Design, synthesis and characterization of novel gamma-aminobutyric acid type A receptor ligands

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We dedicate this work to Professor Jan Bergman for his outstanding contributions to heterocyclic and organic chemistry as a whole

Abstract

Antinociceptive ligand HZ-166 is a GABA$_A$ $\alpha2/\alpha3$ receptor subtype-selective potentiator. It has been shown to exhibit anxiolytic-like effects in rodent and rhesus monkeys, as well as reduced sedative/ataxic liabilities. In order to improve the metabolic stability of HZ-166, the ethyl ester moiety was bioisosterically replaced with 2,4-disubstituted oxazoles and oxazolines. The new analogs of HZ-166 were synthesized, characterized, and evalutated for their biological activity and docked in the human full-length heteromeric α1β3γ2L GABA$_A$ receptor subtype CyroEM structure (6HUO). Importantly no sedation nor ataxia was observed on the rotorod for LKG-I-70 (**6**) or KPP-III-51 (**6c**) at 100 and 120 mg/kg, respectively. There was also no loss of righting response for either ligand.
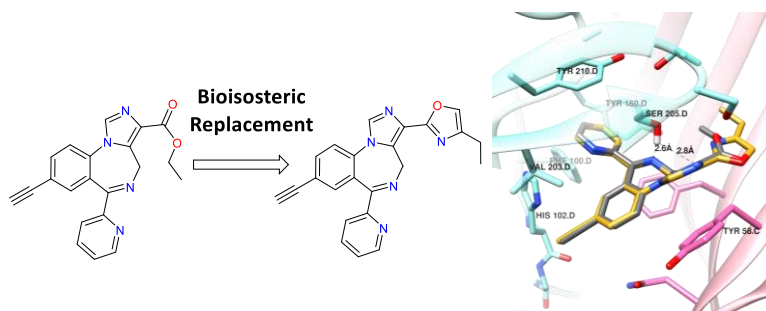
Introduction

Benzodiazepines (BDZs) act on gamma-amino-butyric acid A (GABA$_A$) receptor ion channels and allosterically potentiate the influx of chloride ions through the channel, imparting a hyperpolarized state to the neuron. The pharmacological action exerted by a BZD is dependent on the discrete subunits of the receptor complex. Convergent evidence from transgenic animals and molecules with selectivity for the proteins required for ligand gating has suggested that $\alpha_1\beta_{2/3}\gamma_2$ comprised GABA$_A$ receptors mediate the tolerance and sedative/ataxic effects of the drugs, whereas the α2 and α3 GABA$_A$ receptors mediate the anticonvulsant anxiolytic and antinociceptive effects of ligands distinct from sedative/ataxic effects.[1-3] A compelling clinical opportunity exists in the development of selective α1-sparing subtype GABA$_A$ receptor ligands. These new ligands are expected to result in superior treatments for seizures and anxiety without causing amnesia, sedation, ataxia, or the propensity for addiction/dependence.

The ligand HZ-166 (ethyl 8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate) is an α2/α3-subtype selective imidazodiazepine that has been reported to produce anticonvulsant activity at non-motor-impairing doses in both mice (maximal electroshock, and PTZ) and rats (maximal electroshock, PTZ, and hippocampal kindling).[4-9] Imidazodiazepines have very different structures as compared to BZDs diazepam, alprazolam and chlorodiazepoxide. However, the ester functionality of HZ-166 is metabolically labile creating reduced bioavailability when compare to the oxazole KRM-II-81 (5-(8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)oxazole).[6] It is well known that molecules which contain ester functional groups are susceptible to hydrolysis. Hydrolysis of the ester function results in the formation of the corresponding carboxylic acid and alcohol. Ester bioisosteres have been utilized commonly in drug design in order to render the ester topology metabolically stable with less adverse effects and provide better clinical candidates. Prior results indicated that replacement of ester functions by a five membered ring (oxazole or oxadiazole) significantly increased the *in vitro* and *in vivo* stability.[6, 10-12] Moreover, the efficacy at GABA$_A$ BZ receptors was enhanced, as compared to the corresponding ester ligands. Encouraged by the previous results, the substituted novel oxazoles and oxazolines were designed, synthesized and characterized.
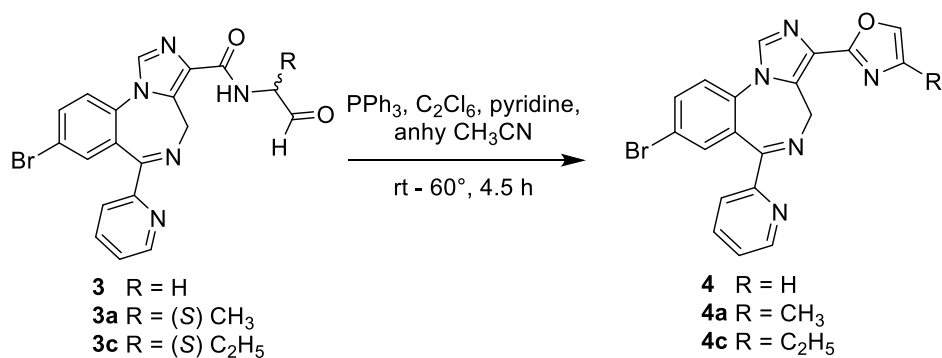
Results and Discussion

Chemistry

Recently our research group published an improved large-scale synthesis of the ethyl ester **1**.[13] The ester **1** was then heated to 90 °C with substituted ethanolamines (individually) in a sealed tube to afford α-hydroxy amides **2a-2d** that were purified by chromatography and in 80-98% yield. The α-hydroxy amides **2**, **2a** and **2c** were then oxidized by treatment with the Dess-Martin reagent to provide the corresponding α-acylaminoaldehydes **3**, **3a** and **3c**, which were purified by crystallization, in 75-81% yield (Scheme 1).

