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The Imidazodiazepine Anticonvulsant, KRM-II-81, Produces Novel, Non-diazepam-like Antiseizure Effects[1]

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Footnote

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Abstract

The need for improved medications for the treatment of epilepsy and chronic pain is essential. Epileptic patients typically take multiple antiseizure drugs without complete seizure freedom and chronic pain is not fully managed with current medications. A positive allosteric modulator (PAM) of α2/3-containing $GABA_A$ receptors (5-(8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazole[1,5-α][1,4]diazepin-3-yl) oxazole or KRM-II-81 (**8**) is a lead compound in a series of imidazodiazepines. We previously reported that KRM-II-81 produces broad-based anticonvulsant and antinociceptive efficacy in rodent models and provides a wider margin over motoric side effects than that of other $GABA_A$ receptor PAMs. The present series of experiments was designed to fill key missing gaps in prior preclinical studies assessing whether KRM-II-81 could be further differentiated from non-selective $GABA_A$ receptor PAMs using the anticonvulsant diazepam (DZP) as a comparator. In multiple chemical seizure provocation models in mice, KRM-II-81 was either equally or more efficacious than that of DZP. Most strikingly, KRM-II-81 but not DZP blocked the development of seizure sensitivity to the chemoconvulsants cocaine and pentylenetetrazol in seizure kindling models. These and predecessor data have placed KRM-II-81 into consideration for clinical development requiring the manufacture of kilogram amounts of GMP material. We describe here a novel synthetic route amenable to kilogram quantity production. The new biological and chemical data provide key steps forward in the development of KRM-II-81 (**8**) as an improved treatment option for patients suffering from epilepsy.

Keywords: KRM-II-81, $GABA_A$ receptor PAMs, α2/3-containing $GABA_A$ receptors, diazepam, epilepsy, seizure kindling

Introduction

Epilepsy is a chronic neurological disorder suffered by millions of people world-wide with large negative health outcomes and life disruption.[1] Many antiseizure drugs of different structural and pharmacological classes have been approved for medical use.[2] Nonetheless, seizures are often not fully controlled despite the daily administration often of more than one antiseizure medication. In up to 70% of epileptic patients, standard of care medicines are not efficacious.[3-5] The uncontrolled seizures can also increase the probability of subsequent epileptic events through sensitization mechanisms termed seizure kindling.[6-8] Chronic, uncontrolled seizures and the side effects arising from seizure medications have a negative impact on the developing and adult brain and can lead to severe impairment of neurocognitive function.[9, 10] Uncontrolled seizures can also increase the liklihood of neuronal cell death and patient lethality.[11] Therefore, there continues to be an urgent need for improved antiseizure medicines.[12, 13]

The ligand, 5-(8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)oxazole or KRM-II-81 (**8**, **Figure 1**) is a positive allosteric modulator (PAM) of α2/3-containing $GABA_A$ receptors with potential for medicinal use in epilepsy based upon preclinical data acquired over the past several years (**Table 1**). The excitement over this molecule arises from the fact that although GABA is a long-known modulator of epilepsy, $GABA_A$ receptor PAMs like DZP are not used as daily antiseizure agents due to the sedation and motoric side effects occurring at anticonvulsant doses.[2] DZP does not discriminate among $GABA_A$ receptors composed of different alpha subunits, whereas

KRM-II-81 is selective for α2/3-containing $GABA_A$ receptors.[14, 15] Genetic mutation experiments combined with pharmacological studies with alpha subtype-selective molecules have long suggested eliminating potentiation of α1-containing $GABA_A$ receptors as one means of reducing sedation and motor impairment.[16] KRM-II-81 exhibits reduced sedation and motoric burden compared to DZP.[17, 18] As such there is the opportunity for achieving higher drug exposures with KRM-II-81 to modulate $GABA_A$ receptors for improved therapeutic advantage. This gain in efficacy has been demonstrated with KRM-II-81 in models of pain where DZP could not be dosed sufficiently high enough to achieve efficacy, whereas KRM-II-81 was at least as efficacious and more potent than tramadol.[19] In rodent antiseizure models, KRM-II-81 has also shown greater efficacy than DZP under some assay conditions (**Table 1**). The present study was undertaken to further differentiate DZP from KRM-II-81.

A recent licensing option agreement from RespireRx to acquire this molecule from the University of Wisconsin-Milwaukee established increased prioritization toward the development of KRM-II-81 for epileptic patients. Although the case for development of KRM-II-81 as an antiseizure agent is gaining traction (**Table 1**), there are some gaps in the biology. As an anticonvulsant against chemically induced seizures, KRM-II-81 has only been studied against pentylenetetrazol (PTZ). However, since PTZ is a $GABA_A$ receptor antagonist, the blockade of seizures with a $GABA_A$ receptor PAM like KRM-II-81 or DZP is expected on pharmacological grounds; blockade of seizures might be due to receptor pharmacology rather than to suppression of the seizurogenic effects of PTZ per se. The present study addresses this deficiency by studying a broader array of chemoconvulsants with diverse non-$GABA_A$ receptor mechanisms,

thus enabling a pure test of the antiseizure hypothesis and side-by-side comparisons with diazepam.

KRM-II-81 (**8**) has also shown an ability to block seizures in rodent models where seizures have been developed over time by sensitizing seizure networks by daily brain stimulation, a process known as seizure kindling. Seizure kindling can be modeled in rodents and is often used as another means of differentiating antiseizure drugs.[20, 21] The data in **Table 1** summarize the ability of KRM-II-81 to suppress the expression of seizures that have already been developed (blockade of seizure expression). What remains unknown is whether KRM-II-81 can block the development of the seizure sensitization process (kindling). In contrast to other $GABA_A$ receptor PAMs like some neuroactive steroids, DZP has limited impact on this process.[22, 23] In order to determine the relative efficacy of KRM-II-81 on seizure kindling, the effects of DZP and KRM-II-81 were compared in two kindling models in the present study.

Another obstacle to the development of KRM-II-81 (**8**) for epileptic patients is the synthesis of GMP material on large scale for IND-enabling toxicology and for first human dose studies. Previous work has created synthetic routes from the precursor molecule HZ-166 (**6**, **Scheme 1**). The first route employed a three step approach which involved a low yielding LAH reduction of the ester (HZ-166) to an alcohol which was then subsequently oxidized by manganese dioxide to the aldehyde **7**.[15] A modification of this synthesis[24] improved these final steps of the synthesis by converting HZ-166 (**6**) to its respective ester bioisostere KRM-II-81 (**8**) in two steps by switching the reduction reagent to PDBBA to afford the key aldehyde **7** directly. However, a process conducive to larger scale chemistry was still required to synthesize the ester precursor

(HZ-166) to KRM-II-81. We sought to replace the chromatographic purification steps employed in all five steps of the earlier discovery process for the synthesis of HZ-166 with crystallizations. In addition, to address scale up issues including poor stirring, side product formation, long process times and yield losses on large scale. Provided herein is a method amenable for production of kilogram quantities of HZ-166 (**6**) and KRM-II-81 (**8**).

Results and Discussion

KRM-II-81 has Broader Efficacy Against Chemical Convulsant Agents than DZP. In order to extend the observations on the anticonvulsant activity of KRM-II-81 (**8**) to other, non-GABA convulsants, chemicals that act upon monoamine transporters (cocaine), potassium channels (4-aminopyridine or 4-AP), N-methyl-D-aspartate (NMDA) receptors (NMDA), glycine receptors (strychnine), and muscarinic cholinergic receptors (pilocarpine)(acute seizures, not status epilepticus) were studied. In addition, PTZ and another antagonist of $GABA_A$ receptors, picrotoxin, were also studied. The comparative efficacy of DZP (1 mg/kg, i.p.) and KRM-II-81 (30 mg/kg, i.p.) are summarized in **Error! Reference source not found.**. The doses of DZP and KRM-II-81 were based upon prior data showing that these doses represent ~ED_{95} values for seizure blockade against PTZ-induced clonus in mice.[18, 22] The doses of both DZP and KRM-II-81 are further justified for this single dose comparison study based upon the equivalent efficacy of these two anticonvulsants against PTZ-induced clonus, tonus, and lethality (**Table 2**).

For PTZ, both DZP and KRM-II-81 significantly attenuated clonic convulsions, as previously reported in rats.[18] Prior studies with PTZ[18] did not study doses inducing tonic seizures and lethality. Here we show that both DZP and KRM-II-81 (**8**) also significantly reduce these toxic endpoints induced by PTZ. Although a similar profile of comparative protection occurred when studying picrotoxin, the statistical preference for KRM-II-81 might be due to the relatively small percentage of tonic convulsions and lethality that were produced by the dose of picrotoxin employed. As with the $GABA_A$ receptor antagonists PTZ and picrotoxin, both DZP and KRM-II-81 were also equally effective against the acute seizures evoked by the muscarinic cholinergic receptor agonist pilocarpine.

