSRLNQYDLLGQTVDSGIVQSSTGEYVVM
THFHLKRKIGYFVIQTYLPCIMTVILSQVSFWLNRESVPARTVFGVTTVLTMTLSISAR
NSLPKVAYATAMDWFIACVYAFVFSALIEFATVNYFTKRGYAWDGKSVVPEKPKKVKDPL
IKKNNTYAPTATSYTPNLARGDPGLATIAKSATIEPKVETKPKPEPKKTFNSVSKIDR
LSRIAFLPLFGIFNLVYWATYLNREPQLKAPTPHQ

Data Table 4. Amino acid sequence of the gamma 2 subunit of the rat GABA(A) receptor

>sp|P18508|GBRG2_RAT Gamma-aminobutyric acid receptor subunit gamma-2 OS=Rattus norvegicus GN=Gabrg2 PE=1 SV=1
MSSPNTWSTGSTVYSPVFSQKMTLWILLLLSLYPGFTSQKSDDDDYEDYASNKTWVLT PKV
PEGDVTVILNNLLEGYDNKLRPDIGVKPTLIHTDMYVNSIGPVNAINMEYTIIDIFFAQTW
YDRRLKFNSTIKVLRNLNSNMVGKIWIPDTFFRNSKKADAHWITTPNRMLRIWNDGRVLYT
LRLTIDAECQLQLHNFPMDEHSCPLEFSSYGYPREEIVYQWKRSSVEVGDRSWRLYQFS
FVGLRNTTEVVKTTSGDYVVMSSVYFDLSRRMGYFTIQTYPCTLIVVLSWVSFWINKDAV
PARTSLGITTTLTMTTLSTIARKSLPKVSYVTAMDLFVSVCFIVFSALVEYGTLYHYFVS
NRKPSKDKDKKKKNPAPTIDIRPRSATIQMNNATHLQERDEEYGYECLDGKDCASFFCCF
EDCRTGAWRHGRIHIRIAKMDSYARIFFPTAFCLFNLVYWVSYL

Data Table 5. Amino acid sequence of the alpha 1 subunit of the human GABA(A) receptor

>sp|P14867|GBRA1_HUMAN Gamma-aminobutyric acid receptor subunit alpha-1 OS=Homo sapiens GN=GABRA1 PE=1 SV=3
MRKSPGLSDCLWAWILLSTLTGRSYGQPSLQDELKDNTTVFTRILDRLLDGYDNRLRPG
LGERVTEVKTDIFVTSFGPVSDHDMYETIDVFFRQSWKDERLKFKGPMTVLRLNNLMASK
IWTPDTFFHNGKKSVAHNMTMPNKLRLITEDGTLTYMRLTVRAECPMHLEDFPMDAHAC
PLKFGSYAYTRAEEVVEWTREPARSVVVAEDGSRLNQYDLLGQTVDSGIVQSSTGEYVVM
TTHFHLKRKIGYFVIQTYLPCIMTVILSQVSFWLNRESVPARTVFGVTTVLTMTTLSISA
RNSLPKVAYATAMDWFIACVAFVFSALIEFATVNYFTKRGYAWDGKSVVPEKPKKVKDP
LIKKNNTYAPTATSYPNLRAGDPGLATIAKSATIEPKVKPETKPPEPKKTFNSVSKID
RLSRIAFPLLFGIFNLVYWATYLNREPQLKAPTPHQ

Data Table 6. Amino acid sequence of the gamma 2 subunit of the human GABA(A) receptor

>sp|P18507|GBRG2_HUMAN Gamma-aminobutyric acid receptor subunit gamma-2 OS=Homo sapiens GN=GABRG2 PE=1 SV=2
MSSPNIWSTGSSVYSTPVFSQKMTVWILLLSLYPGFTSQKSDDDYEDYASNKTWVLTTPK
VPEGDVTVLNNLLEGYDNKLRPDIGVKPTLIHTDMYVNSIGPVNAINMEYTDIFFAQT
WYDRRLKFNSTIKVLRLNSNMVGGKIWIPTDFFRNSKKADAHWITTPNRMLRIWNDGRVLY
TLRLTIDAECQLQLHNFPMDEHSCPLEFSSYGYPREEIVYQWKRSSVEVGDTRSWRLYQF
SFVGLRNTTEVVKTTSGDYVVMVSYFDLSRRMGYFTIQTYIPCTLIVVLSWVSFWINKDA
VPARTSLGITTVLTMTTSLTIARKSLPKVSYVTAMDLFVSVCFIFVFSALVEYGTLYHYFV
SNRKPSKDKDKKKKNPAPTIDIRPSATIQMNNATHLQERDEEYGYECLDGKDCASFFCC
FEDCRTGAWRHGRIHIRIAKMDSYARIFFPTAFCLFNLVYWVSYYLYL

Data Table 7. Amino acid sequence of the Beta 2 Subunit of the Human GABA(A) Receptor

>sp|P47870|GBRB2_HUMAN Gamma-aminobutyric acid receptor subunit beta-2 OS=Homo sapiens GN=GABRB2 PE=1 SV=1
MWRVRKRGYFGIWSFPLIIAAVCAQSVNDPSNMSLVKETVDRLLKGYDIRLRPDFGGPPV
AVGMNIDIASIDMVSEVNMDYTLTMYFQQAWRDKRLSYNVIPLNLTLDNRVADQLWVPDT
YFLNDKKSFVHGVTVKNRMIRLHPDGTVLYGLRITTTAACMMDLRRYPLDEQNCTLEIES
YGYTTDDIEFYWRGDDNAVTVGVTKIQLPQFSIVDYKLITKKVVFSTGSYPRLSLSFKLKR
NIGYFILQTYMPSILITLSWVSFWINYDASAARVALGITTVLTMTTINTHLRETLPKIP
YVKAIDMYLMGCFVVFVFMALLEYALVNYIFFGRGPQRQKKAEEKAASANNEKMRLDVNKM
DPHENILLSTLEIKNEMATSEAVMGLGDPRTMMLAYDASSIQYRKAGLPRHSFGRNALER
HVAQKKSRLLRRRASQLKITIPDLTDVNAIDRWSRIFFPVVFSFFNIVYWLYYVN

Appendix II.

Data Table 8. Coordinates of the Pharmacophore

REMARK File generated by Swiss-PdbViewer 4.00b0
REMARK <http://www.expasy.org/spdbv/>
HETATM 1 * hip 0 2.246 2.113 1.413 0.00100.00
HETATM 2 O hip 0 -5.691 -0.267 -4.151 0.00100.00
HETATM 3 H1 hip 0 0.742 3.154 -3.474 0.00100.00
HETATM 4 H2 hip 0 -2.472 -1.062 0.681 0.00100.00
SPDBVT 1.0000000000 0.0000000000 0.0000000000
SPDBVT 0.0000000000 1.0000000000 0.0000000000
SPDBVT 0.0000000000 0.0000000000 1.0000000000
SPDBVT 0.0000000000 0.0000000000 0.0000000000
SPDBVT 0.0000000000 0.0000000000 0.0000000000
SPDBVV default;
SPDBVV 15.426431672954 1678.699418606842 20.000000000000
SPDBVV 0.4758893755 0.5102135228 -0.7163877885
SPDBVV 0.3084772896 0.6659613576 0.6792181034

```

SPDBVV    0.8236328455    -0.5442220423    0.1595346499
SPDBVV    -1.7224999666    1.0460000038    -1.3689999580
SPDBVV    0.0000000000    0.0000000000    0.0000000000
SPDBVf 27
SPDBVi 1.00 1.00 1.00
SPDBVb 0.00 0.00 0.20
SPDBVg 64
SPDBVi 1 1 1 0 1 1 1 1 0 1 0 0 1 0 0
SPDBVp 0
END

```

Appendix III.

Structures of compounds, as reported in Clayton.²¹

STRUCTURE	COOK CODE	a1	a2	a3	a4	a5	a6
<chem>CCOC1=CC=C2C(=C1)C(=CN2)c3ccccc3</chem> $C_{13}H_{12}N_2O$	3 EBC	6.43	25.1	28.2		826	1000
<chem>CCOC1=CC=C2C(=C1)C(=CN2)c3ccccc3</chem> $C_{14}H_{14}N_2O$	3 PBC	5.3	52.3	68.8		591	1000
<chem>CC1=CC=C(C=C1)COc2cc3c(cc2)C(=Nc4ccccc43)</chem> $C_{18}H_{14}N_2O$	3-BENZOXY bC	830	3000	3000		10000	10000
<chem>CC(C)CCOC1=CC=C2C(=C1)C(=CN2)c3ccccc3</chem> $C_{15}H_{16}N_2O$	3-isobutoxy bC	24.9	123.6	139.2		1000	10000
<chem>CCCCCOC1=CC=C2C(=C1)C(=CN2)c3ccccc3</chem> $C_{16}H_{18}N_2O$	3-isopentoxy bC	350.2	3000	3000		3000	10000
<chem>COC1=CC=C2C(=C1)C(=CN2)c3ccccc3</chem> $C_{12}H_{10}N_2O$	3MBC
<chem>CCOC(=O)c1cc(OC)c(cc1-c2cc3c(cc2)C(=Nc4ccccc43)CCCCC5=CC=C(C=C5)OCC)OC</chem> $C_{18}H_{20}N_2O_4$	6-PBC	0.49	1.21	2.2		2.39	1343