        To afford oxazoles **4**, **4a** and **4c** from α-acylaminoaldehydes **3**, **3a** and **3c**, a cyclodehydration reaction (Robinson–Gabriel synthesis) was employed. At first, the Burgess reagent was employed to dehydrate analog **3**. The aldehyde **3** was dissolved in anhydrous THF and then the Burgess reagent was added after which the mixture was heated to reflux for 1.5 hours. The above reaction resulted in poor yields (20-24%, entries 1 and 2). The yield of the reaction was highest with newly purchased Burgess reagent and decreased with storage over time, as expected. The Wipf group had reported the Burgess reagent was oxidative but moisture sensitive; they also obtained the best yields with freshly prepared Burgess reagent.[14] This group also

demonstrated that 5-unsubtituted oxazoles can be synthesized from α-acylaminoaldehydes by using triphenylphosphine and iodine.[15] Unfortunately, reaction of triphenylphosphine and iodine gave a poor yield of the oxazole **4** (entry 3) from **3**. Examination of a publication by Morwick, T., *et. al*. indicated modified reaction conditions, in which triphenylphosphine and hexachloroethane were employed, to synthesize 5-unsubtituted oxazoles from α-acylaminoaldehydes looked promising.[16] Fortunately, the triphenylphosphine and hexachloroethane-mediated reaction conditions gave better yields of the oxazole **4** (entries 4 and 5, Scheme 2).
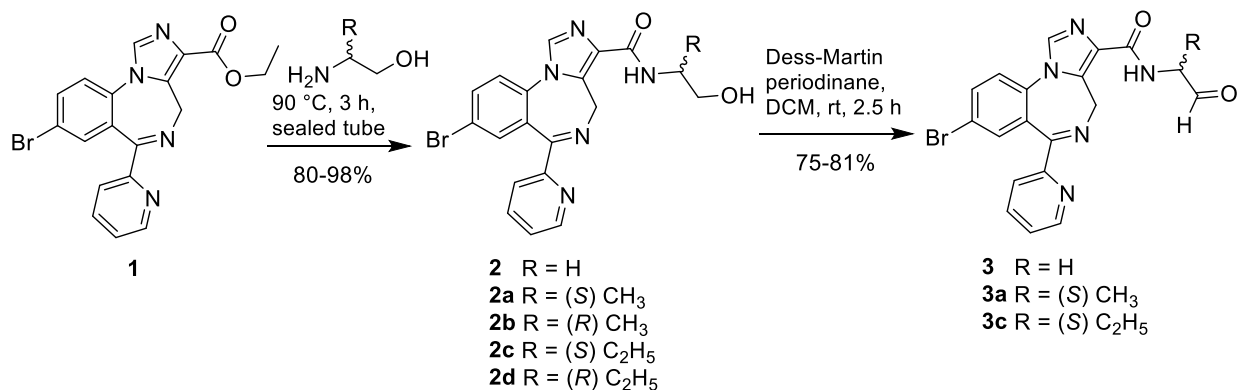
**Scheme 1.** Synthesis of α-acylaminoaldehydes **3**, **3a** and **3c**.

Scheme 2. Synthesis of oxazoles **4**, **4a** and **4c**.

Entry	Reagents and conditions	Yield
1	Burgess reagent (2 eq), THF (anhyd), 1.5 h, reflux	20%
2	Burgess reagent (3 eq), THF (anhyd), 1.5 h, reflux	24%
3[15]	PPh$_3$ (2 eq), I$_2$ (2 eq), CH$_3$CN (anhyd), Et$_3$N (4.5 eq), 1.5 h, rt	28%
4[16]	PPh$_3$ (2 eq), C$_2$Cl$_6$ (2 eq), CH$_3$CN (anhyd), pyridine (4 eq), 2 h, 60 °C	55%
5[16]	PPh$_3$ (3 eq), C$_2$Cl$_6$ (3 eq), CH$_3$CN (anhyd), Et$_3$N (6 eq), 2 h, 0 °C-rt	71-74%

The five membered heterocyclic oxazolines **5-5d** were also employed as bioisosteric replacement of carboxylic acid esters.[17] The α-hydroxy amides **2a-2d** reacted with thionyl chloride in dichloromethane to provide α-halo amides (not shown). The crude α-halo amides were then heated with sodium hydroxide in ethanol to afford the cyclized five-membered oxazolines **5-5d** in 75-85% yield (Scheme 3).[18-19] The optical rotation of the chiral oxazolines **5a-5d** were measured. The chiral oxazolines **5c** and **5d** were also evaluated by

chiral HPLC to detect any racemization. Examination of the data obtain indicated that there was no racemization (Supplementary material).
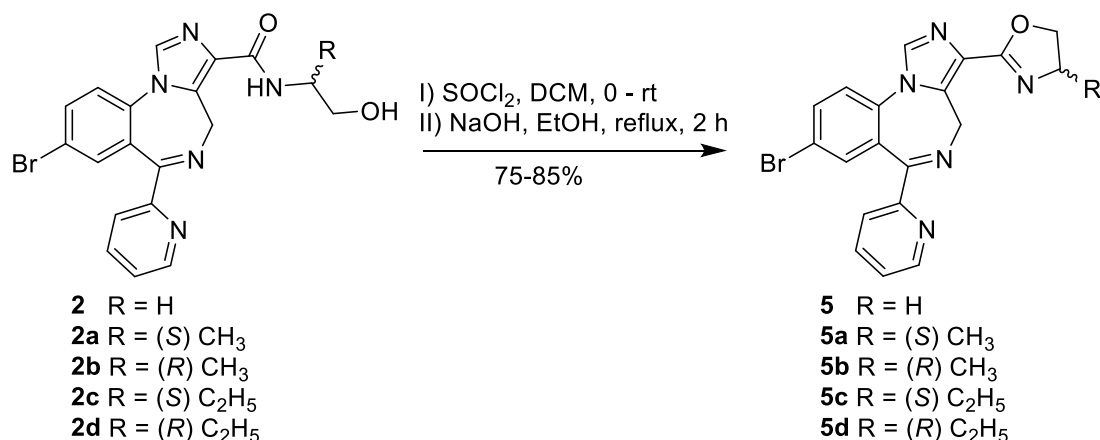
Scheme 3. Synthesis of oxazolines **5-5d.**