In contrast, for cocaine (COC), 4-AP, NMDA, and strychnine, KRM-II-81 was efficacious when DZP was not. Clonic convulsions were blocked by KRM-II-81 for these convulsant compounds. The differential efficacy of KRM-II-81 over DZP extended also to tonic seizures and lethality in the case of 4-AP, strychnine, and NMDA. The findings reported here are consistent with the literature on the anticonvulsant effects of DZP.[25-30] Overall, the data support the conclusion that KRM-II-81 has broad ranging anticonvulsant efficacy against chemoconvulsants (**Error! Reference source not found.**). Further, the data in **Error! Reference source not found.** support the conclusion that KRM-II-81, in the present study, has a superior efficacy profile over that of DZP as shown earlier with other seizure provoking stimuli (**Table 1**). Statistically significant blockade (Fisher's Exact probability test) were observed for KRM-II-81 but not DZP against COC (clonus), 4-AP (clonus and tonus), NMDA (clonus and lethality), picrotoxin (tonus and lethality), and strychnine (clonus, tonus, and lethality). This differentiation is most striking for 4-AP, NMDA, and strychnine especially since doses of DZP and KRM-II-81 produced equivalent anticonvulsant effects against the GABA-based chemoconvulsants PTZ and picrotoxin (**Table 2**). However, full dose-response comparisons (both of chemoconvulsant and of anticonvulsant) will be ideally required to fully appreciate precise quantitative differences between DZP and KRM-II-81 as has been reported earlier in some anticonvulsant-detecting assays.[18]

KRM-II-81 but Not DZP Blocks the Development of PTZ Kindling. When PTZ, in a subconvulsant dose (45 mg/kg), is given every other day for 4 days, the percentage of mice exhibiting clonic seizures increases (**Figure 2A**) as previously reported.[22] On the 5th experimental session, either vehicle, DZP (1 mg/kg, i.p.), or KRM-II-81 (30 mg/kg, i.p.) was given prior to PTZ.

Both DZP and KRM-II-81 prevented clonic seizures in these PTZ-sensitized mice (**Figure 2A**) thus demonstrating efficacy to block seizures in fully kindled animals. In order to test whether these compounds also prevent the expression of seizure kindling, either vehicle, DZP or KRM-II-81 was administered prior to each dose of PTZ. Under these conditions, both drugs fully protected mice from the occurrence of seizures and the increased sensitivity that develops to PTZ in the absence of treatment (**Figure 2B**). Another experiment was conducted in order to assess whether these compounds can prevent or attenuate the development of kindling. In this study, vehicle, DZP, or KRM-II-81 was given on each of the first 4 experimental sessions prior to PTZ. Then, on day 10 (experimental session 5), mice were given PTZ alone (with vehicle pretreatments). While prior exposure to KRM-II-81 + PTZ inoculated mice from kindling, DZP did not (**Figure 2C**). Thus, despite the ability of DZP to block both acute and sensitized effects of PTZ, DZP was not able to dampen the seizure kindling process where KRM-II-81 was protective.

Another study was conducted in order to extend these observations to another seizure kindling agent. Cocaine (COC) was chosen since the DZP and KRM showed differential efficacy against PTZ and COC convulsant challenge. Whereas in the case of PTZ, DZP and KRM-II-81 were equally effective in preventing seizures induced by acutely administered PTZ, only KRM-II-81 was efficacious against acutely-administered COC (**Error! Reference source not found.**). Seizure kindling with COC was studied as with PTZ kindling with the exception that COC kindling was conducted every day[31, 32] vs. the every other day dosing method that is established for PTZ kindling.[22] In this study, COC given daily for 5 days produced significant and large changes in the percentage of mice exhibiting clonic convulsions on day 5 vs. day 1 (**Figure 3A**) demonstrating seizure kindling. On day 6, either vehicle, DZP (1 mg/kg, i.p.) or KRM-II-81 (30 mg/kg, i.p.) was

given prior to COC challenge. KRM-II-81 but not DZP significantly attenuated clonic seizure incidence in mice (**Figure 3B**) as was observed after acute COC challenge (**Error! Reference source not found.**). Thus, both in non-kindled mice and fully kindled mice, KRM-II-81 but not DZP is an effective seizure protectant. The expression of kindled seizures over the 6-day period of COC dosing was assessed next. KRM-II-81 significantly attenuated the expression of COC seizures across 6 days of COC dosing; DZP was not active (**Figure 3B**).

A final seizure kindling study was initiated to determine whether DZP or KRM-II-81 (**8**) could prevent the development of COC seizure kindling. In this study, vehicle, DZP, or KRM-II-81 was given prior to each COC kindling session on days 1-5. On day 6, COC alone was given (+ vehicle) to ascertain whether kindling was suppressed by the prior drug treatments. On the day 6 test, KRM-II-81 but not DZP significantly attenuated the development of kindling (**Figure 3C**). The blockade of kindling by KRM-II-81 appeared to be greater for PTZ kindling (**Figure 2C**) than that observed with COC kindling (**Figure 3C**) perhaps due to the more prominent effects of KRM-II-81 on acute PTZ vs. COC seizures (**Error! Reference source not found.**) and the greater ability to block expression of PTZ vs. COC kindling (**Figure 2B** vs. **Figure 3B**). However, it is critical to emphasize that even though DZP is an effective anticonvulsant against acute seizures induced by PTZ (**Error! Reference source not found.**) and is efficacious as a suppressor of the expression of PTZ kindling, DZP is still not effective as a blocker of the development of kindling while KRM-II-81 is. The data for DZP reported here are consistent with prior findings.[22, 23, 31, 32] Overall, the kindling experiments document the differential ability of KRM-II-81 to significantly suppress the development of the seizure sensitization process and that this effect is not confined to the $GABA_A$ receptor antagonist convulsant PTZ.

A Synthetic Method Amenable for the Production of Kilogram Quantities of KRM-II-81. Given the potential therapeutic value of KRM-II-81 for patients as discussed above and elsewhere,[17, 18, 33] a novel method for the synthesis of KRM-II-81 with efficiency on a large scale was developed. The synthesis of KRM-II-81 (**Scheme 1**) has been reported previously[15, 24], but the discovery chemistry was not well-suited for scale up in a potential GMP manufacturing setting. All five steps of the synthesis to the key intermediate HZ-166[34, 35] required purification by column chromatography, while several steps exhibited typical scaleup issues such as inefficient stirring, side product formation, prolonged process times and yield loss upon scale up.

The challenge in the first step of the synthesis was to improve upon the stirring difficulties of the reaction media and the lack of an adequate purification procedure for multigram quantities. The discovery route developed by Li et al.[36] based on the work of Selnick et al.[37] called for cooling the mixture of 2'-pyridyl ketone **1**, sodium bicarbonate and dichloromethane to 0^{o}C prior to the addition of bromoacetyl bromide. Early into the addition process an extremely thick red gelatinous-like solid developed that made efficient stirring of the reaction mixture nearly impossible. Upon warming to room temperature, the gelatinous solid would slowly dissipate, which resulted in a more readily stirred reaction mixture. Any procedure that is being developed for large-scale synthesis cannot involve agitation issues. Similar research in our chemistry group utilized the same reaction protocol,[34, 38] but those analogs lacked the 2'-pyridine function and therefore lacked the stirring difficulties encountered herein. Upon consideration of a possible reaction mechanism (**Scheme 2**), it was proposed that formation of the desired 2-halooacetamide product **2c** must proceed through the insoluble acetyl pyridinium intermediate **2b** for which the

red, gelatinous-like solid can be attributed. As a remedy, the initial reaction temperature was raised to 25 - 30°C prior to the addition of bromoacetyl bromide, while the bromo acetyl reagent was added over a 60minute time period. Utilization this new approach completely avoided the agitation issue while the red, insoluble pyridinium intermediate **2b** was not observed to a significant amount. Presumably, the elevated process temperature increased the reaction rate enough, so the reaction did not stall at the pyridinium salt stage. Analysis of this reaction progress immediately after the addition of the bromide by TLC confirmed this observation for there was no evidence of the 2'-pyridyl ketone **1** nor pyridinium intermediate **2b**. Upon completion of the reaction, the inorganic salts were partitioned into the aqueous layer, after which the solvent was exchanged to ethanol to precipitate the pure halooacetamide product **2a** or **2c** (individually). No further purification was required. The employment of 2-chloroacetyl chloride as an economical substitute for 2-bromoacetyl bromide proved to perform equally as well in the reaction protocol with no detrimental effects to the chemistry in the following steps. In summary, the modifications employed to the synthesis provided 125.6g of **2c** in 98.5% yield (2L reaction flask).

The second step of the synthesis furnished the diazepine core **3** via the conversion of the chloroacetamide **2c** into a β-amino amide intermediate **3b** prior to condensation of the amine with the carbonyl function. The process problems presented in this reaction sequence included the lack of a reliable and efficient method for purification of the amide **3** and the formation of detrimental side products observed on scale up. The discovery route (**Scheme 3**) utilized saturated methanolic ammonia to furnish the β-amino amide intermediate **3b** *in situ* by nucleophilic aliphatic substitution of the alkyl halide **2a** which then should cyclize via a carbinolamine condensation to yield the 2'-pyridyldiazepine **3**. As is typical with scale-up chemistry, process times (heating and

cooling) and reaction times are prolonged, as compared to smaller scale procedures and a subsequent drop in yield of the amide **3** was observed. An investigation of the causes of the yield reduction revealed the formation of a significant side product that was observed to a small extent by TLC on small scale but increased substantially as the scale was increased. The isolation of the impurity (m/z = 314.0167) indicated a mass unit one less than that of the desired product **3** (m/z = 315.0007). A thorough analysis of the structure of the impurity using ^{1}H NMR, ^{13}C NMR, HSQC, ^{1}H-^{13}C-HMBC, ^{1}H-^{15}N-HMBC spectroscopy experiments provided evidence which supported the proposed imidate **3c** as the structure of the impurity. The ^{1}H-^{15}N-HMBC experiment was especially revealing because examination of the spectrum indicated the identity of the impurity **3c** contained 4 nitrogen atoms in the structure (**Figure S1**). The pyridine nitrogen atom (N13) correlated to pyridine ring protons (H11 and H12), while the remaining nitrogen atoms (N15, N18 and N19) correlated to the aliphatic CH_2 protons (H16). Finally, the iminium nitrogen atom (N18) correlated to the A-ring proton, H6. The logical explanation for the formation of the imidate (**3c**) would be through a dehydration mechanism first involving addition of ammonia into the diazepine amide carbonyl via nucleophilic acyl substitution, which was followed by the elimination of water (**Scheme S1**). The mechanism seems reasonable because of the extended reaction times under high concentrations of ammonia. Options to mitigate the formation of the imidate **3c** included shorter reaction times and lower ammonia concentrations, but the application of these solutions were ruled out due to concern for low yields attributed to incomplete consumption of the starting material, which would then have to be removed.