$C_{24}H_{24}N_2O_4$	ABECARNIL	12.4	15.3	7.5		6	1000
$C_{17}H_{13}ClN_4$	alprazolam	0.8	0.59	1.43		1.54	10000
$C_{14}H_{12}N_2O_2$	BCCE	1.2	4.9	5.7		26.8	2700
$C_{16}H_{16}N_2O_2$	BCCt	0.72	15	18.9		110.8	5000
$C_{19}H_{20}BrN_3O_3$	BRETazenil	0.35	0.64	0.2		0.5	12.7
$C_{18}H_{19}ClN_2O$	C-383	1000		1000	1000	1000	1000
$C_{16}H_{14}ClN_3O_3$	CD-214	16.4	48.2	42.5		9.8	168

$C_{16}H_{11}N_3O$	CGS8216	0.13					46
$C_{17}H_{13}N_3O_2$	CGS9895	0.21					9.3
$C_{16}H_{10}ClN_3O$	CGS9896	0.28					181
$C_{13}H_9F_3N_4$	CL 218,872	57	1964	1161		561	10000
$C_{16}H_{15}IN_2O_2$	CM-A100	14.49	44.91	123.8	1000	65.31	1000
$C_{16}H_{16}N_2O_2$	CM-A49(R)	7.7	32.5	43		69	1000

$C_{16}H_{16}N_2O_2$	CM-A50(S)	17	59	88		144	1000
$C_{15}H_{13}FN_2O$	CM-A57	3.7	27	40		254	1000
$C_{15}H_{15}FN_2$	CM-A58	16	120	184		1000	1012.2Nm
$C_{16}H_{16}N_2O_2$	CM-A64	18	60	116		216	1000
$C_{16}H_{13}F_3N_2O_2$	CM-A69	1000	1000	1000		1000	1000
$C_{20}H_{16}N_2O_2$	CM-A71	333.57	1000	1000	1000	1000	3000

$C_{17}H_{18}N_2O$	CM-A77	11.51	51.9	105.16	1000	42.62	1000
$C_{22}H_{30}N_2O$	CM-A77a	27.4	111.5	301.3	1000	61.61	1000
$C_{20}H_{24}N_2O_2$	CM-A82a	2.78	8.93	24.51	1000	7.49	1000
$C_{16}H_{15}FN_2O_2$	CM-A87	1.62	4.54	14.73	1000	4.61	1000
$C_{19}H_{20}BrN_3O_3$	CM-A95(ss)	141.5	225	59.45	30.36	146.3	107.1
$C_{19}H_{20}BrN_3O_3$	CM-A97(sr)	70.81	89.48	30.64	40.73	67.01	38.12

$C_{14}H_{11}IN_2O_2$	CM-B01	4.8	31	34	1000	286	1000
$C_{19}H_{20}ClN_3O_3$	CM-B31c(ss)	118	319	173	37	15	137
$C_{19}H_{20}IN_3O_3$	CM-B31i(ss)	90	184	78	18	4.9	121
$C_{19}H_{17}BrF_3N_3O_3$	CM-B34	472	451	223	114	17	175
$C_{19}H_{20}FN_3O_3$	CM-B44(ss)	32	43	12	379	4.3	485
$C_{19}H_{17}ClF_3N_3O_3$	CM-B45	230	557	336	265	15	230

$C_{19}H_{17}F_4N_3O_3$	CM-B47	32	63	34	2007	4.4	717
$C_{19}H_{17}BrF_3N_3O_3$	CM-C28(SR)	176	752	244	290	14	141
$C_{15}H_{11}F_3N_2O_2$	CM-D30(R)	27	343.3	453	3000	1847	2000
$C_{15}H_{11}F_3N_2O_2$	CM-D30(S)	90	931	172	3000	1000	3000
$C_{17}H_{17}N_3O_4$	CM-D44	34.3	56.3	20.7	0.33	0.57	0.92
$C_{19}H_{21}N_3O_4$	CM-D45	90.5	65.5	30.3	0.15	1.65	0.23
$C_{18}H_{16}F_3N_3O_4$	CM-E09a	176	192	122	490	9.2	718

<chem>C19H21N3O4</chem>	CM-E09b	20	22	19	55	0.45	69
<chem>C23H21N3O4</chem>	CM-E10	23	26	14	215	0.51	96
<chem>C20H23N3O4</chem>	CM-E11	333	308	161	394	14	750
<chem>C16H13ClN2O</chem>	diazepam	14	20	15		11
<chem>C21H26N4O3</chem>	DM-139	5.8		169		9.25	325
<chem>C22H28N4O3</chem>	DM-146	6.44		148		4.23	247
<chem>C19H24N4O3</chem>	DM-173	13.1		38.1		0.78	118

$C_{18}H_{21}N_3O_4$	DM-215	6.74		7.42		0.293	8.28
$C_{17}H_{19}N_3O_4$	DM-239	1.5		0.53		0.14	6.89
$C_{17}H_{18}N_2O_4$	DMCM	5.69	8.29	4		1.04	134
$C_{49}H_{46}N_6O_4Si_2$	DMH-D-048 (C49H46N6O4Si2)	5000	5000	5000		5000	5000
$C_{43}H_{30}N_6O_4$	DMH-D-053 (C43H30N6O4)	236	7.4	272	5000	194.2	5000
$C_{39}H_{28}Br_2N_6O_4$	DMH-D-070 (C39H28N6O4Br2)	5000	1901			5000	5000
$C_{44}H_{32}N_6O_5$	DMH-III-96 (C44H32N6O5)	460	5000			5000	5000

$C_{20}H_{16}BrN_3O_2$	DM-I-070
$C_{26}H_{21}N_3O_2$	DM-I-81	2000	2000	2000	2000	176	2000
$C_{22}H_{14}F_3N_3O_2$	DM-II-20 (C22H14N3O2F3)	54.3	27.14	35.68		15.35	5000
$C_{20}H_{14}F_3N_3O_2$	DM-II-30 C20H13N3O2BrF3)	17.6	13.4	28.51		7.8	5000
$C_{20}H_{13}BrCl_3N_3O_2$	DM-II-33 (C20H13N3O2BrCl3)	88.6	85	11.6		26.2	5000
$C_{20}H_{13}Br_4N_3O_2$	DM-II-34

<p><chem>C25H22F3N3O2Si</chem></p>	DM-II-35 (C25H22N3O2SiF3)	11380	327			380	5000
<p><chem>C15H10BrClN2O</chem></p>	DM-II-72 (C15H10N2OBrCl)	5000	1.37			2.02	5000
<p><chem>C17H12BrClN4</chem></p>	DM-II-90 (C17H12N4BrCl)	0.505	1	0.63		0.37	5000
<p><chem>C18H12BrN3O2</chem></p>	DM-III-01 (C18H12N3O2Br)	5000	12			4.73	5000
<p><chem>C40H30Br2N6O5</chem></p>	DM-III-93 (C40H30N6O5Br2)	351	5000			179	5000
<p><chem>C50H48N6O5Si2</chem></p>	DM-III-94 (C50H48N6O5Si2)	240	5000	5000		5000	5000

$C_{45}H_{34}N_6O_4$	DM-III-97 (C45H34N6O4)	280	5000			5000	5000
$C_{21}H_{13}N_3$	EDC-I	165		797		382.3	10000
$C_{27}H_{24}N_2O_3$	EDC-I-042	183	20.5	300		270	10000
$C_{11}H_6N_4O_4$	EDC-I-071	12.9	83.1			314	5000
$C_{13}H_6N_4S_2$	EDC-I-093	13.6	423			2912	5000
$C_{24}H_{32}N_2O_4$	EDC-II-012	1000	3000	3000		3000	3000

EDC-II-015	3000	3000	3000		3000	3000
EDC-II-024	3647	29749	35060		12683	30000
EDC-II-031	1000	1000	1000		1000	1000
EDC-II-044	15.4		293		323	1000
EMJ-I-025	288	474	316		86	5000

EMJ-I-026	5000	135	1027		152	5000
FG8205	0.4	2.08	1.16		1.54	227
FLUNITRAZEPAM	2.2	2.5	4.5		2.1	2000
HDA-343	25	82.6	58.8		159	10000
HDA-90-III	25.6	105.4	163.6		160.6	10000
HDA-II-96	236	3000	3000		3000	10000

$C_{21}H_{15}FN_4O$	HJ-I-037	15.07	8.127	28.29		0.818
$C_{20}H_{15}N_5O$	HJ-I-040	1070	869.4	1123.6		1065
$C_{20}H_{16}ClN_3O_2$	Hz111 C20H16N3ClO2	46.65	34	77.6		24.6	842
$C_{20}H_{16}IN_3O_2$	Hz120 ANX2	5000	35	78.5		20.6	32.2
$C_{16}H_{14}BrN_3$	Hz135 C16H14N3Br	94.03	182			97	5000
$C_{21}H_{23}N_3Si$	Hz141 C21H23N3Si	6480	5000	5000		737	5000