The aryl bromide moiety in imidazodiazepines **4** and **4c** was subjected to a copper-free Sonogashira coupling[20-21] to afford the triisopropylsilyl (TIPS) protected acetylenes (not shown). Finally, deprotection of the TIPS group with fluoride anion provided the 8-ethinyl imidazodiazepines **6** and **6c** in 72-75% yield (Scheme 4).
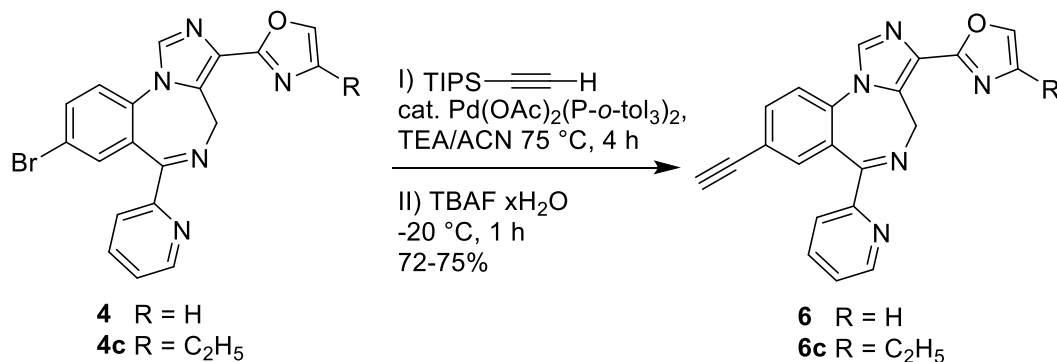
**Scheme 4.** Synthesis of 8-ethinyl imidazodiazepines **6** and **6c.**

Molecular modeling

To determine if the ester replacement in **4, 4a, 4c, 5-5d, 6** and **6c** could undergo a similar binding pose as HZ-166, molecular docking was performed using AutoDock Vina 1.5.6.[22] A recently published CryoEM structure of the human full-length α1β3γ2L GABA$_A$ receptor ion complex with alprazolam (PDB: 6HUO) by Masiulis S. *et al*[23] was used. The molecular docking scores (binding affinity) of the designed ligands were determined and noted (Table 1). The binding poses of all ligands described here was found to be similar to HZ-166 (not shown). Illustrated in Figure 1 is the overlay of the binding pose of oxazole **6c** and HZ-166. The binding affinities (based on docking scores) of all ligands was found to be within ±1 kcal/mol difference from the lead compound HZ-166 (-9 kcal/mol), which suggested all bioisosteres would bind with the receptor complex with a similar affinity to the lead compound ester HZ-166. The halogen substituted benzodiazepines e.g. diazepam and alprazolam undergo a halogen bond interaction with the carbonyl oxygen of the backbone of the α1His102 amino acid in the CryoEM structure (Figure S1 in Supplementary material). Examination of the docking of 8-bromo

substituted bioisosteres **4**, **4a**, **4c** and **5-5d** shows a similar halogen bond interaction with the carbonyl oxygen of the backbone of the α1His102 amino acid, but most of the docking software (including AutoDock Vina)[24] does not include halogen bonding in their scoring functions and, therefore, are unable to successfully predict the correct docking score of such complexes. Therefore, it was expected the ligands with bromine at position 8 would bind stronger with the α1β3γ2L GABA$_A$ receptor complex, as compared to the 8-ethinyl substituted ligands (**6** and **6c**) and would exert increased motor side effect. A general method for the molecular modeling is provided in the Supplementary material.

Table 1. Docking score (binding affinity) of bioisosteric ligands

| Comp	Binding affinity kcal/mol	Comp	Binding affinity kcal/mol
4	-9	**5b**	-9.8
4a	-9.8	**5c**	-9.3
4c	-9.1	**5d**	-9.5
5	-9.6	**6**	-9.9
5a	-10	**6c**	-9.6
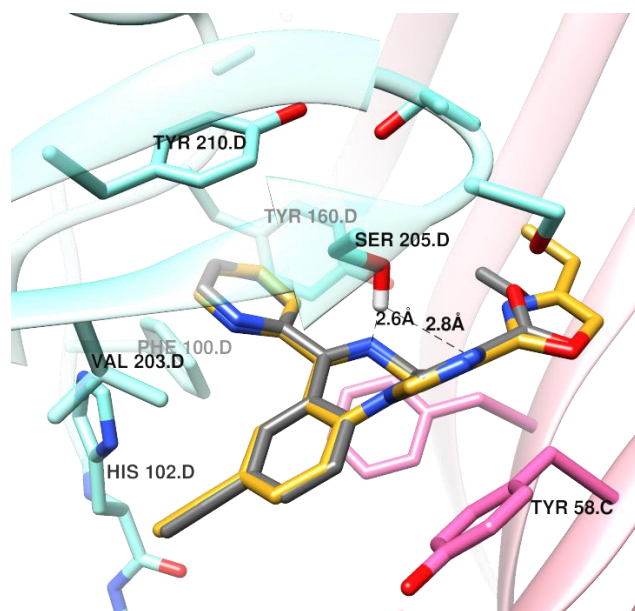
Figure 1. Overlay of the docked conformations of HZ-166 (gray) and **6c** (gold), in the complex with the α1β3γ2L GABA$_A$ receptor 6HUO at the $\alpha^+\gamma^-$ interface BZ binding site [α1 (represented as chain D, color: aquamriane) and γ2 ((represented as chain C, color: orchid)], dashed lines indicate hydrogen bonds.