Clearly, a new approach to synthesis of the key 2'-pyridyl-diazepine core (**3**) was warranted. Here, one turned to the Delépine reaction developed by Stéphane Marcel Delépine.[39] It entails

incorporation of the hexamethylenetetramine-(HMTM)-based cyclization reaction first developed by Blažević and Kajfež[40] and further refined by Cepanec, et al.[41] The HMTM-based cyclization (**Scheme 4**) involves first, the formation of quaternary ammonium salt **3a** by nucleophilic aliphatic substitution of the alkyl halide **2** and this was followed by *in situ* hydrolysis to the β-amino amide intermediate **3b**, which was then condensed with the carbonyl function to provide the 2'-pyridyl-diazepine **3**. This new approach performed well albeit with slightly lower than expected yields (60-65%) than the literature report.[41] An investigation into the side products revealed a 5-10 % impurity identified as β-alkoxy amide **3d**. Presumably, when a primary alcohol (methanol or ethanol) was used as the solvent this undesired Sn2 addition of the alcohol to displace the tetramine to provide to **3d** was possible. Modification of the reaction solvent to the secondary alcohol, 2-propanol (IPA) eliminated this side reaction evidenced by the lack of detection of the corresponding β-alkoxy amide impurity **3d**; importantly, the corresponding yield increase was realized. On large-scale (2L flask) the utilization of the Delépine reaction delivered 75g (75% yield) of 2'-pyridyl-benzodiazepine **3**.

The third step of the synthesis of HZ-166 (**6**) was to install the imidazole ring C to produce imidazodiazepine **4**. The previous procedure[42] involved formation of the iminophosphate **4a** (**Scheme 5**) by deprotonation of the starting amide using potassium *t*-butoxide followed by the addition of diethylchlorophosphate. Then, iminophosphate **4a** subsequently underwent a nucleophilic addition with the enolate of ethyl isocyanoacetate to furnish the desired imidazodiazepine **4**. Several improvements to the original chemistry were required in order to provide a reliable and scalable procedure. Initially, the purification was conducted using column chromatography. Secondly, the reagents were added in an "all in one" fashion and specifically as

a solid in the case of the portions of potassium *t*-butoxide. Thirdly, each subsequent reagent addition involved cooling of the reaction mixture to below -35°C. Then, the next reagent was added and finally the mixture was allowed to warm to 0°C for each constituent of the process to react. With those challenges in mind we sought to develop a purification method by crystallization and to determine a reaction temperature in which the reagents could be added in a controlled fashion at a temperature at which they would react. By careful monitoring of the reaction progress by TLC it was determined that the iminophosphate **4a** did not form until the reaction temperature reached -20°C. Similarly, it was determined that the required reaction temperature of the condensation of the enolate of ethyl isocyanoacetate with the iminophosphate **4a** was -35 to -30°C. Consequently, it was felt that addition of the reagents in a controlled, dropwise fashion would allow one to simply hold the reaction mixture at -20 to -15°C throughout the sequence of additions. This procedure first involved dissolving diazepine **3** in tetrahydrofuran (THF) and cooling that solution to -20°C. Then, a solution of potassium *t*-butoxide (1.3 eq) in THF was added dropwise over 30 minutes to the reaction mixture. The reaction mixture was then stirred for 30 minutes at -20 to -15°C to ensure complete deprotonation of the amide starting material. Next, diethylchlorophosphate (1.4 eq) was added over a 15-minute time span. Within 2 hours after the addition of the phosphate reagent, consumption of the amide starting material **3** was observed to generate the iminophosphate intermediate **4a**. Then, ethyl isocyanoacetate (1.3 eq) was added over 15 minutes, and this was followed by a dropwise addition of a solution of potassium *t*-butoxide (1.3 eq) in THF at -20 to -15°C. The isocyanoacetate / butoxide addition sequence was designed in this manner so that the enolate would react with the iminophosphate as readily as it formed. As predicted, the formation of imidazodiazepine **4** was complete immediately after the *t*-butoxide addition with no detectable iminophosphate intermediate **4a** observed. Upon completion of the

reaction procedure the imidazodiazepine product **4** was partitioned into the organic layer using dichloromethane. An aqueous bicarbonate work-up removed the potassium salts and unreacted butoxide. The purification of the product was achieved by crystallization from *t*-butyl methyl ether (*t*BME), which adequately removed any traces of side products related to the phosphate related byproducts. The trituration of the crystals with hot ethanol was then employed to remove any residual diazepine starting material **3**, if necessary. With the goal of eliminating the column chromatography and a more robust and scalable procedure in hand, successful execution of the large-scale synthesis provided 61.0 g (52 % yield) of imidazodiazepine **4** (5 L flask).

The next step in the reaction sequence employed the copper-free Sonogashira coupling[43, 44] to covert aryl bromide **4** into the silyl-protected acetylene **5**. The earlier discovery chemistry used TMS-acetylene (1.5 eq) and bis(triphenylphosphine)palladium(II) diacetate ($Pd(OAc)_2(PPh_3)_2$) as the catalyst, as well as a large excess of triethylamine. Under these conditions the reaction time was typically 15 hours at reflux (75^{o}C) to achieve the desired consumption of the aryl bromide **4** (**Table 3**, **entry 1**). These reaction conditions presented an opportunity for improvement. First, the catalyst was prone to poisoning and the procedure required an extensive de-gassing protocol prior to initiating the reaction. The degree of proper degassing (to remove dissolved oxygen in the solvents) played a direct role on the success or failure of the coupling reaction. Secondly, the reaction conditions employed an excess of TMS-acetylene (1.5 eq) to drive the reaction to completion, while most often catalytic poisoning and perhaps degradation of the TMS-reagent stalled the reaction with 5-10% of aryl-bromide **4**, which remained. Typically, additional TMS-acetylene and fresh catalyst were added to complete the reaction. Thirdly, solvent quantities of triethylamine (coupled with acetonitrile in a 1:1 mixture) were used to dissolve the

imidazodiazepine **4** starting material in the reaction medium. Finally, difficult column chromatography was required to purify the product to remove the closely eluting aryl bromide **4** starting material from the crude mixture. This process was also further complicated by the triethylammonium bromide side product, which remained in the crude residue by the previous processing protocol. Therefore, in order to develop a reliable large-scale procedure for the copper-free Sonogashira coupling, the catalyst poisoning, the acetylene reagent, the isolation process and the purification of the silyl target **5** must be improved.

To remedy the problem of the poisoning of the catalyst, which was caused by the oxidation of triphenylphosphine to the corresponding phosphine oxide, a ligand less prone to oxidation was required. A trial with tri-ortho-tolylphosphine (P-*o*-tol_3) developed by Greg Fu was first attempted because it has a larger cone angle (194°) than that of triphenylphosphine (PPh_3, 145°)[45, 46] and should exhibit improved resistance to oxide formation and subsequent catalyst poisoning. As mentioned, the ligand in the process was altered to P-*o*-tol_3, which significantly shortened the reaction time to 6 hours (from 15 hours) and no cumbersome degassing was required (**Table 3**, **entry 2**). The starting TMS-acetylene (1.5 eq) could be replaced with the inherently more stable TIPS-acetylene reagent (1.2 eq), which shortened the reaction time further to 4 hours, even with reduced equivalents of the protected-acetylene reagent. (**Table 3**, **entry 3**). Finally, reduction of triethylamine from the solvent quantities to stoichiometric quantities (2.0 eq) exhibited no detrimental impact on the reaction time (**Table 3**, **entry 4**). With the optimal reaction conditions in hand the product isolation and purification procedure were then refined. The spent and undissolved catalyst was filtered from the reaction media through a small pad of silica gel. The filtrate, which resulted, was concentrated and then partitioned between dichloromethane and

aqueous sodium bicarbonate to remove triethylammonium bromide. Finally, the concentrated residue was run through a short flash column to remove the baseline impurities. No other purification was needed in order to carry the product **5b** forward into the next step since the aryl bromide starting material **4** was completely consumed using the refined reaction conditions. In a 3L flask, the improved copper-free Sonogashira coupling process produced 65.0g of the TIPS-protected acetylene **5b** in 85% yield.

The final step of this development process provided the key intermediate HZ-166 (**6**) via deprotection of the TIPS-acetylene function using fluoride anion. The improvements to this process included optimization of the reaction temperature and replacement of the chromatographic purification with crystallization. The discovery process utilized tetrabutylammonium fluoride (TBAF) as the fluoride source. This reagent performed, as required, but the isolation and purification of HZ-166 target (**6**) required removal of the tetrabutylammonium hydroxide byproduct, adequately. Additionally, the purification protocol would also need to efficiently remove the TIPS-fluoride side product. The discovery route cooled the reaction media to -78^{o}C prior to the addition of TBAF. Careful monitoring of the reaction by TLC indicated that deprotection did not initiate, however, until the temperature reached -20^{o}C. Consequently, the process temperature was modified to – 20 to -15^{o}C, at which point the TBAF·xH_2O (1.0M in THF) was added over a 30-minute time period. The process was monitored by TLC, which indicated complete deprotection had occurred upon completion of the TBAF addition.

Evaluation of a variety of solvents led to the selection of 2-propanol (IPA) as the ideal crystallization solvent, which exhibited minimal product solubility with efficient side-product

(TBA-OH and TIPS-F) solubility. The improved final step of the HZ-166 (**6**) synthesis employed the optimum temperature, while eliminating the chromatographic purification providing the 38.4g (85.0%) of HZ-166 (3L flask).