<chem>C[Si](C)(C)C#Cc1ccc2c(c1)c(c3ccccc3n2)N(C)C=NO</chem> $C_{21}H_{23}N_3OSi$	Hz146 $C_{21}H_{23}N_3OSi$	5000	5000			5000	5000
<chem>CN1C=NC2=CC=CC=C2C(=C1C#C)C3=CC=CC=C3N3O</chem> $C_{18}H_{15}N_3O$	Hz147 $C_{18}H_{15}N_3O$	5000	5000			5000	5000
<chem>CN1C=NC2=CC=CC=C2C(=C1C#C)C3=CC=CC=C3N3O</chem> $C_{18}H_{15}N_3$	Hz148 $C_{18}H_{15}N_3$	10.98	5000			256	5000
<chem>N[C@@H]1C=NC2=CC=CC=C2C(=C1CBr)C3=CC=CC=C3N3O</chem> $C_{16}H_{14}BrN_3O$	Hz150 $C_{16}H_{14}N_3OBr$	485	469	5000		419	5000
<chem>C[Si](C)(C)C#Cc1ccc2c(c1)c(c3ccccc3n2)N(C)C=NO</chem> $C_{19}H_{19}N_3OSi$	Hz157 $C_{19}H_{19}N_3OSi$	5000	5000	5000		5000	5000
<chem>C[Si](C)(C)C#Cc1ccc2c(c1)c(c3ccccc3n2)N(C)C=NO</chem> $C_{20}H_{21}N_3OSi$	Hz158 $C_{20}H_{21}N_3OSi$	5000	5000	5000		5000	5000

<chem>C17H13N3O</chem>	Hz160 C17H13N3O	492	2633	334		87.3	5000
<chem>C19H15BrN4O2</chem>	Hz164 C19H15N4O2Br	99.18	150	459		108.2	494
<chem>C24H24N4O2Si</chem>	Hz165 C24H24N4O2Si	5000	5000			5000	5000
<chem>C21H16N4O2</chem>	Hz166 C21H16N4O2	118	148	365	5000	76.88	5000
<chem>C13H9BrN2OS</chem>	JC184 C13H9BrN2OS	9.606	10.5			6.709
<chem>C18H18N2OSSi</chem>	JC207 C18H18N2OSSi	104.8	169.7			68.16
<chem>C15H10N2OS</chem>	JC208 C15H10N2OS	22.42	18.89			5.039

$C_{19}H_{20}N_2OSSi$	JC209 C19H20N2OSSi	294.6	334.7			249.5
$C_{20}H_{15}N_3O_2S$	JC221 ANX1	106.2	49.4	182		7.75	362
$C_{16}H_{12}N_2OS$	JC222 C16H12N2OS	86.7	45.11			17.63
$C_{20}H_{15}N_3O_2S$	JIE-I-12	3576	454.8	768.3		4416
$C_{19}H_{20}BrN_3O_3$	JYI-01(C19H20N3O3Br)	59.2	159	96		10.6	2.88
$C_{21}H_{21}N_3O_3$	JYI-03 (C21H21N3O3)	185.4	107			0.954	3.34

C ₂₁ H ₂₃ N ₃ O ₃	JYI-04 (C ₂₁ H ₂₃ N ₃ O ₃)	28.3	16		0.51	1.57

C ₂₃ H ₂₃ N ₃ O ₄	JYI-06 (C ₂₃ H ₂₃ N ₃ O ₄)	16.5	5.48	5000	12.6	5000

C ₁₇ H ₁₃ BrF ₃ N ₃ O ₃	JYI-10 (C ₁₇ H ₁₃ N ₃ O ₃ F ₃ Br)	5000	368		12.3	23

C ₂₂ H ₂₂ F ₃ N ₃ O ₃ Si	JYI-11 (C ₂₂ H ₂₂ N ₃ O ₃ F ₃ Si)	5000	5000		648	3.97

C ₁₉ H ₁₆ F ₃ N ₃ O ₃	JYI-12 (C ₁₉ H ₁₆ N ₃ O ₃ F ₃)	91	39		4.5	6.8

C ₂₁ H ₁₆ F ₃ N ₃ O ₄	JYI-13 (C ₂₁ H ₁₆ N ₃ O ₄ F ₃)	5000	63.7		16	8.38

C ₁₇ H ₁₄ F ₃ N ₃ O ₃	JYI-14 (C ₁₇ H ₁₄ N ₃ O ₃ F ₃)	32	25		13	565

$C_{19}H_{14}F_3N_3O_3$	JYI-15 (C ₁₉ H ₁₄ N ₃ O ₃ F ₃)	205	812			4.8	22
$C_{23}H_{23}N_3O_3S$	JYI-19 (C ₂₃ H ₂₃ N ₃ O ₃ S)	2.2	205			34	12.7
$C_{25}H_{25}N_3O_3$	JYI-20 (C ₂₅ H ₂₅ N ₃ O ₃)	20.96	611			54	40.46
$C_{23}H_{18}F_3N_3O_3$	JYI-21 (C ₂₃ H ₁₇ N ₃ O ₃ F ₃)	113.7	5000			209	60.96
$C_{20}H_{15}BrFN_3O_2$	JYI-32 (C ₂₀ H ₁₅ N ₃ O ₂ BrF)	3.07	4.96			2.92	52.24
$C_{25}H_{24}FN_3O_2Si$	JYI-38 (C ₂₅ H ₂₄ N ₃ O ₂ FSi)	5000	314			55.4	5000

<chem>C15H10BrFN2O</chem>	JYI-42	0.26	0.15	0.28		0.26
<chem>C18H11BrFN2O2</chem>	JYI-47	2.8	2.3	0.5		0.4
<chem>C24H18FN3O3</chem>	JYI-48	75.59	90.68	12.78		31.28
<chem>C20H12BrF4N3O2</chem>	JYI-49 (C20H12N3O2F4Br)	1.87	2.38			6.7	3390
<chem>C24H18FN3O2S</chem>	JYI-50 (C24H18N3O2SF)	106.4	200			37.6	5040

$C_{25}H_{21}F_4N_3O_2Si$	JYI-53 (C ₂₃ H ₂₁ N ₃ O ₂ F ₄ Si)	77.4	40.4	183		133	3650
$C_{24}H_{15}F_4N_3O_3$	JYI-54 (C ₂₄ H ₁₅ N ₃ O ₃ F ₄)	2.89	172	6.7		57	1890
$C_{20}H_{19}FN_2OSi$	JYI-55	41.39		0.504		24.75
$C_{24}H_{18}FN_3O_3$	JYI-56 (C ₂₄ H ₁₈ N ₃ O ₄ F)	95.2	346	131		34.6	4140
$C_{17}H_{12}BrFN_4$	JYI-57	0.08	0.08	0.13		0.04
$C_{22}H_{13}F_4N_3O_2$	JYI-59 (C ₂₂ H ₁₃ N ₃ O ₂ F ₄)	1.08	2.6	11.82		11.5	5000

<chem>C17H11FN2O</chem>	JYI-60 (C17H11N2OF)	3.7	1.6	4.3		1.7	5000
<chem>C17H12BrFN4</chem>	JYI-64 (C17H12N4FBr)	0.3	1.11	0.62		0.9	5000
<chem>C19H13FN4</chem>	JYI-70 (C19H13N4F)	6.3	2.1			0.56	5000
<chem>C22H21FN4Si</chem>	JYI-72 (C22H21N4SiF)	48.5	18.5			11.5	5000
<chem>C22H16FN3O2</chem>	JY-XHE-053	22	12.3	34.9		0.7
<chem>C22H19N3O2</chem>	LD-9	156				3000	10000

C ₁₇ H ₁₆ N ₂ O	LJD-I-0036A	245	818	869		10000	10000

C ₁₆ H ₉ ClN ₄	LJD-II-87	3000	3000	3000		10000	10000

C ₁₆ H ₁₀ N ₄	LJD-III-15E	1.93	14	19		70.8	1000

C ₁₆ H ₁₀ N ₄	LJD-III-22A	105	227.5	284		3000	10000

C ₁₄ H ₁₄ N ₂ O	MA-3-PROPOXYL	5.3	52.3	68.8		591	1000

C ₁₃ H ₁₄ F ₃ N ₃ O ₂	MH-1 (Parkistan)	3000	3000	3000	3000	3000	3000

C ₁₃ H ₁₃ F ₃ N ₂ OS	MH-2 (Parkistan)	3000	3000	3000	3000	3000	3000

$C_{19}H_{15}N_3$	MJP-II-34	240		391		1360	10000
$C_{17}H_{11}N_3$	MLT-I-70	1.1	1.2	1.1		40.3	1000
$C_{17}H_{10}FN_3$	MLT-II-16	5.05	10.41	18.4		260	10000
$C_{17}H_{10}ClN_3$	MLT-II-18	3.9	12.2	24.4		210	10000
$C_{18}H_{13}N_3O$	MLT-II-18	3.4	11.7	11		225	10000
$C_{17}H_{10}BrN_3$	MLT-II-29

<chem>C17H11N3O</chem>	MLT-II-34	7.04	15.95	22.3		158	1000
<chem>C24H17N3O</chem>	MLT-II-87	432	3000	3000		10000	10000
<chem>C18H15BrF3N3O3</chem>	MMB-II-37	872	2602	1003	95	98	115
<chem>C18H15BrF3N3O3</chem>	MMB-II-43	1353	5173	1535	98	132	78
<chem>C15H11F3N2O2</chem>	MMB-II-57	90	931	172	3000	1000	3000
<chem>C15H11F3N2O2</chem>	MMB-II-68A	27	343.3	453	3000	1847	3000