Rotarod study

The ethinyl analog **6c** was evaluated for potential motor side effects. The motor/sensory study was carried out in CFW mice on a rotating rod (rotarod). The experiment was conducted by placing mice on the rotarod for a maximum of 3 minutes after oral administration of **6c** at doses of 40, 80 and 120 mg/kg. The mice were also observed for loss of righting reflex, an indication of undesired CNS effects. The mice exhibited no sedation nor ataxia, or loss of righting reflex; in contrast diazepam (5mg/kg) significantly impaired rotarod performance (Figure 2). The ligand (**6c**) exhibited sensorimotor steadiness at all three time-points, which indicated no sedative/ataxic effects (Figure 2). The rotarod experiment was performed according to the previously

published protocol.[25] In addition, **LKG-I-70** (**6**) was assayed on the rotarod and no sign of sedation nor ataxia was observed even up to 100 mg/kg. There was also no loss of righting response. Based on this early data both 1,3-oxazoles have potential clinical use. There is much work to be done to determine if this holds up in other cases.
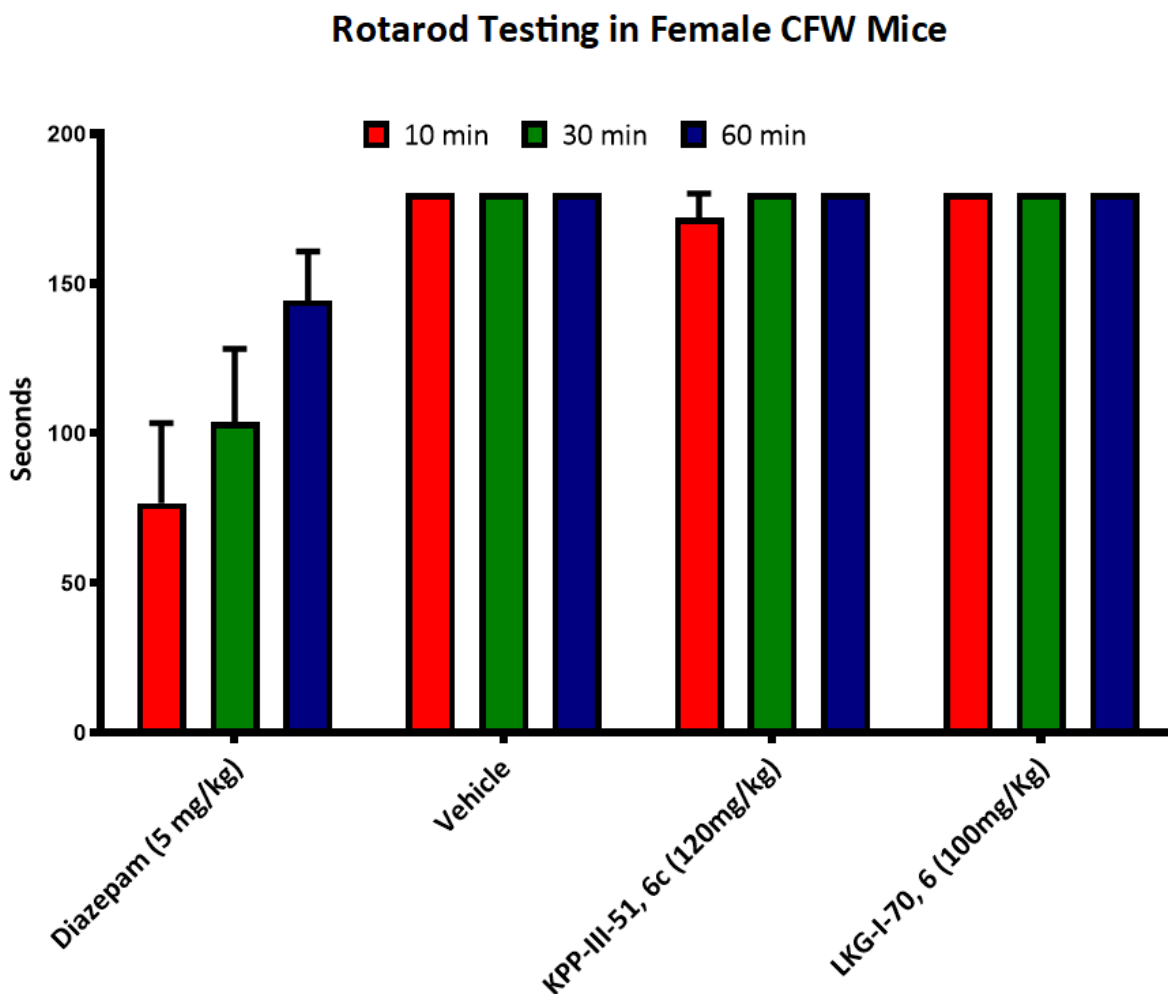
Figure 2. The rotarod study in CFW mice.