In summary, the five-step synthesis of HZ-166 (**6**) was refined to provide a more reliable and robust procedure (**Scheme 6**) with a 27.6% overall yield. As noted, the chromatographic purification steps were replaced with large-scale crystallizations in four steps, while importantly, the overall reaction times were reduced. The conversion of HZ-166 (**6**) on 20 gram scale into KRM-II-81 (**8**) involved first the reduction of the ester with the hindered reducing agent potassium diisobutyl-*tert*-butoxyaluminum hydride (PDBBA) at 0 ^{o}C to provide the aldehyde (**7**) in 80% yield. This was followed by the reaction of the aldehyde (**7**) with toluenesulfonylmethyl isocyanide (TosMIC) to afford the oxazole, KRM-II-81 (**8**) in 89% yield. This optimized conversion was previously carried out by Li, et al,[24] and the experimental of which is fully detailed in the Supporting Information.

As already discussed, the need for improved antiseizure drugs is critical to the medical and life needs of epileptic patients. The ability of KRM-II-81 (**8**) to produce anticonvulsant effects in multiple preclinical models and to generate, in some cases, superior efficacy to that of another $GABA_A$ receptor PAM, DZP, supports the proposition that KRM-II-81 represents a new lead candidate for development. KRM-II-81 was also recently shown to block the cortical hyperactivity of neurons after traumatic brain injury in a mouse model,[17] which suggests potential efficacy against the development of post-traumatic epilepsy.[47, 48] The data in models of pharmacoresistant epilepsy (**Table 1**) combined with the current findings in kindling experiments (**Figure 2C** and **Figure 3C**) provide important new data on compound superiority. Ganaxolone is a neuroactive

steroid that has shown similar antiepileptogenic activity against COC and PTZ kindling under conditions where DZP is not effective.[22, 23, 32] Ganaxolone was recently assessed in patients with difficult to treat status epilepticus, a life-threating condition. In this study, ganaxolone was efficacious, thus translating preclinical predictions into patients in this emergency situation.[49]

In addition to the data in rodent seizure models presented here and elsewhere,[17, 18] additional patient translational assurance comes from the efficacy of KRM-II-81 (**8**) to dampen hyperexcitabity of neural networks in cortical tissue slices from epileptic patients (**Table 1**). PF-06372865 is another compound that has selectivity for α2/3-containing $GABA_A$ receptor although, unlike KRM-II-81, PF-96372865 also potentiates α5-containing $GABA_A$ receptors.[50] PF-06372865 is well-tolerated in people[50-52] and was efficacious in inhibiting electrical activity in patients with photosensitive epilepsy.[51]

The more benign sedative and motor-impacting effects of KRM-II-81[15, 18, 33] are an additional feature of its pharmacology that bode well for clinical superiority. Recent docking studies of KRM-II-81 (**8**) with the CryoER structure 6HUP (α1β3γ2L $GABA_A$ receptor in complex with DZP)[53] provided structural support for the reduced impact of KRM-II-81 at the α1His102 side chain implicated in sedation and motor-impairment.[17] KRM-II-81 also showed less respiratory depression than alprazolam.[18] Additional data suggest that α2/3-selective PAMs will have less liability than non-selective $GABA_A$ receptor PAMs to produce memory impairment, tolerance, or abuse.[54-58] KRM-II-81 is also predicted to have efficacy in other therapeutic domains that are both stand-alone diseases and are comorbid with epilepsy including anxiety,[15, 59] depression,[60] and pain.[14, 19]

In conclusion, the data presented in the present manuscript along with literature reports are consistent with KRM-II-81 (**8**) showing superiority over diazepam and being a lead compound for clinical development for pharmacoresistant epilepsy and other epileptic states. The newly designed synthesis amenable to kilogram quantities of material presented here provides another key step in the drug development process.

Experimental Section

Biology

Compounds. KRM-II-81 (**8**) was synthesized (the laboratory of James M. Cook) as previously described.[15, 24] The other compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA). KRM-II-81 was suspended in 1% carboxymethylcellulose and dosed at 1 ml/kg in rats below doses of 30 mg/kg; 30 mg/kg (dosed at 3 ml/kg), 60 mg/kg (dosed at 6 ml/kg). Mice were dosed in a volume of 10 ml/kg. The other compounds were dissolved in sterile water with sonication as needed. Compounds were dosed i.p. with the exception of pentylenetetrazol (PTZ), which was given by s.c. injection in the acute convulsant tests.

Rodent Assays. All studies were performed in accordance with the guidelines of the National Institutes of Health and by local animal care and use committees. The local animal care and use committee and veterinary staff provided direct oversight of the animals by inspections, protocol reviews, laboratory site visits, and animal health monitoring. Male, CD1 mice were used and weighed 28-33g at the time of testing. The mice were allowed to acclimate to the vivarium for at least 4 days prior to testing and for at least 45 min in the testing room prior to experimentation. Animals were housed in a temperature- and humidity-controlled room with a 12 h light/dark cycle (on at 0600). Mice were group housed in large plastic containers with sawdust bedding. Standard mouse food pellets and water were continuously available except during experimental sessions. Mice used in the acute convulsant studies were used only once and then euthanized. Mice in the kindling studies were used only for their respective test group and then euthanized after the last test session.

Chemoconvulsant Studies. The experiments were conducted with doses and routes of administration per the general protocol of Witkin et al. [61] with the exception that PTZ was given at 75 mg/kg by the s.c. route and that the mice were a different outbred strain than previously employed. Mice were given either vehicle, diazepam (DZP) (1 mg/kg, i.p.) or KRM-II-81 (30 mg/kg, i.p.) and placed into a holding cage for 30 min. DZP and KRM-II-81 studies for each chemoconvulsant were run in side-by-side experiments on the same day. The doses of DZP and KRM-II-81 have been shown previously to produce nearly full protection against PTZ-induced clonic convulsions.[18, 22, 23] After 30 min, the mice were given the convulsant agent and placed into individual observation chambers where they were observed for 60 min for clonic convulsions, tonic convulsions and lethality by trained observers. The percentage of mice exhibiting clonus, tonus, and lethality were recorded. Statistically significant differences from vehicle treatments were assessed by Fisher's Exact Probability test with $p<0.05$ as an a priori level of significance.

Seizure Kindling. Seizure kindling was established in separate groups of mice by giving close to subconvulsant doses of cocaine (COC) (60 mg/kg, i.p.) or PTZ (45 mg/kg), as previously described for COC[31, 32] where cocaine was given every day and for PTZ,[22, 23] where PTZ was given every other day. Each experiment was independently conducted twice with groups of 6 mice each. For PTZ seizure kindling, PTZ was given on days 1,3,5, and 8 and then the mice were tested with PTZ again on day 10. For COC, COC was given on days 1,2,3,4, and 5 and then tested with COC on day 6. Three separate groups of mice were studied in each of three separate experiments to study drug effects on 1) fully kindled seizures, 2) the expression of kindled seizures, and on 3) the development of kindling where either vehicle, DZP (1 mg/kg, i.p.), or KRM-II-81 (30 mg/kg, i.p.) was studied. Fully-Kindled Seizures: vehicle + PTZ or COC were given on kindling days/vehicle, DZP, or KRM-II-81with PTZ or COC given on test day; Expression of Kindling: Vehicle + PTZ or COC, DZP + PTZ or COC, or KRM-II-81 + PTZ or COC was given for each kindling day and for the test day; Development of Kindling: Vehicle, DZP, or KRM-II-81 + PTZ or COC was given on each kindling day/Vehicle + PTZ or COC was given on the test day. Data presented are the percentage of mice exhibiting convulsions; mice were not scored for seizure severity. The data were analyzed by two-way ANOVA analyzing treatment and treatment day with repeated measures on subjects. Bonferonni post-hoc tests compared each treatment day to its vehicle control ($p<0.05$ considered to be statistically significant a priori).

Chemistry

General Procedures for the Synthesis of HZ-166 (6). All reactions were performed in round-bottom flasks with magnetic stir bars or overhead mechanical stirrers under an argon atmosphere. Organic solvents were purified when necessary by standard methods or purchased from Sigma-Aldrich Chemicals were purchased from either Sigma Aldrich, Oakwood Chemical, Alfa Aesar, Matrix Scientific, Admiral Chemical Company, or Acros Organic. The ^{1}H and ^{13}C NMR data were obtained on Bruker Spectrospin 300 MHz and 500 MHz instruments with the chemical shifts in δ (ppm). The HRMS spectral data was obtained on a LCMS-IT-TOF by Shimadzu Scientific.

N*-(4-Bromo-2-picolinoylphenyl)-2-chloroacetamide (2c).** To a mixture of (2-amino-5-bromophenyl)(pyridine-2yl)methanone (**1**, 100 g, 360.9 mmol), sodium bicarbonate (60.6 g, 721.7 mmol), and dichloromethane (1000 mL), chloroacetyl chloride (43.0 mL, 541.3 mmol) was added dropwise over 60 min while keeping the temperature between 25 – 30°C. The bright red reaction mixture, which resulted, was then allowed to stir for more than 1 h at rt. The completion of the reaction was verified by analysis by TLC using silica gel and 50% ethyl acetate / hexanes. The reaction mixture was then slowly diluted over 30 min with water (500 mL) as carbon dioxide gas evolved. The biphasic mixture, which resulted, was allowed to stand for 15 min and the layers were separated. The aq layer was extracted with dichloromethane (500 mL) and the combined organic layers were washed with 5% aq sodium bicarbonate solution (500 mL) and then 10% aq sodium chloride solution (500 mL). The organic layer was dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was slurried with ethanol (500 mL) at 50 – 55°C for 30 min. Upon cooling to rt and after holding the temperature for 1 h, the solid was filtered and washed with ethanol (100 mL x 3). The solid was dried under vacuum at 40°C to afford the product **2c** as an off-white solid (115.2 g, 90.3%). Additional product (**2c**) was obtained as a 2nd crop by concentrating the filtrate (10.4 g, 8.2%). Total yield: 125.6g, 98.5%: ***Rf = 0.6 (EtOAc-hexanes, 1:1 and 1% of TEA); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 11.66 (s, 1H), 8.76 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1H), 8.58 (d, *J* = 9.0 Hz, 1H), 8.03 (dt, *J* = 7.8, 1.1 Hz, 1H), 8.01 (d, *J* = 2.4 Hz, 1H), 7.96 (td, *J* = 7.7, 1.7 Hz, 1H), 7.72 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.21 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.29, 165.34, 154.76, 148.83, 138.80, 137.43, 137.19, 136.88, 126.70, 124.99, 124.62, 122.99, 115.81, 43.15; **HRMS** (ESI/IT-TOF): m/z [M + H]+ calcd for $C_{14}H_{11}BrClN_2O_2$: 352.9693; found: 352.9690.