<chem>C14H9F3N2O2</chem>	MMB-II-74	3	24.5	41.7	500	125.7	1000
<chem>C18H17BrF3N3O3</chem>	MMB-II-87	200	333	107	109	5.4	333
<chem>C18H12BrF6N3O3</chem>	MMB-II-89	333	333	333	333	43	333
<chem>C18H18BrN3O3</chem>	MMB-II-90	20	24	5.7	9	0.25	36
<chem>C19H21N3O4</chem>	MMB-II-99	>333	333	333	333	333	333
<chem>C18H19N3O3</chem>	MMB-III-016	3	1.97	2	1074	0.26	211
<chem>C18H13F6N3O3</chem>	MMB-III-018	117	140	78	3500	14	976

$C_{18}H_{20}N_2O_4$	MSR-I-032	6.2	18.7	4		3.3	74.9
$C_{17}H_{20}N_4O_3$	MSR-I-036	46	422	400		107.5	300
$C_{16}H_{19}N_3O_2$	MSR-I-046	45	540	700		380	3000
$C_{16}H_{18}N_4O_3$	MSR-I-049	896	1000	570		91.5	3000
$C_{19}H_{24}N_2O_3$	MSR-I-056	24.6	214.4	270.4		331.9	1000
$C_{17}H_{20}N_2O_3$	MSR-I-057	>300	300	300		300	300

<p>C₁₆H₁₈N₂O₃</p>	MSR-I-100	300	300	300		300	300
<p>C₁₇H₁₇ClN₂O</p>	N-111	691		4209	3000	3439	3000
<p>C₁₇H₁₈ClN₃O</p>	N-112	296		5260	3000	3000	3000
<p>C₁₈H₁₉ClN₂O₂</p>	N-120	422		1000	3000	932	3000
<p>C₁₇H₁₇ClN₂O</p>	N-26	1000		3000	3000	3000	3000
<p>C₁₉H₂₁ClN₂O</p>	N-30	1491		2000	2000	2000	2000
<p>C₁₉H₂₁ClN₂O₂</p>	N-80	1614.4		2000	2000	2000	2000

<chem>C18H13FN2O</chem>	OMB-18	3.9	1.2	3.4	1733	0.8	5
<chem>C19H15FN2O</chem>	OMB-19	22	4.6	20	3333	3.5	40
<chem>C23H17N5O</chem>	PS-1-26 C23H17N5O	933.5	366	260		393.5	5000
<chem>C21H16BrN5O</chem>	PS-1-27 C21H16N5BrO	182	294			86	5000
<chem>C26H25N5OSi</chem>	PS-1-28 C26H25N5OSi	5000	5000			5000	5000
<chem>C20H17BrN4O</chem>	PS-1-34B		4.198	3.928

$C_{23}H_{22}BrN_5O$	PS-1-35		16.03	24.41
$C_{28}H_{31}N_5OSi$	PS-1-36			939.1
$C_{25}H_{23}N_5O$	PS-1-37	193	35	435		22	5000
$C_{13}H_9BrN_2OS$	PS-I-68	3134	481.3	679.5		101.3
$C_{20}H_{20}N_4SSi$	PS-I-71	145.8	122.8	214.2		119.2

C ₁₇ H ₁₂ N ₄ S	PS-I-72	123	31	386		34	5000

C ₁₆ H ₁₅ N ₃ O ₂	PWIII-012	1000	1000	1000		166	1000

C ₁₆ H ₁₀ ClN ₃ O	PWZ-0071	0.23	0.17	0.12		0.44	17.31

C ₁₇ H ₁₃ N ₃ O ₂	PWZ-007A	0.11	0.1	0.09		0.2	10

C ₁₇ H ₁₃ N ₃ O ₂	PWZ-009A1	1.34	1.31	1.26		0.84	2.03

C ₁₄ H ₁₄ ClN ₃ O ₂	PWZ-029	300	300	300		38.8	300

$C_{13}H_{11}Cl_2N_3O$	PWZ-031A	300	300	300		28.5	300

$C_{13}H_{12}ClN_3O_2$	PWZ-034A	300	300	300		300	300

$C_{15}H_{16}ClN_3O_2$	PWZ-035A	300	300	300		82.7	300

$C_{17}H_{18}ClN_3O_2$	PWZ-049	1581	2865	2739		166	2930

$C_{14}H_{12}ClN_3O_2$	PWZ-051	17535	33834	22125		2612	29500

$C_{17}H_{21}ClN_4O$	PWZ-057	9483	30000	15409		2583	30160

C ₁₉ H ₂₅ ClN ₄ O	PWZ-058	4201	12590	6266		1346	8600

C ₁₄ H ₁₄ ClN ₃ O	PWZ-066	408	1527	1125		182	3648

C ₁₇ H ₂₀ ClN ₃ O	PWZ-068	2050	2900	2907		369	960

C ₁₆ H ₁₇ N ₃ O ₄	PWZ-085	4.86	13	8.5		0.55	40

C ₁₇ H ₁₉ N ₃ O ₄	PWZ-096	11.1	36	16.9		1.07	51.5

C ₁₇ H ₁₂ ClN ₃ O ₂	PZII-028	0.2		0.2		0.32	1.9

<chem>C18H15N3O3</chem>	PZII-029	0.34		0.79		0.52	10
<chem>C15H10N8O</chem>	QH-133	2164		4620		1635	1000
<chem>C16H12N4O5</chem>	QH-146	0.49		0.76		7.7	10000
<chem>C16H12N8O</chem>	QH-149
<chem>C13H9FN2OS</chem>	QH-27	175	335	405		150	300
<chem>C14H11FN2OS</chem>	QH-28	141	215	205		65.5	300
<chem>C15H10N4O5</chem>	QH-65

C ₁₅ H ₁₂ N ₂ O	QH-II-058b	288	233	350		140	3000

C ₁₆ H ₁₄ N ₂ O	QH-II-059	81	138	318		98	3000

C ₁₆ H ₁₃ BrN ₂ O	QH-II-063	9.4	9.3	31		7.7	3000

C ₂₁ H ₂₂ N ₂ OSi	QH-II-065	94	73	203		63	3000

C ₁₈ H ₁₄ N ₂ O	QH-II-066	76.3	42.1	47.4	2000	6.8	3000

C ₁₅ H ₁₀ BrN ₃ O ₃	QH-II-067a	16	31	52		199	3000

<chem>Cc1c2c(c3ccccc3n2C(=O)N1)C#N</chem> $C_{17}H_{13}N_3O$	QH-II-072	320	310	350		265	3000
<chem>Brc1ccc(cc1)N2C(=O)N=C3C=CC=C4C3=NC(=O)N4C2</chem> $C_{16}H_{10}BrN_3O$	QH-II-075	0.18	0.21	0.25		1.3	40
<chem>Brc1ccc(cc1)N2C(=O)N=C3C=CC=C4C3=NC(=O)N4C2c5ccccc5</chem> $C_{16}H_{10}BrN_3O$	QH-II-077	0.06	0.08	0.05		0.12	4
<chem>C[Si](C)(C)C#Cc1ccc(cc1)N2C(=O)N=C3C=CC=C4C3=NC(=O)N4C2</chem> $C_{21}H_{19}N_3OSi$	QH-II-080b	3	3.7	4.7		24	1000
<chem>C[Si](C)(C)C#Cc1ccc2c(c1)nc3c2c(c4ccccc4n3C(=O)N2)C#N</chem> $C_{21}H_{19}N_3OSi$	QH-II-082	1.7	1.8	1.6		6.1	100
<chem>c1ccc(cc1)N2C(=O)N=C3C=CC=C4C3=NC(=O)N4C2C#Cc5ccccc5</chem> $C_{18}H_{11}N_3O$	QH-II-085	0.08	0.06	0.02		0.08

$C_{18}H_{11}N_3O$	QH-II-090(CGS-8216)	0.05	0.08	0.12		0.25	17
$C_{18}H_{11}N_3O$	QH-II-092	0.07	0.03	0.04		0.17
$C_{23}H_{22}N_2OSi$	QH-IV-014	3000	3000	3000		3000	3000
$C_{20}H_{14}N_2O$	QH-IV-016	3000	3000	3000		3000	3000
$C_{22}H_{24}N_2OSi$	QH-IV-019	3000	3000	3000		3000	3000
$C_{19}H_{16}N_2O$	QH-IV-021	3000	3000	3000		3000	3000

C ₁₅ H ₁₄ FN ₃ O ₃	Ro15-1788	0.8	0.9	1.05		0.6	148

C ₁₅ H ₁₄ N ₆ O ₃	Ro15-4513	3.3	2.6	2.5		0.26	3.8

C ₁₈ H ₁₇ N ₃ O ₃	RY-008	3.75	7.2	4.14		1.11	44.3

C ₂₃ H ₂₉ N ₃ O ₃ Si	RY-020	275	387	337		23	301

C ₂₂ H ₂₇ N ₃ O ₃ Si	RY-023	C22H27N3O3Si	197	142.6	255	7.8	2.61	58.6

C ₁₉ H ₁₉ N ₃ O ₃	RY-024	C19H19N3O3	26.9	26.3	18.7		0.4	5.1

<chem>CCOC(=O)c1nc2c(c1)nc(=O)n2CCc3ccc(C)cc3</chem> $C_{17}H_{19}N_3O_3$	RY-031 (RY-10)	20.4	27	26.1		1.5	176
<chem>CCOC(=O)c1nc2c(c1)nc(=O)n2CCc3ccc(C)cc3</chem> $C_{18}H_{21}N_3O_3$	RY-033	14.8	56	25.3		1.72	22.9
<chem>c1ccc2c(c1)nc(=O)n2Cc3ccsc3</chem> $C_{17}H_{12}N_2OS$	RY-035	980	590	775		92.5	3000
<chem>CN1C(=O)N=C2C(=C1)c3ccccc3C2Cc4ccsc4</chem> $C_{18}H_{14}N_2OS$	RY-037	300	300	300		300	300
<chem>CCOC(=O)c1nc2c(c1)nc(=O)n2C3CCCC3c4ccc(C#CC5(C)C(C)C(C)C5)cc4</chem> $C_{22}H_{25}N_3O_3Si$	RY-047	200	124	79		4	340
<chem>c1ccc2c(c1)nc3c(c2)nc(=O)n3C4CCCC4c5ccc(C#C)cc5</chem> $C_{24}H_{17}N_5O_2$	RY-049
<chem>CCOC(=O)c1nc2c(c1)nc(=O)n2C3CCCC3c4ccc(Br)cc4</chem> $C_{17}H_{16}BrN_3O_3$	RY-053	49	29	15		1	46