Conclusion

The 2-substituted novel oxazole and oxazoline analogs of α2/α3-subtype selective imidazodiazepine HZ-166 were synthesized and characterized. The new chiral oxazolines were analyzed for any racemization by measuring the optical rotation, as well as chiral HPLC; no racemization was detected. These new analogs (especially **6** and **6c**) should be more metabolically stable than HZ-166 with potentially less adverse effects and provide better clinical candidates. Examination of the molecular docking study suggests these molecules can bind with the α1β3γ2L GABA$_A$ receptor in a similar pose as HZ-166 but not as tightly. Ligands **6** and **6c"** were also evaluated for motor side effects (rotarod) and neither exhibited sedative nor ataxic effects. Therefore, 2-substituted oxazole and oxazoline bioisosteres of ethyl ester HZ-166 appear to be novel targets for the potential treatment of epilepsy, anxiety and neuropathic pain, by acting via selective positive allosteric

amplification of GABA$_A$ signaling via α2/α3-containing GABA$_A$ receptors. From examination of the data further modification of 8-bromo oxazolines **5-5d** to 8-ethinyl oxazolines is suggested and ongoing. We are also in the process of analyzing these new analogs in various animal models (anxiolytic, anticonvulsant and antinociception).

Experimental Section

General: For all reactions oven-dried round-bottom flasks or screw-cap test tubes were used, unless otherwise specified. All chemicals were purchased from commercial suppliers and purified by standard methods, if required. For all organic reactions anhydrous solvents were employed unless specified. The progress of reactions was visualized with TLC plates from Dynamic Adsorbents, Inc. under a UV light. The flash column chromatography was done for purification of some analogs on silica gel (230-400 mesh, Dynamic Adsorbents). The melting points were determined on a Stuart melting point SMP3 apparatus manufactured by Barloworld Scientific US Ltd. The CHIRALPAK® IB-N3 HPLC Column from Daicel Corporation was used for confirmation of enantiomeric excess. The ^{1}H and ^{13}C NMR spectra were obtained on Bruker Spectrospin 500 MHz or 300 MHz instruments in CDCl$_3$ and chemical shifts were reported in δ (ppm). Multiplicities are represented as follows: singlet (s), broad signal (br), doublet (d), triplet (t), quartet (q), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets) and multiplet (m). The technique employed for HRMS was carried out on a LCMS-IT-TOF at the Milwaukee Institute for Drug Discovery in the Shimadzu Laboratory for Advanced and Applied Analytical Chemistry.

8-Bromo-*N*-(2-hydroxyethyl)-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide　　(2). The mixture of **1** (2 g, 4.8 mmol) and excess 2-aminoethan-1-ol (8 mL) was heated in a sealed tube at 90 °C for 20 h. Analysis by TLC (silica gel, EtOAc/hexane/methanol 2:1:0.5 and a few drops of 14% aq NH$_4$OH) indicated the disappearance of starting material and the appearance of a new spot (R$_f$ = 0.3). After which the reaction mixture was cooled to rt and diluted by addition of EtOAC (30 mL) and water (20 mL). The organic layer was separated, and the aq layer was extracted with EtOAc (20 mL x 3). The combined organic layer was washed with brine (20 mL) and dried (Na$_2$SO$_4$). The solvent was removed under reduced pressure and the residue was purified by crystallization (20% MeOH in EtOAc) to afford **2** as light-yellow colored needle shape crystals (1.94 g, 94%). mp 265-266 °C (20% MeOH in EtOAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.61 − 8.56 (m, 1H), 8.09 (d, *J* 7.9 Hz, 1H), 7.92 (s, 1H), 7.83 − 7.76 (m, 2H), 7.57 (d, *J* 2.2 Hz, 1H), 7.48 (d, *J* 8.6 Hz, 1H), 7.39-7 (m, *J* 7.5, 4.8, 1.1 Hz, 1H), 6.07 (d, *J* 12.2 Hz, 1H), 4.47 − 4.43 (m, 2H), 4.17 (d, *J* 12.2 Hz, 1H), 4.11 (t, *J* 19.0, 6.0 Hz, 2H). ^{13}C NMR (126 MHz, CDCl$_3$): δc 167.0 (s), 160.1 (s), 156.3 (s), 148.7 (s), 136.9 (s), 135.4 (s),135.3 (s) 134.9 (s), 134.6 (s), 128.5 (S), 127.3 (s), 124.8 (s), 124.3 (s), 124.2 (s), 124.2 (s), 120.3(s), 64.9 (s), 54.7 (s), 45.0 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{19}$H$_{16}$BrN$_5$O$_2$ 426.0566; found 426.0543.

(*S*)-8-Bromo-*N*-(1-hydroxypropan-2-yl)-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (2a). The carboxamide **2a** was prepared from **1** by following the same procedure described for synthesis of **2** from **1**. The (*S*)-2-aminopropan-1-ol (8 mL) was used instead of 2-aminoethan-1-ol. The crude residue was purified by crystallization from 15% MeOH in EtOAc to provide **2a** as light-yellow colored needle shape crystals (1.64g, 98%). mp 259-260 °C (20% MeOH in EtOAc). ^{1}H NMR (300 MHz, CDCl$_3$): δ$_H$ 8.55 (d, *J* 4.7 Hz, 1H), 8.14 (d, *J* 7.9 Hz, 1H), 7.90 − 7.81 (m, 1H), 7.77 (dd, *J* 8.6, 2.7 Hz, 2H), 7.59 (d, *J* 2.3 Hz, 1H), 7.50 − 7.39 (m, 1H), 7.39 − 7.32 (m, 1H), 7.24 (d, *J* 7.2 Hz, 1H), 6.24 (d, *J* 10.8 Hz, 1H), 4.22 (qd, *J* 6.7, 3.2 Hz, 1H), 4.12 (s, 1H), 3.75 (d, *J* 9.3 Hz, 1H), 3.64 (dd, *J* 11.0, 6.4 Hz, 1H), 1.98 (s, 1H), 1.28 (d, *J* 6.8 Hz, 3H). ^{13}C NMR (75 MHz,

CDCl$_3$): δ$_C$ 166.8 (s), 163.4 (s), 156.3 (s), 148.5 (s), 136.9 (s), 135.9 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.2 (s), 128.6 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.4 (s), 67.6 (s), 48.0 (s), 44.8 (s), 17.1(s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{18}$BrN$_5$O$_2$ 440.0722; found 440.0694.