7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one (3). A mixture of *N*-(4-bromo-2-picolinoylphenyl)-2-chloroacetamide (**2c**, 115 g, 325.2 mmol), hexamethylenetetramine (HMTM, 100.3 g, 715.5 mmol), ammonium acetate (55.2 g, 715.5 mmol), and isopropanol (2000 mL) was heated to reflux (82°C). The reaction mixture was held at reflux for 4 h at which point the reaction was deemed complete by analysis by TLC (silica gel and 50% ethyl acetate / hexanes). The reaction mixture was then cooled to 0 – 5°C using an ice bath. The solid, which resulted, was filtered and washed with cold isopropanol (100 mL x 2) and then water (100mL x 4). The solid was dried under vacuum at 40°C to afford the product **3** as an off-white solid (75.1 g, 75%): **Mp** 228-229 °C; ***Rf*** = 0.4 (EtOAc-hexanes, 1:1 and 1% of TEA); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.57 (dd, *J* = 4.7, 0.7 Hz, 1H), 8.05 (d, *J* = 7.9 Hz, 1H), 7.95 (td, *J* = 7.7, 1.7 Hz, 1H), 7.71 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.51 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H), 7.43 (d, *J* = 2.3 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 4.23 (s, 2H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 170.36, 168.13, 156.32, 148.88, 139.31, 137.60, 134.43, 134.17, 127.93, 125.41, 123.93, 123.57, 114.50, 57.58; **HRMS** (ESI/IT-TOF): m/z [M + H]+ calcd for $C_{14}H_{11}BrN_3O$: 316.0080; found: 316.0076.

Ethyl 8-bromo-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (4). A mixture of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one (**3**, 90.5 g, 286.2 mmol) and tetrahydrofuran (1200 mL) was cooled to -20°C using a dry ice / IPA bath. A solution of potassium *t*-butoxide (41.8 g, 372.1 mmol) and tetrahydrofuran (300 mL) was added dropwise to the reaction mixture over a 30 min period, while maintaining the temperature at -20 to -15°C. Upon completion of the addition, the reaction mixture was allowed to stir for an additional 60 min at -20°C. Diethyl chlorophosphate (57.9 mL, 400.7 mmol) was then added

dropwise to the reaction mixture over 15 min while maintaining the temperature at -20 to -15°C. Upon completion of the addition, the reaction mixture was allowed to stir for an additional 2 h at -20 °C at which point the reaction was deemed complete by analysis by TLC (silica gel and 1% triethylamine / ethyl acetate). Ethyl isocyanoacetate (40.7 mL, 372.1 mmol) was then added dropwise to the reaction mixture over a 15 min period, while maintaining the temperature at -20 to -15°C. Immediately, a solution of potassium t-butoxide (41.8 g, 372.1 mmol) and tetrahydrofuran (300 mL) was added dropwise to the reaction mixture over 30 min while maintaining the temperature at -20 to -15°C. Upon completion of the addition, the reaction mixture was allowed to warm to rt and stirred for an additional 12 h at which point the reaction was deemed complete by analysis by TLC (silica gel and 1% triethylamine / ethyl acetate). The reaction mixture was then diluted with 5% aq sodium bicarbonate (1000 mL). The biphasic mixture, which resulted, was allowed to stand for 15 min and the layers were separated. The aq layer was then extracted twice with dichloromethane (1000 mL x 2) and the combined organic layers were washed with 5% aq sodium bicarbonate solution (1000 mL) and then 10% aq sodium chloride solution (1000 mL). The organic layer was dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was slurried with *t*-butyl methyl ether (1000 mL) at 50 – 55°C for 30 min. Upon cooling to rt and after holding for 12 h, the solid was filtered and washed with *t*-butyl methyl ether (100 mL x 3). The solid was then purified by stirring with ethanol (300 mL) at reflux for 1h. Upon cooling to -20°C and after holding at -20°C for 12 h, the solid was filtered off and washed with cold ethanol (50 mL x 2). The solid was dried under vacuum at 40°C to afford the product **4** as a light brown powder (61.0 g, 51.8%): **MP** 212-213 °C; ***Rf*** = 0.4 (EtOAc with 1% TEA); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 4.9, 0.9 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.89 (s, 1H), 7.81 (td, *J* = 7.8, 1.8 Hz, 1H), 7.78 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.59 (d, *J* = 2.2 Hz, 1H), 7.47 (d, *J* =

8.6 Hz, 1H), 7.37 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 1H), 6.12 (d, *J* = 12.6 Hz, 1H), 4.51 – 4.36 (m, 2H), 4.15 (d, *J* = 12.6 Hz, 1H), 1.43 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.08, 162.93, 156.20, 148.73, 138.43, 136.94, 135.32, 134.99, 134.57, 134.44, 129.37, 128.54, 124.90, 124.29, 123.99, 120.57, 60.81, 45.02, 14.45; **HRMS** (ESI/IT-TOF): m/z [M + H]+ calcd for $C_{19}H_{16}BrN_4O_2$: 411.0451; found: 411.0454.

Ethyl-6-(pyridin-2-yl)-8-((triisopropylsilyl)ethynyl)-4*H*-benzo[*f*]imidazo[1,5-*a*] [1,4]diazepine-3-carboxylate (5b). A mixture of palladium acetate (1.67 g, 7.4 mmol), tri-*o*-tolylphosphine (4.51g, 14.8 mmol) and acetonitrile (400 mL) was stirred for 30 min at rt. To the reaction mixture was then added in sequence ethyl 8-bromo-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (**4**, 61.0 g, 148 mmol), triethylamine (41.3 mL, 297 mmol), (triisopropylsilyl)acetylene (39.9 mL, 178 mmol) and additional acetonitrile (500 mL). The reaction mixture was then heated to reflux (75^{o}C) and held for 4 h at which point the reaction was deemed complete by analysis by TLC (silica gel and 1% triethylamine / ethyl acetate). Upon completion of the reaction, the mixture was cooled to rt and silica gel (25 g) was added. After stirring for 30 min, the spent silica gel was removed by filtration and washed with acetonitrile (100 mL x 2). The solvents were removed under reduced pressure and the residue was dissolved in dichloromethane (900 mL) and 5% aq sodium bicarbonate (900 mL). The biphasic mixture, which resulted, was allowed to stand for 15 min and the layers were separated. The aq layer was then extracted with dichloromethane (900 mL) and the combined organic layers were washed with 5% aq sodium bicarbonate solution (900 mL) and then 10% aq sodium chloride solution (900 mL). The organic layer was dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was purified by flash chromatography using silica gel (750 g) and 50% ethyl acetate /

hexanes with 5% triethylamine. The pure fractions were pooled and the solvents were removed under reduced pressure. The oil, which resulted, was dried under reduced pressure at 40°C for 2 h to afford the product **5b** as a clear, light brown oil (65.0 g, 85%): ***Rf*** = 0.6 (EtOAc with 1% TEA) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.55 (dt, *J* = 4.7, 1.4 Hz, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.89 (s, 1H), 7.78 (td, *J* = 7.8, 1.8 Hz, 1H), 7.70 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 1.8 Hz, 1H), 7.33 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 6.07 (d, *J* = 12.5 Hz, 1H), 4.42 (ddt, *J* = 30.1, 14.8, 7.4 Hz, 2H), 4.15 – 4.10 (m, 1H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.08 (d, *J* = 3.6 Hz, 21H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.74, 162.94, 156.47, 148.67, 138.48, 136.80, 135.70, 135.30, 134.93, 134.50, 129.24, 126.97, 124.74, 123.99, 122.71, 122.59, 104.89, 93.78, 60.71, 45.02, 18.58, 14.42, 11.20.