$C_{19}H_{17}N_5O_3$	RY-054	59	44	27		1.3	126
$C_{21}H_{19}N_5O_2$	RY-057	73	85	97		4.8	333
$C_{20}H_{17}N_5O_2$	RY-058	86	40	85		2.4	150
$C_{19}H_{15}N_5O_2$	RY-059	89	70	91		3.7	301
$C_{17}H_{12}N_2O_2$	RY-060	1000	1000	1000		1000	1000
$C_{16}H_{14}BrN_5O_3$	RY-061	17	13	6.7		0.3	31

$C_{19}H_{15}BrN_4O_4$	RY-062	1000	1000	500		172	2000
$C_{18}H_{14}N_2O_2$	RY-062	300	300	300		300	300
$C_{21}H_{23}N_3O_3Si$	RY-066	83	60	48		2.6	180
$C_{18}H_{15}N_3O_3$	RY-067	21	12	10		0.37	42
$C_{24}H_{24}N_4O_4Si$	RY-068	500	877	496		37	1000

$C_{21}H_{16}N_4O_4$	RY-069	692	622	506		19	1000
$C_{18}H_{13}N_5O_2$	RY-071	19	56	91		7.2	266
$C_{19}H_{15}N_5O_2$	RY-072	220	150	184		12.7	361
$C_{20}H_{17}N_5O_2$	RY-073	156	88	122		8.5	267
$C_{23}H_{15}N_5O_2$	RY-075
$C_{15}H_{14}BrN_3O_3$	RY-076	26	27	13		0.7	22

C ₂₀ H ₂₃ N ₃ O ₃ Si	RY-079	121.1	141.9	198.4	159	5	113.7
C ₁₇ H ₁₅ N ₃ O ₃	RY-080	C ₁₇ H ₁₅ N ₃ O ₃	28.4	21.4	25.8	5.3	0.49	28.8
C ₂₁ H ₂₅ N ₃ O ₃ Si	RY-097		300	300	300		300	300
C ₁₈ H ₁₇ N ₃ O ₃	RY-098		10.1	22.2	16.5		1.68	100
C ₁₇ H ₁₆ BrN ₃ O ₃	RY-I-26		1000	1000	1000		1000	1000
C ₂₂ H ₂₅ N ₃ O ₃ Si	RY-I-27		1000	1000	1000		1000	1000

RY-I-28	283	318	102		7.2	61
RY-I-29	1000	1000	1000		157	1000
RY-I-31	10	45	19		6	1000
SA-01-031	2013	1887			609.3
SA-SH-053-2'N-R-CH3	406	73565	3392		16843
SH-053-2'F (JY-XHe-053)	21.99	12.34	34.9		0.671
SH-053-2'F-R-CH3	759.1	948.2	768.8		95.17

$C_{23}H_{18}FN_3O_2$	SH-053-2'F-S-CH3	468.2	33.27	291.5		19.2
$C_{21}H_{16}N_4O_2$	SH-053-2'N	300	160	527		82	5000
$C_{22}H_{18}N_4O_2$	SH-053-2'N-S-CH3	6951	2331	2655		744.1
$C_{23}H_{19}N_3O_2$	SH-053-R-CH3	2026	2377	1183		949.1
$C_{23}H_{19}N_3O_2$	SH-053-S-CH3	1666	1263	1249		206.4
$C_{23}H_{19}N_3O_2$	SH-053-S-CH3	5000	551	540.1		201	5000

<chem>C46H34F2N6O4</chem>	SH-222A	2058	292.3			51.24
<chem>C46H32F2N6O4</chem>	SH-223A	1231	2008	3609		261.3
<chem>C36H28FN3O4</chem>	SH-223B	4117	4785	10661		911
<chem>C16H14N2O</chem>	SH-I-029B	489	576.5	706.1		208.8
<chem>C14H10BrN3O</chem>	SH-I-02B	29.82	1315	18		74.05
<chem>C16H13BrN2O</chem>	SH-I-030	14.42	11.04	19.09		1.89

$C_{19}H_{15}BrN_4O_2$	SH-I-031A	84.05	141.3	150.5		151.2
$C_{24}H_{24}N_4O_2Si$	SH-I-031B	981.9	1004	2046		356.2
$C_{21}H_{18}BrN_3O_2$	SH-I-034(S)	793.4	746.8	544.6		1564
$C_{16}H_{14}N_2O$	SH-I-035A	7389	6049	16811		15793
$C_{16}H_{13}BrN_2O$	SH-I-035B	7439	11084	3021		4043
$C_{21}H_{18}BrN_3O_2$	SH-I-036	587	587.9	280.2		150.8

SH-I-038
SH-I-04	7.3	6.1	5.1		7.7
SH-I-040 R	774.4	334.1	584		355.9
SH-I-041
SH-I-044	5633	2034	6658		3521
SH-I-047	1710	17.52	1222		1519

$C_{16}H_{12}BrFN_2O$	SH-I-048A	0.77	0.17	0.38		0.11
$C_{21}H_{17}BrFN_2O_2$	SH-I-048B
$C_{15}H_{12}BrN_3O$	SH-I-049R
$C_{15}H_{12}BrN_3O$	SH-I-049S	155.6	137.2	88.5		11094
$C_{26}H_{26}FN_3O_2Si$	SH-I-055	5109	1638	1394		223.4
$C_{21}H_{21}N_5Si$	SH-I-06

$C_{26}H_{26}FN_3O_2Si$	SH-I-064	4249	3970	3412	1215
$C_{21}H_{15}N_3O_2$	SH-I-067
$C_{20}H_{12}BrFN_4O$	SH-I-085	11.08	4.866	13.75	0.24
$C_{22}H_{17}N_3O_2$	SH-I-75	1487	989.9	773	0.1825
$C_{18}H_{15}BrN_4$	SH-I-89S	12.78	8.562	8.145	3.23

<p>C₂₃H₁₆F₃N₃O₂</p>	SH-I-R66	219.5	169.1	87.01	47.97
<p>C₂₃H₁₆F₃N₃O₂</p>	SH-I-S66	22.93	30.36	55.26	0.69
<p>C₁₅H₁₂BrN₃O</p>	SH-O53-2'N-S-CH3
<p>C₂₃H₁₈FN₃O₂</p>	SH-O53-S-CH3-2'F	350	141	1237	16	5000
<p>C₁₈H₁₃N₅</p>	SH-TRI-108 ANX4	143.9	81.29	103.9	45.64
<p>C₁₄H₁₄ClN₃O₂</p>	SH-TSC-1(PWZ-029)	362.4	180.3	328.2	6.185

$C_{16}H_{16}N_2O_2$	SH-TSC-2(BCCT)	0.03	0.0419	0.035		69.32
$C_{33}H_{26}N_6O_6$	SH-TSC-3(XLI-093)	1295	744.9	1814		33.99
$C_{21}H_{17}BrFN_3O_2$	SH-TSC-5(SH-I-047)	356.7	169.4	285		75.31
$C_{21}H_{18}BrN_3O_2$	SH-TSC-6(SH-I-034)	913.2	283	300		436.8
$C_{21}H_{18}BrN_3O_2$	SH-TSC-7(SH-I-040)	709	1997	1256		727.4
$C_{20}H_{16}N_4$	SH-TS-CH3	107.2	50.09	20.95		8.068

$C_{18}H_{14}BrN_3O$	SHU-1-07	108	260	175	940	155	2535
$C_{18}H_{14}ClN_3O$	SHU-1-15	81	63	86	2175	174	2167
$C_{18}H_{15}N_3O$	SHU-1-19	4	12	7	48	14	84
$C_{20}H_{20}N_2OSi$	SHU-221 (XLI-250)	492	439	339	3000	118	3000
$C_{17}H_{12}N_2O$	SHU-221-1	66	41	43	3000	9	3000
$C_{20}H_{22}N_2Si$	SHU-223	1000	1000	1000	1000	1000	1000

SHU-224	1000	1000	1000	1000	1000	1000
SHU-525	667	667	667	667	667	429
SHU-528	667	667	667	667	667	429
SHU-530	667	667	667	667	667	429
SHU-537	333	333	333	333	667	429
SHU-547	667	667	667	667	667	429
SHU-550	333	333	333	333	333	429