(*R*)-8-Bromo-*N*-(1-hydroxypropan-2-yl)-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (2b). The carboxamide **2b** was prepared from **1** by following the same procedure described for synthesis of **2** from **1**. The (*R*)-2-aminopropan-1-ol (8 mL) was used instead of 2-aminoethan-1-ol. The crude residue was purified by crystallization from 15% MeOH in EtOAc to provide **2b** as light-yellow colored needle shape crystals (1.53 g, 85%). mp 261-262 °C (20% MeOH in EtOAc). ^{1}H NMR (300 MHz, CDCl$_3$): δ$_H$ 8.56 (d, *J* 4.4 Hz, 1H), 8.15 (d, *J* 7.9 Hz, 1H), 7.83 (dd, *J* 7.7, 1.8 Hz, 1H), 7.77 (dd, *J* 8.5, 2.3 Hz, 2H), 7.59 (d, *J* 2.2 Hz, 1H), 7.43 (d, *J* 8.6 Hz, 1H), 7.39 – 7.32 (m, 1H), 7.23 (d, *J* 7.2 Hz, 1H), 6.26 (d, *J* 12.6 Hz, 1H), 4.22 (qd, *J* 6.9, 3.4 Hz, 1H), 4.13 (d, *J* 13.2 Hz, 1H), 3.75 (d, *J* 10.9 Hz, 1H), 3.64 (dd, *J* 11.1, 6.5 Hz, 1H), 1.87 (s, 1H), 1.28 (d, *J* 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ$_C$ 166.8 (s), 163.5 (s), 156.3 (s), 148.5 (s), 136.9 (s), 136.0 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.2 (s), 128.6 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.5 (s), 67.7 (s), 48.1 (s), 44.8 (s), 17.1 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{18}$BrN$_5$O$_2$ 440.0722; found 440.0699.

(*S*)-8-Bromo-*N*-(1-hydroxybutan-2-yl)-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (2c). The carboxamide **2c** was prepared from **1** by following the same procedure described for synthesis of **2** from **1**. The (*S*)-2-amino-1-butanol (8 mL) was used instead of 2-aminoethan-1-ol. The crude residue was purified by crystallization from 15% MeOH in EtOAc to provide **2c** as light-yellow colored needle shape crystals (1.35 g, 83%). mp 255-256 °C (20% MeOH in EtOAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.54 (d, *J* 3.6 Hz, 1H), 8.11 (d, *J* 7.9 Hz, 1H), 7.79 – 7.71 (m, 3H), 7.53 (s, 1H), 7.36 – 7.33 (m, 2H), 7.21 (d, *J* 7.8 Hz, 1H), 6.21 (t, *J* 12.5 Hz, 1H), 4.10 – 3.96 (m, 2H), 3.7 (1H), 3.65 – 3.62 (m, 1H), 1.66 (s, 1H), 1.59 – 1.53 (m, 1H), 0.98 (br, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 166.8 (s), 163.3 (s), 156.3 (s), 148.5 (s), 137.0 (s), 135.8 (s), 135.3 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.2 (s), 128.5 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.4 (s), 65.4 (s), 53.4 (s), 44.7 (s), 24.3 (s), 10.7 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{21}$H$_{20}$BrN$_5$O$_2$ 454.0879; found 454.0866.

(*R*)-8-Bromo-*N*-(1-hydroxybutan-2-yl)-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (2d). The carboxamide **2d** was prepared from **1** by following the same procedure described for synthesis of **2** from **1**. The (*R*)-2-amino-1-butanol (8 mL) was used instead of 2-aminoethan-1-ol. The crude residue was purified by crystallization from 15% MeOH in EtOAc to provide **2d** as light yellow-colored needle shaped crystals (1.35 g, 80%). mp 250-251 °C (20% MeOH in EtOAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.56 – 8.51 (m, 1H), 8.11 (d, *J* 8.0 Hz, 1H), 7.83 – 7.70 (m, 3H), 7.55 (d, *J* 2.3 Hz, 1H), 7.34 (m, 2H), 7.23 (d, *J* 7.9 Hz, 1H), 6.23 (t, *J* 11.1 Hz, 1H), 4.10 (dd, *J* 9.3, 4.9 Hz, 1H), 4.03 – 3.92 (m, 1H), 3.76 (t, *J* 8.5 Hz, 1H), 3.66 (s, 1H), 3.46 (s, 1H), 1.68 (s, 1H), 1.59 (m, 1H), 0.98 (br, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 166.8 (s), 163.5 (s), 156.3 (s), 148.5 (s), 137.0 (s), 135.9 (s), 135.3 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.2 (s), 128.5 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.5 (s), 65.7 (s), 53.6 (s), 44.7 (s), 24.3 (s), 10.7(s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{21}$H$_{20}$BrN$_5$O$_2$ 454.0879; found 454.0861.