Ethyl-8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (HZ-166, 6). A mixture of ethyl 6-(pyridin-2-yl)-8-((triisopropylsilyl)ethynyl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate (**5b**, 65.0 g, 126.8 mmol), water (6.5 mL) and tetrahydrofuran (650 mL) was cooled to -20°C using a dry ice / IPA bath. Tetrabutylammonium fluoride hydrate, 1M in THF (145.4 mL, 145.4 mmoL) was added dropwise to the reaction mixture over a 30 min period, while maintaining the temperature at -20 to -15°C. Upon completion of the addition, the reaction mixture was allowed to warm to rt and stir for an additional 60 min at which point the reaction was deemed complete on analysis by TLC (silica gel and 1% triethylamine / ethyl acetate). The reaction mixture was then diluted with ethyl acetate (650 mL) and 10% aq sodium chloride (650 mL). The biphasic mixture, which resulted, was allowed to stand for 15 min and the layers were separated. The aq layer was then extracted with ethyl acetate (650 mL) and the combined organic layers were washed with 10% aq sodium chloride solution (650 mL). The

organic layer was dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was slurried with 2-propanol (IPA, 250 mL) at 70 – 75°C for 30 min. Upon cooling to -20°C and after holding for 2 h, the solid was filtered and washed with cold IPA (50 mL x 3) and then hexanes (50 mL x 3). The solid was dried under vacuum at 40°C to afford the product **6** as an off-white crystalline solid (38.4 g, 85%): **Mp** 204-205 °C; ***Rf*** = 0.4 (EtOAc with 1% TEA); **^{1}H NMR** (500 MHz, CDCl3) δ = 8.58 (d, *J* = 4.3 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 8.05 (s, 1H), 7.83 (dd, *J* = 11.8, 4.4 Hz, 1H), 7.78 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.57 (d, *J* = 1.0 Hz, 1H), 7.38 (dd, *J* = 6.7, 5.3 Hz, 1H), 6.16 (d, *J* = 11.4 Hz, 1H), 4.29 (d, *J* = 11.2 Hz, 1H), 3.19 (s, 1H), 2.85 (q, *J* = 7.6 Hz, 2H), 1.44 (t, *J* = 7.6 Hz, 3H); **^{13}C NMR** (126 MHz, CDCl3) δ 171.92, 170.75, 167.87, 156.29, 148.73, 137.08, 136.33, 136.15, 135.92, 135.46, 135.25, 127.08, 124.97, 124.78, 124.08, 122.87, 121.45, 81.60, 79.75, 44.93, 19.79, 11.56; **HRMS** (ESI/IT-TOF): m/z [M + H]+ calcd for $C_{21}H_{17}N_4O_2$: 357.1346; found: 357.1344.

Associated Content

Supporting Information: Proposed Imidate (**3c**) Formation Mechanism, NMR Evidence (1H-15N-HMBC) for Imidate Impurity **3c**, and Experimental for Aldehyde **7** and KRM-II-81 (**8**)

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Abbreviations Used

COC, cocaine; GABA, gamma-aminobutyric acid; PAM, positive allosteric modulator; PTZ, pentylenetetrazol

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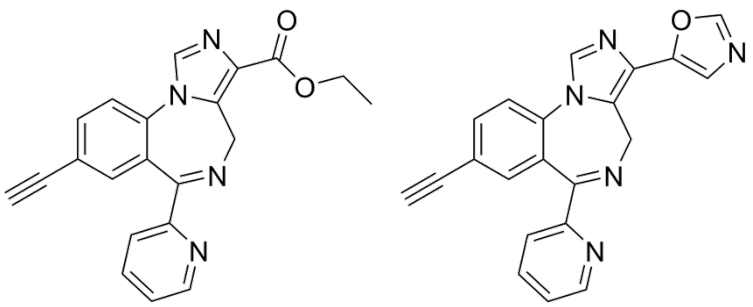
Figure 1: The Lead α2/3-Selective Ligands of this Work



HZ-166, 6 **KRM-II-81, 8**

Table 1: Effects of KRM-II-81 in Antiseizure-Detecting Models in Rodents and in Human Epileptic Brain Tissue Slices

Model System	Species	Activity	Efficacy	Reference
NEURONAL CULTURE				
Dissociated cortical neurons	Rat	Yes	ND	Witkin et al., 2018
CHEMOCONVULSANT MODELS				
Pentylenetetrazol – clonic seizures	Rat	Yes	= Diazepam	Witkin et al., 2018
ELECTRICAL SEIZURE PROVOCATION MODELS				
6Hz stimulation – 44mA	Mouse	Yes	ND	Witkin et al., 2018
Electroconvulsive Shock*	Mouse	Yes	= Diazepam	Witkin et al., 2018
SEIZURE KINDLING				
Corneal kindling	Mouse	Yes	>Tpm	Witkin et al., 2020
Amygdala kindling-ADT	Rat	Yes	> Diazepam	Witkin et al., 2018
Amygdala kindling-ADD	Rat	Yes	= Diazepam	Witkin et al., 2018
Amygdala kindling-Seizure Severity	Rat	Yes	= Diazepam	Witkin et al., 2018
PHARMACORESISTANT MODELS				
Mesial temporal lobe epilepsy	Mouse	Yes	>Ltg, Val	Witkin et al., 2020
Ltg-insensitive kindling	Rat	Yes	>Ltg, Tpm	Witkin et al., 2020
Kainate-induced chronic epilepsy	Rat	Yes	>Ltg, Lev	Witkin et al., 2020
HUMAN EPILEPTIC TISSUE				
Picrotoxin stimulation	Human	Yes	ND	Witkin et al., 2018
4-Aminopyridine stimulation	Human	Yes	ND	Witkin et al., 2018

ADT – After-discharge threshold, ADD- After-discharge duration, Tpm – topiramate, Ltg – lamotrigine,Val – valproate, Lev – levetiracetam, ND – no comparative data

*Current was delivered to corneal electrodes at 10μA for 0.2 s. Under these conditions in mice, seizures (tonic extension) were induced in 94 ± 2.5% of the mice tested in the absence of antiseizure drugs (vehicle control).

Table 2: Comparative effects of diazepam and KRM-II-81 as anticonvulsants against some chemoconvulsants[a]

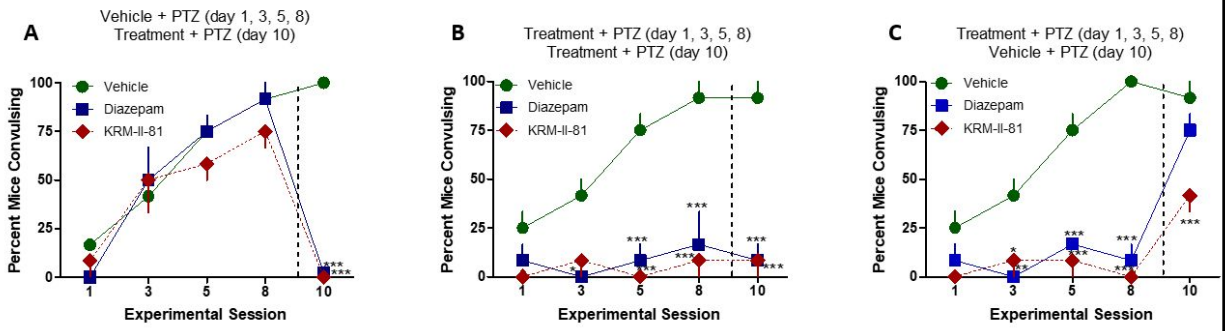
Drug	Event	Vehicle	Diazepam	KRM-II-81
Cocaine	Clonus	8/8	6/10	4/10*
75 mg/kg, i.p.	Tonus	0/8	0/10	0/10
	Lethality	0/8	0/10	0/10
PTZ	Clonus	8/8	2/8*	1/8*
75 mg/kg, s.c.	Tonus	6/8	2/8*	1/8*
	Lethality	7/8	0/8*	0/8*
4-AP	Clonus	8/8	5/8	2/8*
14 mg/kg, i.p.	Tonus	7/8	4/8	1/8*
	Lethality	7/8	1/8*	0/8*
NMDA	Clonus	7/8	4/8	2/8*
200 mg/kg, i.p.	Tonus	0/8	0/8	0/8
	Lethality	7/8	4/8	2/8*
Pictrotoxin	Clonus	8/8	2/8*	1/8*
6 mg/kg, i.p.	Tonus	4/8	2/8	0/8*
	Lethality	5/8	2/8	0/8*
Strychnine	Clonus	8/8	6/8	3/8*
2 mg/kg, i.p.	Tonus	8/8	6/8	3/8*
	Lethality	8/8	7/8	4/8*
Pilocarpine[b]	Clonus	8/8	2/8*	1/8*
300 mg/kg, i.p.	Tonus	3/8	1/8	0/8
	Lethality	7/8	2/8*	0/8*

[a]Studies were conducted in male, C57BL/6 mice using ~ED95 doses of the chemoconvulsants to induce clonus. Diazepam: 1 mg/kg, s.c., 30 min prior to chemoconvulsant; KRM-II-81: 30 mg/kg, i.p., 30 min prior to chemoconvulsant

[b]Pilocarpine was given acutely like the other chemoconvulsants and induced acute seizures and lethality (status epilepticus was not studied).

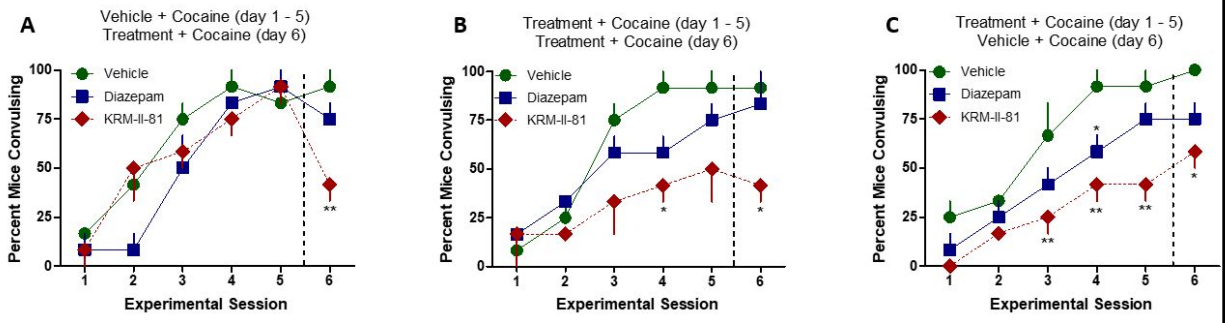
*Significantly different than effects of vehicle for each of the endpoints (clonus, tonus, lethality) by Fisher's Exact Probability test, $p < 0.05$.

Figure 2: Effects of Vehicle, Diazepam (1.0 mg/kg), or KRM-II-81 (30 mg/kg) on Seizure Kindling from Every Other Day Dosing with PTZ (45 mg/kg).