C ₁₀ H ₆ O ₂	SHU-III-032	1000	1000	1000	1000	1000	1000

C ₂₀ H ₁₈ FN ₃ O	SHU-III-24	1000	1000	1000	1000	1000	1000

C ₁₂ H ₁₀ O ₂	SHU-III-32	1000	1000	1000	1000	1000	1000

C ₁₄ H ₁₂ O ₃	SHU-III-33	1000	1000	1000	1000	1000	1000

C ₁₄ H ₁₀ O ₂	SHU-III-34	1000	1000	1000	1000	1000	1000

C ₁₄ H ₁₄ O ₂	SHU-III-36	1000	1000	1000	1000	1000	1000

C ₁₅ H ₁₂ O ₂	SHU-III-37	1000	1000	1000	1000	1000	1000

<chem>C12H8N2</chem>	SHU-III-40	1000	1000	1000	1000	1000	1000

<chem>C10H8O3</chem>	SHU-III-42	1000	1000	1000	1000	1000	1000

<chem>C10H10O3</chem>	SHU-III-42A	1000	1000	1000	1000	1000	1000

<chem>C8H8O2</chem>	SHU-III-42B	1000	1000	1000	1000	1000	1000

<chem>C11H10O2</chem>	SHU-III-44	1000	1000	1000	1000	1000	1000

<chem>C10H12O2</chem>	SHU-III-45	1000	1000	1000	1000	1000	1000

$C_{12}H_{12}O_2$	SHU-III-66	1000	1000	1000	1000	1000	1000

$C_{13}H_{13}NO_2$	SHU-III-87	1000	1000	1000	1000	1000	1000

$C_{11}H_9NO_2$	SHU-III-88	1000	1000	1000	1000	1000	1000

$C_{13}H_{11}NO_2$	SHU-III-90	1000	1000	1000	1000	1000	1000

$C_{17}H_{15}NO_2$	SHU-III-93	1000	1000	1000	1000	1000	1000

$C_{17}H_{13}NO_2$	SHU-III-94	1000	1000	1000	1000	1000	1000

SLL-II-25	244	500	532		1560	10000
SPH-121	0.14	1.19	1.72		4	479
SPH-165	0.63	2.79	4.85		10.4	1150
SPH-166	20.8	78.3	58.7		67.3	10000
SPH-178	1000	1000	1000		1000	1000
SPH-195	7.2	168.5	283.5		271	10000

$C_{16}H_{16}N_2O_4$	SPH-38	2	5.4	10.8		18.5	3000
$C_{19}H_{12}N_4O_2$	SR-II-54	174.1	363.2	946.4		966.6
$C_{18}H_{19}N_3O_3$	SVO-8-14	8	25	8	6.9	0.9	14
$C_{20}H_{23}N_3O_4$	SVO-8-20	11	40	28	19	8.6	138
$C_{19}H_{17}N_3O_4$	SVO-8-30	1.1	5.3	5.3	2.8	0.6	15
$C_{21}H_{19}N_3O_3$	SVO-8-67	7	41	26	15	2.3	191

<chem>C16H13ClN2O3</chem>	TC-T-JG-24	746.5	8081	1543		2364
<chem>C18H19N3O3</chem>	TC-YT-II-76	101.1	1.897	5.816		11.99
<chem>C34H36N6O4</chem>	TC-YT-III-19
<chem>C30H26Cl2N6O6</chem>	TC-YT-TC-2	2657	737.8	643		1973
<chem>C17H17N3O3</chem>	TG-4-29	8.3	10.2	6.9		0.4	7.61
	TG-4-29	2.8	3.9	2.7	2.1	0.18	3.9

<chem>C19H17N3O3S</chem>	TG-4-39	1.6	34	24	5.6	1.4	23
<chem>C16H16N2O3</chem>	TG-I-97
<chem>C17H18N2O3</chem>	TG-II-82	1.6	2.9	2.8		1	1000
<chem>C13H9N5O2</chem>	TJH-6AZ
<chem>C20H19N3O2</chem>	TJH-IV-43	5.42	30.19	48.9		475	10000

C ₂₄ H ₂₁ N ₃ O ₂	TJH-IV-50	30.7	205	271	814	10000

C ₁₉ H ₁₃ N ₃ O ₂	TJH-IV-51	2.39	17.4	14.5	316	10000

C ₁₄ H ₉ N ₃ O ₂ S	TJH-V-88	3.41		30	140.9	10000

C ₁₇ H ₁₂ Cl ₂ N ₄	triazolam

C ₂₅ H ₂₁ N ₃ O	TSC-I-93	5000	5000	5000	304	5000

C ₂₆ H ₂₃ N ₃ O	TSC-I-94

TSC-I-95						
$C_{24}H_{18}ClN_3$	WY-A-99-2(WYS8)	0.972	111	102	2000	208	1980
$C_{18}H_{16}N_2O_2$	WY-B-08	78	301	131	3000	681	3000
WY-B-09-1	3.99	8	32	1000	461	2000
WY-B-09-2	1000	2000	2000	3000	1000	3000
WY-B-14(WYS7)	6.84	30	36	2000	108	1000
$C_{21}H_{24}N_2O_2Si$

<chem>C[Si](C)(C)C#Cc1ccc2c(c1)c(c[nH]2)c3cc(C(=O)OC(C)(C)C)cn3</chem> $C_{21}H_{24}N_2O_2Si$	WY-B-14(WYS7)	8.5	165	245		1786	5000
<chem>CCOC(=O)c1cc2c(c1)c(c[nH]2)c3ccc([N+](=O)[O-])cc3</chem> $C_{14}H_{11}N_3O_4$	WY-B-15	0.92	0.83	0.58	2080	4.42	646
<chem>CC(C)(Cl)COC(=O)c1cc2c(c1)c(c[nH]2)c3ccccc3</chem> $C_{15}H_{11}Cl_3N_2O_2$	WY-B-17	2000	2000	2000	3000	2000	5000
<chem>FC(F)(F)COC(=O)c1cc2c(c1)c(c[nH]2)c3ccc(I)cc3</chem> $C_{14}H_8F_3IN_2O_2$	WY-B-20	12	39	47	2000	122	3000
<chem>ClC(Cl)(Cl)COC(=O)c1cc2c(c1)c(c[nH]2)c3ccccc3</chem> $C_{14}H_9Cl_3N_2O_2$	WY-B-23-1	10	33	43	1000	189	2000
<chem>COC(F)(F)COC(=O)c1cc2c(c1)c(c[nH]2)c3ccccc3</chem> $C_{15}H_{11}F_3N_2O_3$	WY-B-23-2(WYS11)	4.2	37.7	39	2000	176	69.4

$C_{18}H_9F_3N_2O_2$	WY-B-24	22.02	177	44.78	3000	422	3000
$C_{19}H_{17}F_3N_2O_2Si$	WY-B-25	7.6	40	66	2000	263	2000
$C_{18}H_9F_3N_2O_2$	WY-B-26-2	4.45	44.57	42.66	2000	124	2000
$C_{18}H_{11}F_3N_2O_3$	WY-B-27-1	26	143	117	3000	127	2000
$C_{18}H_{11}F_3N_2O_2S$	WY-B-27-2	9.19	111	72	2000	449	2000
$C_{20}H_{13}F_3N_2O_2$	WY-B-29-2	25	137	125	2000	299	2000

<chem>CC(C)[Si](C)(C)C#Cc1ccc2c(c1)c(c[nH]2)C#CC(=O)OCC</chem> $C_{19}H_{20}N_2O_2Si$	WY-B-99-1	4.4	4.5	5.58	2000	47	2000
<chem>CC(C)OC(=O)c1cnc2c(c1)c(c[nH]2)C#Cc3c4c(c[nH]4)cnc3C(=O)OC(C)(C)C</chem> $C_{44}H_{46}N_4O_8$	WYS1 C45H50N4O6	missing
<chem>CC(F)(F)FOC(=O)c1ccc2c(c1)c(c[nH]2)c3ccccc3</chem> $C_{14}H_9F_3N_2O_2$	WYS10 C14H9F3N2O2	0.88	36	25.6		548.7	15.3
<chem>CC(C)OC(=O)c1cnc2c(c1)c(c[nH]2)c3ccc4c(c3)sc(C=C4)</chem> $C_{20}H_{18}N_2O_2S$	WYS12 C20H18N2O2S	37	166	314		2861	5000
<chem>CC(C)(C)OC(=O)c1cnc2c(c1)c(c[nH]2)c3ccc4c(c3)oc(C=C4)</chem> $C_{20}H_{18}N_2O_3$	WYS13 C20H18N2O3	2.442	13	27.5		163	5000
<chem>CC(C)(F)(F)FOC(=O)c1cnc2c(c1)c(c[nH]2)C#Cc3c4c(c[nH]4)cnc3C(=O)OC(C)(C)C</chem> $C_{33}H_{25}F_3N_4O_4$	WYS14 C34H29F3N4O4	798	5000			5000	5000

<chem>C22H20N2O2</chem>	WYS15 C22H20N2O2	3.63	2.02	44.3		76.5	5000
<chem>C15H14N2O2</chem>	WYS16 C18H22N2O2	5000	5000			5000	5000
<chem>C17H12N2O2</chem>	WYS17 C17H18N2O2	missing
<chem>C20H18N2O2</chem>	WYS18 C21H22N2O2	missing
<chem>C26H32N2O4Si</chem>	WYS19 C26H32N2O4Si
<chem>C34H30N4O4</chem>	WYS2 C35H34N4O4
<chem>C34H30N4O4</chem>	WYS2 C35H34N4O4	30	124	100	300	300	4000