8-Bromo-*N*-(2-oxoethyl)-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide (3). To a solution of **2** (3 g, 7.04 mmol) in CH$_2$Cl$_2$ (70 mL), the Dess-Martin periodinane (3.5 g, 8.4 mmol) was added in one portion and the mixture was stirred at rt for 4 h. The reaction mixture was analyzed by TLC (silica gel, EtOAc/hexane/MeOH 2:1:0.5). After the completion of the reaction by TLC, the mixture was diluted with CH$_2$Cl$_2$ (100 mL) and quenched by addition of a sat. aq NaHCO$_3$ solution (80 mL) and a sat. solution of aq Na$_2$S$_2$O$_3$ (80 mL). The mixture was stirred for 20 min and the layers were separated. The aq layer was extracted with CH$_2$Cl$_2$ (100 mL x 2) and the combined organic layer was washed with brine (100 mL) and dried (Na$_2$SO$_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography

(silica gel, 5% MeOH in CH₂Cl₂) to provide amide **3** as a yellow powder (2.4 g, 81%), which was employed directly in the next step without further characterization.

(S)-8-Bromo-N-(1-oxopropan-2-yl)-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxamide (3a). The carboxamide **3a** was prepared from **2a** by following the same procedure described for the synthesis of **3** from **2**. The carboxamide **3a**, so obtained, was purified by flash chromatography (silica gel, 5% MeOH in DCM) to afford **3a** as a white powder (2.83 g, 79%). ^{1}H NMR (500 MHz, CDCl₃): δ$_{H}$ 9.67 (s, 1H), 8.57 (ddd, *J* 4.8, 1.8, 0.9 Hz, 1H), 8.15 (dt, *J* 7.9, 1.1 Hz, 1H), 7.87 – 7.80 (m, 2H), 7.78 (dd, *J* 8.6, 2.2 Hz, 1H), 7.61 (t, *J* 4.5 Hz, 2H), 7.45 (d, *J* 8.5 Hz, 1H), 7.37 (ddd, *J* 7.6, 4.8, 1.2 Hz, 1H), 6.25 (d, *J* 12.5 Hz, 1H), 4.63 (m, 1H), 4.14 (d, *J* 12.7 Hz, 1H)), 1.46 (d, *J* 7.4 Hz, 3H). ^{13}C NMR (75 MHz, CDCl₃): δ$_{C}$ 199.5 (s), 166.8 (s), 162.7 (s), 156.3 (s), 148.6 (s), 136.9 (s), 136.1 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.3 (s), 130.7 (s), 128.6 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.5 (s), 54.0 (s), 44.8 (s), 14.6 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^{+}$ Calcd for C₂₀H₁₆BrN₅O₂ 438.0566; found 438.0549.

(S)-8-Bromo-N-(1-oxobutan-2-yl)-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxamide (3c). The carboxamide **3c** was prepared from **2c** by following the same procedure described for the synthesis of **3** from **2**. The carboxamide **3c**, so obtained, was purified by flash chromatography (silica gel, 5% MeOH in DCM) to afford **3c** as a white powder (2.67 g, 75%). ^{1}H NMR (500 MHz, CDCl₃): δ$_{H}$ 9.68 – 9.62 (m, 1H), 8.54 (dt, *J* 4.7, 1.3 Hz, 1H), 8.15 – 8.09 (m, 1H), 7.88 – 7.71 (m, 3H), 7.63 – 7.55 (m, 2H), 7.43 (d, *J* 8.6 Hz, 1H), 7.34 (ddd, *J* 7.5, 4.8, 1.2 Hz, 1H), 6.22 (d, *J* 12.5 Hz, 1H), 4.57 (s, 1H), 4.11 (q, *J* 7.2 Hz, 2H), 1.78 (dt, *J* 14.4, 7.3 Hz, 1H), 1.05 – 0.96 (m, 3H). ^{13}C NMR (126 MHz, CDCl₃): δ$_{C}$ 199.6 (s), 166.8 (s), 162.9 (s), 156.3 (s), 148.6 (s), 137.0 (s), 136.2 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.4 (s), 130.9 (s), 128.6 (s), 124.9 (s), 124.2 (s), 124.0 (s), 120.5 (s), 59.5 (s), 44.8 (s), 22.6 (s), 9.8 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^{+}$ Calcd for C₂₁H₁₈BrN₅O₂ 452.0722; found 452.0705.

2-(8-Bromo-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepin-3-yl)oxazole (4). The hexachloroethane (2.4 g, 10.5 mmol) was dissolved in anhydrous acetonitrile (20 mL). The carboxamide **3** (1.5 g, 3.5 mmol) was dissolved in anhydrous acetonitrile (12 mL) and added to the above solution. The reaction mixture was cooled to 0 °C, and triethylamine (2.1 g, 21.2 mmol) was added dropwise. Triphenylphosphine (2.76 g, 10.5 mmol) was then added to the above solution in three portions. The ice bath was removed, and the reaction mixture was allowed to stir for 2 h at rt. The reaction progress was monitored by TLC (silica gel, EtOAc/hexane/ methanol 2:1:0.5 and a few drops of 14% aq. NH₄OH). After completion of reaction, water (20 mL) was added. The aq layer was extracted with ethyl acetate (25 mL x 2). The combined organic layer was washed with brine (20 mL) and dried (Na₂SO₄). The solvents were removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane 2:1, and 3% triethylamine) to afford oxazole **4** as a light yellow colored solid (1.0 g, 72%). A small amount of oxazole **4** crystallized in EtoAc for a melting point (white needle shaped crystals). mp 247-248 °C (crystallized from EtoAc). ^{1}H NMR (300 MHz, CDCl₃): δ$_{H}$ 8.59 (d, *J* 4.1 Hz, 1H), 8.12 (d, *J* 7.9 Hz, 1H), 7.96 (s, 1H), 7.81 (t, *J* 8.7 Hz, 2H), 7.73 (s, 1H), 7.60 (s, 1H), 7.51 (d, *J* 8.6 Hz, 1H), 7.42 – 7.32 (m, 1H), 7.27 (s, 1H), 6.17 (d, *J* 12.7 Hz, 1H), 4.31 (d, *J* 12.7 Hz, 1H), 1.63 (s, 2H). ^{13}C NMR (126 MHz, CDCl₃): δ$_{C}$ 165.8 (s), 156.9.0 (s), 155.1 (s), 148.3 (s), 143.7 (s), 137.6 (s), 136.5 (s), 135.8 (s), 136.2 (s), 134.6 (s), 134.1 (s), 133.9 (s), 130.1 (s), 127.2.0 (s), 125.9 (s), 125.1 (s), 122.9 (s), 119.6 (s), 44.2 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^{+}$ Calcd for C₁₉H₁₂BrN₅O 406.0303; found 406.0296.