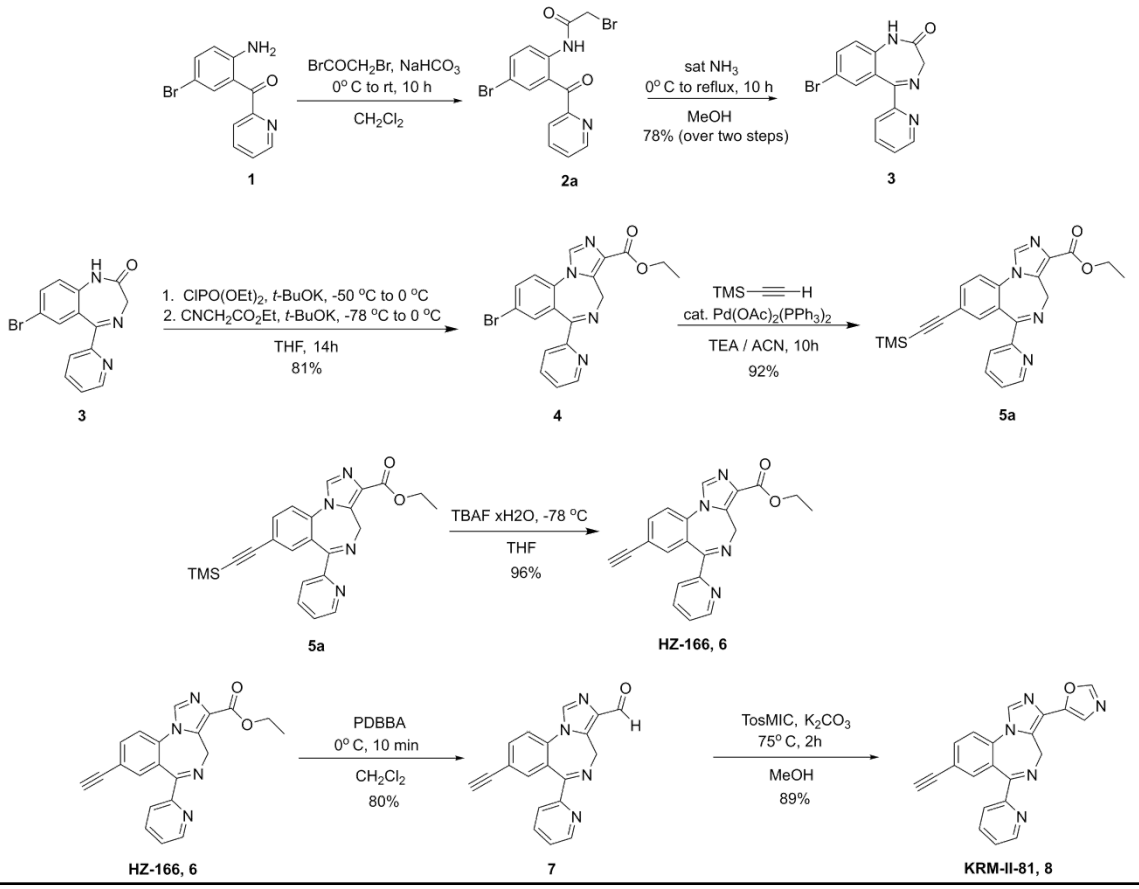
A. Effects of compounds on fully developed PTZ kindled seizures. **B**. Effects of compounds on the expression of PTZ kindling. **C.** Effects of compounds on the development of PTZ kindling. Each point represents the mean ± S.E.M. of 6 mice each. Data presented are the percentage of mice exhibiting convulsions; mice were not scored for seizure severity. Statistical evaluation of the data with two-way ANOVA with repeated measures followed by Bonferroni post-hoc tests comparing vehicle to diazepam or KRM-II-81 – **A.** Effects of compound treatment: $F_{2,12}$=8.25, p=0.06; Experimental session: $F_{4,12}$=49, p<0.001; Interaction: $F_{8,12}$=10.2, p<0.001; Subjects: $F_{3,12}$=1.92, p=0.18. **B.** Effects of compound treatment: $F_{2,12}$=409, p< 0.001; Experimental session: $F_{4,12}$=5.22, p<0.05; Interaction: $F_{8,12}$=3.22, p<0.05; Subjects: $F_{3,12}$=0.16, p=0.92. **C.** Effects of compound treatment: $F_{2,12}$=154, p< 0.001; Experimental session: $F_{4,12}$=30.7, p<0.0001; Interaction: $F_{8,12}$=6.89, p<0.05; Subjects: $F_{3,12}$=0.55, p=0.66.

Figure 3: Effects of Vehicle, Diazepam (1.0 mg/kg), or KRM-II-81 (30 mg/kg) on Seizure Kindling from Daily Dosing with COC (60 mg/kg).

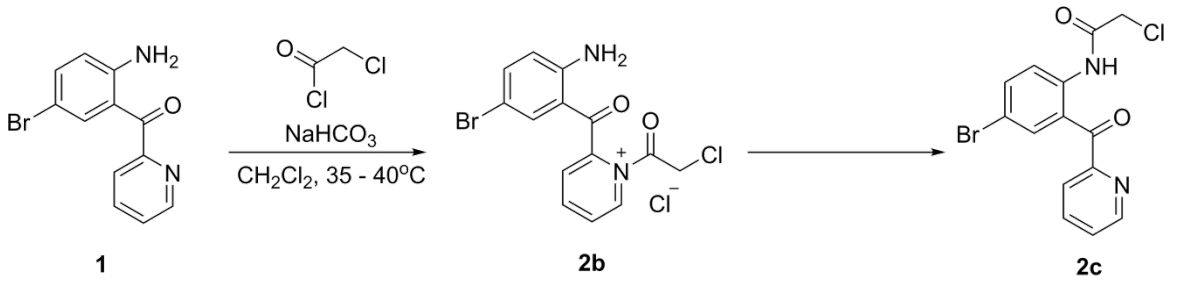


A. Effects of compounds on fully developed COC kindled seizures. **B**. Effects of compounds on the expression of COC kindling. **C.** Effects of compounds on the development of COC kindling. Each point represents the mean ± S.E.M. of 6 mice each. Data presented are the percentage of mice exhibiting convulsions; mice were not scored for seizure severity. Statistical evaluation of the data with two-way ANOVA with repeated measures followed by Bonferroni post-hoc tests comparing vehicle to diazepam or KRM-II-81 – **A.** Effects of compound treatment: $F_{2,15}=2.75$, $p=0.21$; Experimental session: $F_{5,15}=37.8$, $p<0.0001$; Interaction: $F_{10,15}=2.95$, $p<0.05$; Subjects: $F_{3,15}=1.77$, $p=0.20$. **B.** Effects of compound treatment: $F_{2,15}=18.0$, $p<0.05$; Experimental session: $F_{5,15}=17.3$, $p<0.0001$; Interaction: $F_{10,15}=1.61$, $p=0.20$; Subjects: $F_{3,15}=0.74$, $p=0.54$. **C.** Effects of compound treatment: $F_{2,15}=109$, $p<0.05$; Experimental session: $F_{5,15}=28.1$, $p<0.0001$; Interaction: $F_{10,15}=0.78$, $p=0.65$; Subjects: $F_{3,15}=0.26$, $p=0.85$.

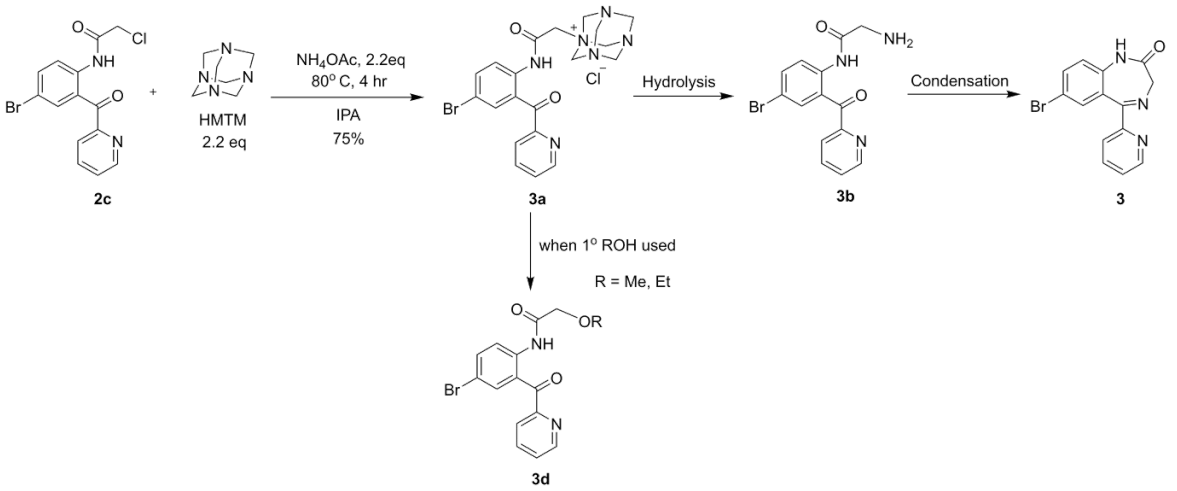
Scheme 1: Discovery Synthesis of KRM-II-81(**8**)[15, 24]