WYS20 C ₂₃ H ₂₄ N ₂ O ₄	450	5000			5000	5000
WYS21 C ₂₁ H ₂₃ IN ₂ O ₄	missing
WYS3 C ₃₅ H ₃₈ N ₄ O ₄	241	5000	1841		5000	5000
WYS4 C ₄₈ H ₅₆ N ₄ O ₈	missing
WYS5 C ₃₇ H ₄₂ N ₄ O ₄	5000	5000			5000	5000
WYS6 C ₃₇ H ₃₅ N ₄ O ₄	120	1059	3942		5000	5000

C ₁₆ H ₁₅ IN ₂ O ₂	WYS9 C16H15IN2O2	2.72	22.2	23.1		562	122

C ₁₆ H ₁₆ N ₂ O ₂	WYSC1 (BCCt)	1.094	5.44	12.3		69.8	21.2

C ₁₅ H ₁₁ F ₃ N ₂ O ₂	WYSC2 C15H11F3N2O2	14.14	113	170		518	61.2

C ₁₈ H ₁₆ N ₂ O ₂	WY-TSC-4(WYS8)	109.431	2374.5	1656.4		2663.5	
	WY-TSC-4(WYS8)	0.007	0.99	1.63		51.04

C ₁₉ H ₁₄ N ₂ O	WZ-030	279	210	200		45.15	1000

<chem>C20H18N2O</chem>	WZ-032	965	345	590		150	3000
<chem>C19H13FN2O</chem>	WZ-069	40	30.5	38.5		12.6	1000
<chem>C20H13FN2O</chem>	WZ-070	72.7	30.7	53.2		18.6	300
<chem>C14H13ClN2OS</chem>	WZ-113	19.2	13.2	13.4		11.5	300
<chem>C14H13ClN2O2</chem>	WZ-148	300	300	300		300	300
<chem>C19H13FN2O</chem>	WZ-153	199	151.3	191.6		73.5	1000
<chem>C20H15FN2O</chem>	WZ-154	>300	>300	>300		>300	>300

C ₁₉ H ₁₃ FN ₂ O	WZ-175	1000	1000	1000		1000	1000

C ₂₀ H ₁₅ FN ₂ O	WZ-176	>300	>300	>300		>300	>300

C ₁₃ H ₁₅ FN ₂ O	WZ-185	>300	>300	>300		>300	>300

C ₁₄ H ₁₃ ClN ₂ OS	WZ-198	>300	>300	>300		>300	>300

C ₁₅ H ₁₁ N ₃ O ₂	WZ-201

C ₂₄ H ₂₅ N ₃ O ₃ Si	XHE-I-027	1000	1000	1000		180

$C_{21}H_{17}N_3O_3$	XHE-I-030	1000	1000	1000	1000	53	1000
$C_{23}H_{19}N_3OSi$	XHE-I-038	7.3	5	34		132	1000
$C_{20}H_{11}N_3O$	XHE-I-043	31	95	272		81	1000
$C_{15}H_{14}BrN_3O_3$	XHE-I-046	1000	1000	1000		1000	1000
$C_{21}H_{19}N_3O_3$	XHE-I-048	220	440	481		50	160
$C_{22}H_{23}N_3O_3Si$	XHE-I-050	254	300	300		195	3000

$C_{19}H_{15}N_3O_3$	XHE-I-051	35	39	42		5.3	979
$C_{17}H_{11}Br_2N_3O_2$	XHE-I-055b	615	1000	1000	2213	268	1524
$C_{24}H_{19}N_3O$	XHE-I-065	7.2	17	18	500	57	500
$C_{21}H_{21}N_3O_3$	XHE-I-066A(SS)	196.66	176.14	95.4	74.26	125.06	186.25
$C_{21}H_{21}N_3O_3$	XHE-I-066B(SR)	76.04	73.56	45.05	22.72	53.42	51.32

$C_{21}H_{19}N_3OSi$	XHE-I-073	62	155	217	1000	393	1000
$C_{19}H_{13}N_3O$	XHE-I-093	2	7.1	8.9	1107	20	1162
$C_{21}H_{17}N_3O_2$	XHE-I-096	3079	1000	4636	1601	567	2712
$C_{24}H_{27}N_3O_3Si$	XHE-I-47	705	2502	2460		480	10000
$C_{19}H_{21}N_3O_3$	XHE-II-002	8.3	18	13	3.9	1.5	11

$C_{20}H_{18}BrN_3O$	XHE-II-006a	4.7	4.4	20	1876	89	3531
$C_{20}H_{18}ClN_3O$	XHE-II-006b	3.7	15	12	1897	144	1000
$C_{22}H_{19}N_3O$	XHE-II-011	9	60	39	3233	90	1000
$C_{20}H_{16}BrN_3O_2$	XHE-II-012	49	24	31	1042	14	2038
$C_{25}H_{27}N_3OSi$	XHE-II-014	329	1098	1156	1000	2462	1000

<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3nc(cc3C(=O)N4C=CC=C4C#C)C5=CC=CC=C5C#C</chem> $C_{22}H_{19}N_3O$	XHE-II-017	3.3	10	7	258	17	294
<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3nc(cc3C(=O)N4C=CC=C4C#CC5=CC=C(C#CC6=CC=C(C(C)(C)C)C6)C5)C7=CC=CC=C7</chem> $C_{28}H_{27}N_3O$	XHE-II-019	273	428	762	1000	1464	1000
<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3nc(cc3C(=O)N4C=CC=C4C5=CC=CC=C5)C6=CC=CC=C6</chem> $C_{20}H_{19}N_3O$	XHE-II-024	0.09	0.18	0.32	14	0.24	11
<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3nc(cc3C(=O)OCC)C4=CC=C(C#C)C=C4C5=CC=CC=C5</chem> $C_{22}H_{17}N_3O_2$	XHE-II-053	287	45	96	1504	13.8	1000
<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3nc(cc3C(=O)OCC)C4=CC=C(C#C)C=C4C5=CC=CC=C5</chem> $C_{17}H_{13}N_5O_2$	XHE-II-065	1000	409	216	37	6.4	175
<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3nc(cc3C(=O)OCC)C4=CC=C(C#CC5=CC=C(CO)C=C5)C6=CC=CC=C6</chem> $C_{19}H_{19}N_5O_4$	XHE-II-070	352	532	454	45%	55	1000

$C_{18}H_{19}N_3O_3$	XHE-II-073A (R ENRICHED)	5.9	11	10	15	1.18	140
$C_{18}H_{19}N_3O_3$	XHE-II-073B (S-ENRICHED)	11	17	12	33	2.1	269
$C_{23}H_{27}N_3O_3$	XHE-II-077	1363	1000	3403	3561	1000	1000
$C_{20}H_{19}N_3O$	XHE-II-087a	1604	1000	1000	1000	1000	1000
$C_{20}H_{18}BrN_3O$	XHE-II-087c	1000	1000	1000	1000	1000	1000
$C_{24}H_{25}N_3O$	XHE-II-098a	1000	1000	1000	1000	1000	1000

<chem>C24H24BrN3O</chem>	XHE-II-098b	1000	1000	1000	1000	1000	1000
<chem>C24H24ClN3O</chem>	XHE-II-098c	1000	1000	1000	1000	1000	1000
<chem>C15H15N3O3</chem>	XHE-III-04	1.2	2	1.1	219	0.4	500
<chem>C16H10BrN3O</chem>	XHE-III-06a	1	2	1	5	1.8	37
<chem>C16H9BrClN3O</chem>	XHE-III-06b	32	33	20	299	28.6	740
<chem>C16H9Br2N3O</chem>	XHE-III-06c	34	44	29	210	23	319

<chem>C[Si](C)(C)C#Cc1ccc2c(c1)c(c[nH]2)c3c(c2)c(c[nH]3)nn(c3ccccc3)c4=O</chem> $C_{21}H_{19}N_3OSi$	XHE-III-12	240		1000	1000	181	536
<chem>CCOC(=O)c1c[nH]c2c1c(c[nH]2)c3c(c2)c(c[nH]3)nn(c3ccccc3)c4=O</chem> $C_{17}H_{17}N_3O_3$	XHE-III-13	7.3		7.1	880	1.6	311
<chem>C#Cc1ccc2c(c1)c(c[nH]2)c3c(c2)c(c[nH]3)nn(c3ccccc3)c4=O</chem> $C_{18}H_{11}N_3O$	XHE-III-14	2.6		10	13	2	7
<chem>CC(C)(C)c1ccc2c(c1)c(c[nH]2)c3c(c2)c(c[nH]3)nn(c3ccccc3)c4=O</chem> $C_{20}H_{18}FN_3O$	XHE-III-24	0.25		8	222	10	328
<chem>CCOC(=O)c1c[nH]c2c1c(c[nH]2)c3c(c2)c(c[nH]3)nn(c3ccccc3)c4=O</chem> $C_{15}H_{14}ClN_3O_3$	XHE-III-49	1.3	5.5	4.2	38.7	11.3	85.1
<chem>Clc1ccc(cc1)c2c(c[nH]2)c3c(c2)c(c[nH]3)nn(c3ccccc3)c4=O</chem> $C_{12}H_8ClN_3O$	XHE-III-54	1071	1624	2048	1000	1158	1000

$C_{12}H_8BrN_3O$	XHE-III-56b	1005	1642	1928	1000	1300	1000
$C_{17}H_{17}N_3OSi$	XHE-III-67	1000	1000	1000	1000	1000	1000
$C_{12}H_8FN_3O$	XHE-III-69	3433	1000	1000	1000	1000	1000
$C_{12}H_9N_3O$	XHE-III-70	997.5	2356	2484	711	500	1000
$C_{14}H_9N_3O$	XHE-III-73	1000	303	942	1000	492	1000
$C_{20}H_{23}N_3O_4$	XHE-III-74	77	105	38	0.42	2.2	5.8

C ₂₀ H ₁₃ N ₃ O ₂	XHE-II-O53-ACID	50.35	11.8	44		5.9	5000

C ₁₃ H ₁₄ N ₂ O ₃	XLI-?