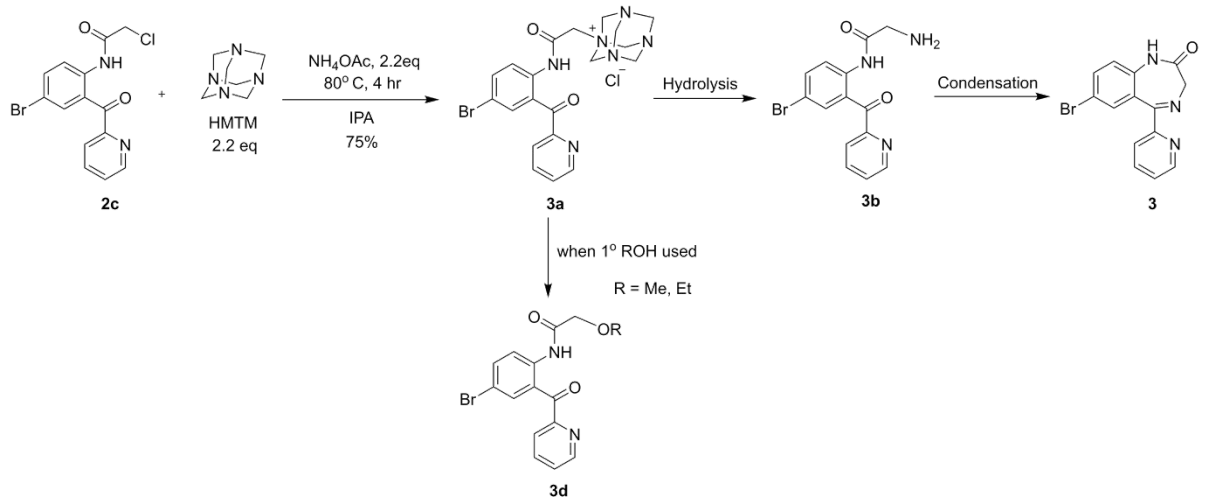
Scheme 2: Proposed Route to Chloroacetamide (**2c**) Via Pyridium Salt (**2b**)



Scheme 3: Discovery Route to Benzodiazepine (**3**) with Imidate Impurity (**3c**)



Scheme 4: Hexamethylenetetramine-(HMTM)-based Cyclization Reaction



Scheme 5: Synthetic Route to Imidazodiazepine **4**

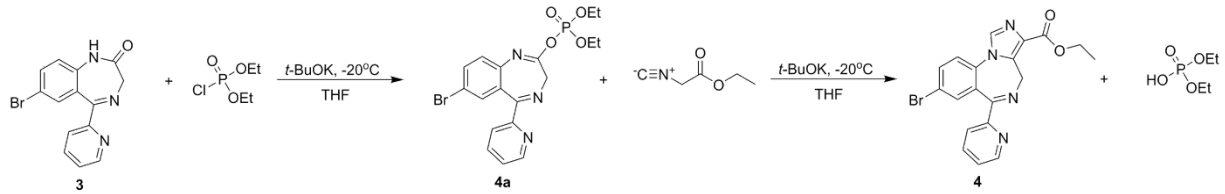
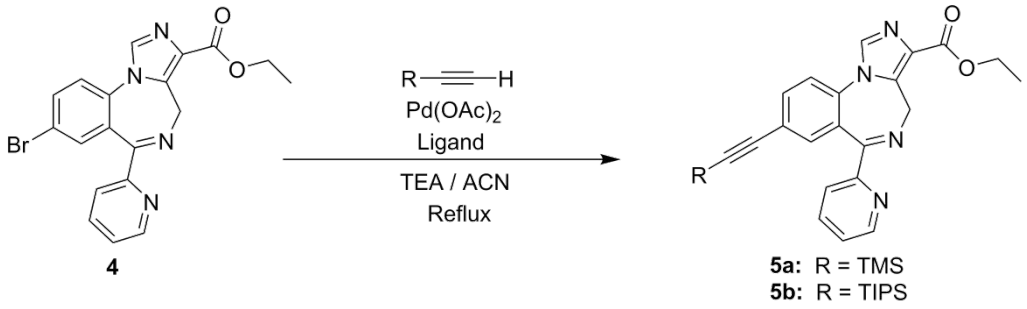


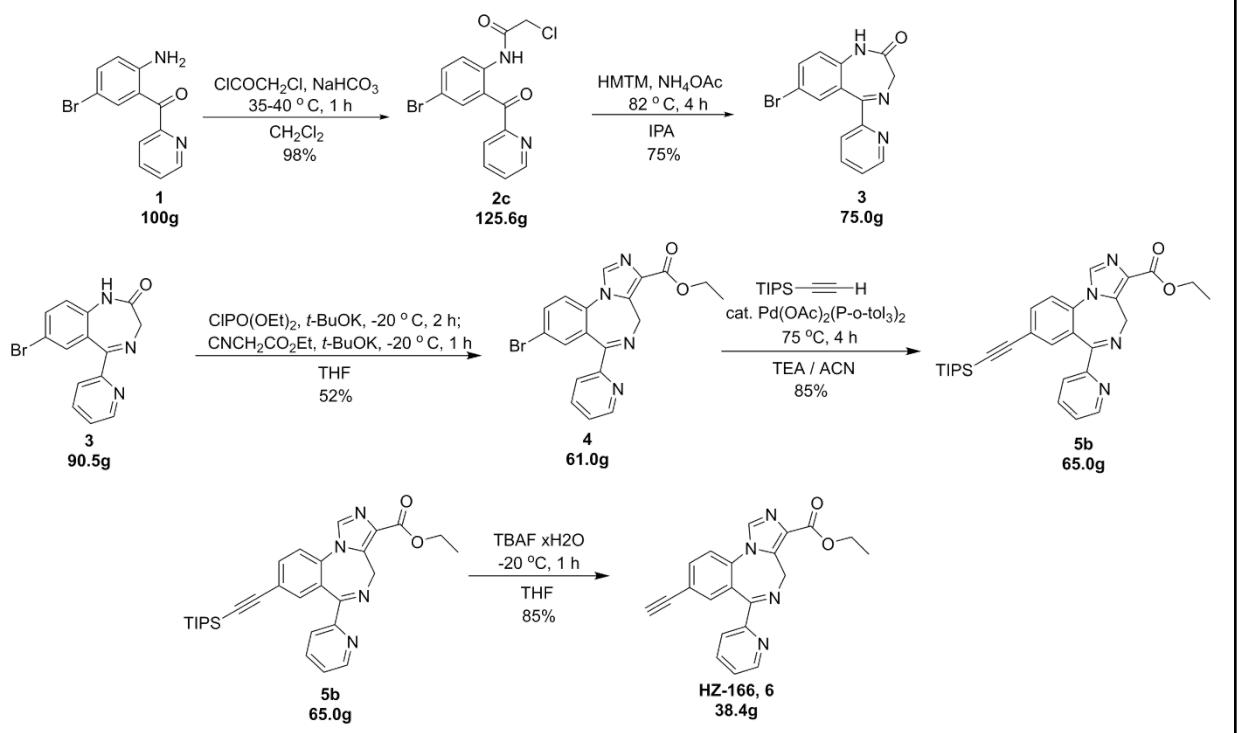
Table 3: Copper-Free Sonogashira Coupling Trials



Entry	Acetylene	Ligand	TEA Qty	Degassing?	Reaction Time
1	TMS (1.5 eq)	PPh_3	Solvent	yes	15 h
2	TMS (1.5 eq)	P-o-tol_3	Solvent	no	6 h
3	TIPS (1.2 eq)	P-o-tol_3	Solvent	no	4 h
4	TIPS (1.2 eq)	P-o-tol_3	2.0 eq	no	4 h

Reactions were monitored for completion using TLC analysis. Samples were pulled every 60 minutes and spotted on silica gel plates. Mobile phase = ethyl acetate with 1% TEA. Respective *Rf's*: **4** = 0.3, **5a** = 0.5, **5b** = 0.7. Disappearance of starter **4** indicated reaction completion.

Scheme 6: Improved Large-Scale Synthesis of HZ-166 (**6**)



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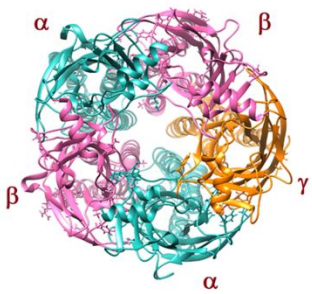
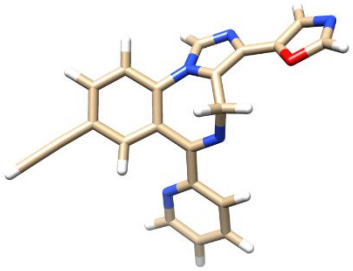
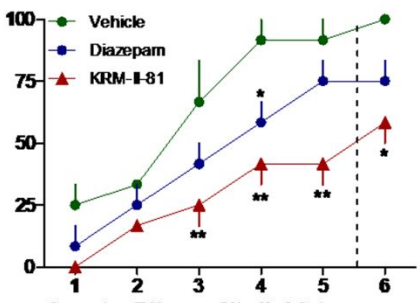


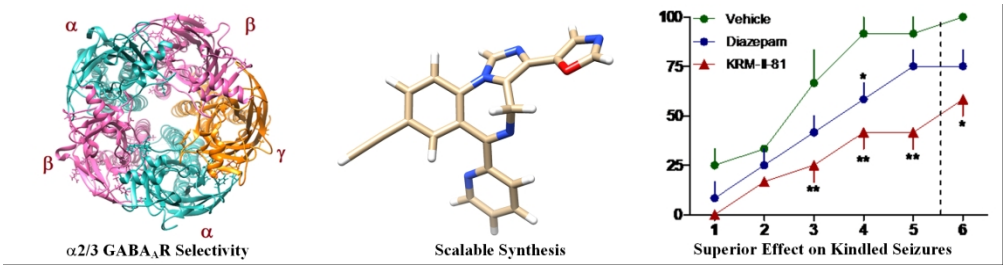
α2/3 GABA$_A$R Selectivity



Scalable Synthesis



Superior Effect on Kindled Seizures



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