C ₃₃ H ₂₆ N ₆ O ₆	XLI-093	1000	1000	858	1550	15	2000

C ₁₀ H ₈ Br ₂ N ₂ O ₂	XLI-1?

C ₁₈ H ₁₂ BrN ₃ O ₂	XLI-12TC

C ₁₂ H ₁₁ BrN ₂ O ₂	XLI-13TC	2078	5289	618.7		8382

C ₁₉ H ₁₉ N ₃ O ₄	XLI-14TC

<chem>C17H13N3O3</chem>	XLI-15TC
<chem>C15H12BrN3O3</chem>	XLI-16TC	7664	3515	787.4		1831
<chem>C19H20BrN3O3</chem>	XLI-17TC	224.8	106.6	120.6		92.43
<chem>C16H15N3O4</chem>	XLI-18TC
<chem>C13H14N2O3</chem>	XLI-19TC
<chem>C17H13BrN4</chem>	XLI-2?
<chem>C35H30N6O6</chem>	XLI-210	231	661	2666		5.4	54.22

<p>C₂₂H₂₀BrN₃O₂</p>	XLI223	C ₂₂ H ₂₀ BrN ₃ O ₂	14	8.7	18	1000	10	2000
<p>C₂₇H₂₉N₃O₂Si</p>	XLI224	C ₂₇ H ₂₉ N ₃ O ₂ Si	333	333	333	3000	333	2000
<p>C₂₄H₂₁N₃O₂</p>	XLI225	C ₂₄ H ₂₁ N ₃ O ₂	333	225	174	3000	110	2000
<p>C₂₃H₂₁F₆N₃O₃Si</p>	XLI-232
<p>C₂₀H₂₀N₂O₂Si</p>	XLI-250 (SHU-221)		1000	1000	339	1000	118	1000
<p>C₁₇H₁₃BrN₄</p>	XLI268	C ₁₇ H ₁₃ BrN ₄	2.81	0.69			0.62

XLI269	C22H22N4Si	221.8	154.2			15.51
XLI270	C19H14N4	36.39	25.81			5.291
XLI275	C20H19N3O3	35.61
XLI-286	C19H10BrClN2O	0.051	0.064	0.118		0.684
xli296	C19H13ClN4
XLI-2TC	C17H13BrN4	3.442	1.673	44.08		1.121
XLI-317	C18H19N3O4	60.24	24.05	4.562		0.295

$C_{23}H_{21}F_6N_3O_3Si$	XLI-332	1390	1507	3405		132.1
$C_{21}H_{23}N_3O_3$	XLI337 $C_{21}H_{23}N_3O_3$
$C_{23}H_{21}ClN_4$	XLI338 $C_{23}H_{21}ClN_4$	380	189	692		5000	5000
$C_{23}H_{22}N_4$	XLI339 $C_{23}H_{22}N_4$	5000	625	5000		5000	5000
$C_{26}H_{25}N_3O_2$	XLI340 $C_{26}H_{25}N_3O_2$
$C_{20}H_{19}ClN_2OSi$	XLI343 $C_{20}H_{19}ClN_2OSi$	6.4	17.7			150.5

$C_{34}H_{28}N_6O_7$	Xli-347 C34H28N6O7	828.05	690.2			13.87
$C_{20}H_{19}ClN_2OSi$	XLI-348	13.56	11.17	1.578		82.05
$C_{17}H_{11}ClN_2O$	XLI350 C17H11ClN2O	1.224	1.188			2.9
$C_{21}H_{21}ClN_2OSi$	XLI351 C21H21ClN2OSi	1.51	0.97			1.98
$C_{18}H_{13}ClN_2O$	XLI352 C18H13ClN2O	1.56	0.99			1.96
$C_{33}H_{34}N_6O_6$	XLI356 C33H34N6O6	1851.5	4702.5	8545		100.5	5000
$C_{22}H_{21}N_3O_2$	Xli366 C22H21N3O2	1

XLI368	C ₂₄ H ₂₅ N ₃ O ₂
XLI-374	C ₃₂ H ₂₄ N ₆ O ₇	3795	2694	1864	76.14
XLI-379	C ₁₃ H ₁₂ BrN ₃ O ₂	1037	482.4	357.5	36.13
XLI-381	C ₁₅ H ₁₂ ClN ₃ O	619.9	285.6	3639	7.1
XLI-382	C ₂₉ H ₂₈ Br ₂ N ₆ O ₄
XLI-385	C ₃₉ H ₄₆ N ₆ O ₄ Si ₂

C ₃₃ H ₃₀ N ₆ O ₄	XLI-387	5454	5000	5000		5000	5000

C ₁₀ H ₁₀ N ₂ O ₂	XLI-4?

C ₁₅ H ₁₁ N ₃ O ₃	XLI-6TC	2371	1421	1840		1169

C ₂₀ H ₁₅ BrFN ₃ O ₂	XLI-8TC	21.52	11.01	2.16		4.06

C ₁₉ H ₁₃ ClN ₄	XLI-JY-DMH ANX3	3.3	0.6	1.9		4.4	5000

C ₂₂ H ₂₁ ClN ₄ Si	XLI-JY-DMH-TMS C22H21N4SiCl)	35	63	99.13		69.7	5000

<chem>C13H14N2O3</chem>	XLI-TC	8155	7528	1036		508.9
<chem>C25H25N2O2Si</chem>	XliXHe-II-048 <chem>C25H25N3O2Si</chem>	5000	5000	5000		5000
<chem>C20H16ClN3O2</chem>	XZ112
<chem>C16H10N4</chem>	YCT-5	2.2	11.46	16.3		200	10000
<chem>C16H10N4</chem>	YCT-7A	3	23.8	30.5		240	10000
<chem>C16H10N4</chem>	YCT-7B	28	118	156		1000	10000

<chem>C14H12BrN3O3</chem>	YT-5	0.421	0.6034	36.06		1.695
<chem>C14H12ClN3O3</chem>	YT-6	15.31	87.8	60.49		1.039
<chem>C18H15N3O3</chem>	YT-I-38	945.9	326.8	245.9		4.07
<chem>C18H14BrN3O</chem>	YT-II	6.93	0.87	3.51		5.12
<chem>C18H19N3O3</chem>	YT-II-76	95.34	2.8	0.05		0.04
<chem>C33H28Br2N6O6</chem>	YT-II-791	1495	2681	527.4		144
<chem>C34H30Br2N6O6</chem>	YT-II-792	789.6	599.5	1698		83.18

<p><chem>C34H30Br2N6O6</chem></p>	YT-II-793	1383	2881	782.6	128.9
<p><chem>C34H30Br2N6O4</chem></p>	YT-II-794	4536	126.2	4981	932.8
<p><chem>C16H16ClN3O4</chem></p>	YT-II-83	32.74	13.22	24.1	3.548
<p><chem>C30H25N3O5</chem></p>	YT-III-1
<p><chem>C31H26BrClN6O6</chem></p>	YT-III-10	1172	1915	1481	282.9
<p><chem>C36H27ClN6O5</chem></p>	YT-III-15	73.19	90.45	141.4	114

<chem>C33H30Cl2N6O6</chem>	YT-III-28	130.7	80.03	82.9		192
<chem>C21H16N4O</chem>	YT-III-31	36.39	67.85	129.7		7.59
<chem>C23H20FN3O2</chem>	YT-III-38	1461	18.21	14.63		3999
<chem>C20H21N5O2</chem>	YT-III-42	382.9	16.83	44.04		9.77
<chem>C29H24Cl2N6O6</chem>	YT-TC-1	441.8	927.4	157.9		122.2
<chem>C30H26Cl2N6O6</chem>	YT-TC-2	839.1	1647	568.1		421.9

<chem>C31H26Cl2N6O6</chem>	YT-TC-3	141.4	11.43	118.1		29.22
<chem>C30H26Cl2N6O4</chem>	YT-TC-4	6291	3315	352.2		2881
<chem>C17H18BrN3O3</chem>	ZG-168	11.2	10.7	9.2		0.47	9.4
<chem>C17H18IN3O3</chem>	ZG-208	9.7	11.2	10.9		0.38	4.6
<chem>C17H18N4O5</chem>	ZG-213	12.8	49.8	30.2		3.5	22.5
<chem>C18H18N4O3S</chem>	ZG-224	17.1	33.7	50		2.5	30.7

ZG-234	7.25	22.14	9.84	0.3	5.25
ZG-63A	17.3	21.6	29.1	0.65	4
ZG69A	6.8	16.3	9.2	0.85	54.6
ZG-69a(Ro15-1310)	6.8	16.3	9.2	0.85	54.6
ZG-88
ZJW-II-040
ZK 93423	4.1	4.2	6	4.5	1000

C ₁₉ H ₂₁ N ₃ O	ZOLPIDEM	26.7	156	383		10000	10000