2-(8-Bromo-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepin-3-yl)-4-methyloxazole (4a). The methyl oxazole **4a** was prepared from **3a** following the same procedure described for the synthesis of **4** from **3**. The methyl oxazole **4a**, so obtained, was purified by flash chromatography (silica gel, 2:1 EtOAc/ hexane, and 3% triethylamine) to afford **4a** as a light yellow colored solid (1.6 g, 71%). A small amount of oxazole **4a** crystallized in EtoAc for a melting point (white needle shaped crystals). mp 241-242 °C (crystallized from

EtoAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ_H 8.57 (q, *J* 2.7 Hz, 1H), 8.08 (d, *J* 6.9 Hz, 1H), 7.95 (s, 1H), 7.82 (d, *J* 2.4 Hz, 1H), 7.79 (q, *J* 1.8 Hz, 1H), 7.77 (p, *J* 2.3 Hz, 1H), 7.72 – 7.65 (m, 1H), 7.57 (d, *J* 2.1 Hz, 1H), 7.50 (d, *J* 8.5 Hz, 1H), 7.36 (ddt, *J* 7.3, 4.9, 1.2 Hz, 2H), 6.14 (d, *J* 12.7 Hz, 1H), 4.27 (d, *J* 12.7 Hz, 1H), 2.25 (d, *J* 1.3 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ_C δ 167.1 (s), 162.7 (s), 156.8 (s), 156.4 (s), 148.7 (s), 137.4 (s), 136.9 (s), 136.2 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.5 (s), 130.9 (s), 128.5 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.3 (s), 45.0 (s), 14.6(s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{14}$BrN$_5$O 420.0460; found 420.0444.

2-(8-Bromo-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-*a*][1,4]diazepin-3-yl)-4-ethyloxazole (KPP-III-34, 4c). The ethyl oxazole **4c** was prepared from **3c** following the same procedure described for the synthesis of **4** from **3**. The ethyl oxazole **4c**, so obtained, was purified by flash chromatography (silica gel, EtOAc/hexanes 2:1 and 3% triethylamine) to afford **4c** as a light-yellow colored powder (1.0 g, 74%). A small amount of oxazole **4c** was crystallized in EtOAc for a melting point (white needle shaped crystals). mp 246-247 °C (crystallized from EtoAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ_H 8.59 – 8.52 (m, 1H), 8.06 (d, *J* 7.9 Hz, 1H), 7.96 (s, 1H), 7.86 – 7.73 (m, 2H), 7.55 (d, *J* 2.2 Hz, 1H), 7.48 (d, *J* 8.6 Hz, 1H), 7.40 (s, 1H), 7.37-7.32 (m, 1H), 6.14 (d, *J* 12.7 Hz, 1H), 4.25 (d, *J* 13.1 Hz, 1H), 2.62 (q, *J* 7.5 Hz, 2H), 1.27 (t, *J* 7.5 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ_C 167.5 (s), 157.0 (s), 156.7 (s), 149.0 (s), 144.1 (s), 137.3 (s), 135.7 (s), 135.4 (s), 135.3 (s), 135.2 (s), 133.2 (s), 133.1 (s), 128.9 (s), 128.0 (s), 125.3 (s), 124.6 (s), 124.4 (s), 120.7 (s), 45.4 (s), 20.3 (s), 13.0 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{21}$H$_{16}$N$_5$OBr 434.0616; found 434.0596.

2-(8-Bromo-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)-4,5-dihydrooxazole (5). The carboxamide **2** (500 mg, 1.2 mmol,) was dissolved in CH$_2$Cl$_2$ (50 mL) and the solution was cooled to 0 °C. Thionyl chloride (837 mg, 7.2 mmol) was added to the solution dropwise and then the reaction mixture was allowed to stir at rt for 16 h. After completion of the process the reaction mixture was cooled to 0 °C and a sat. aq solution of NaHCO$_3$ (100 mL) was added dropwise. The solution was allowed to stir for 30 min at 0 °C. The organic layer was separated and the aq layer was extracted with CH$_2$Cl$_2$ (3 × 10 mL). The combined organic layer was dried (Na$_2$SO$_4$). The solvent was evaporated, and a white solid was obtained directly for the next step without further purification. The white solid was dissolved in in ethanol (25 mL) and NaOH (300 mg, 7.5 mmol) was added. The solution obtained was refluxed for 2 hours. The reaction mixture was cool to rt and ethanol was removed under reduced pressure to afford a solid residue. The solid residue was dissolved in CH$_2$Cl$_2$ (100 mL) and washed with a saturated aq solution of NaHCO$_3$ (100 mL). The organic layer was separated and an aq layer was extracted with CH$_2$Cl$_2$ (3 × 100 mL). The combined organic layer was dried (Na$_2$SO$_4$). The organic solvent was removed and the solid residue obtained was purified by crystallization (5% MeOH in EtOAc) to afford compound **5** as white colored needle shaped crystals (407 mg, 85%). mp 230-231 °C (crystallized from 5% MeOH in EtOAc). ^{1}H NMR (300 MHz, CDCl$_3$): δ_H 8.61 – 8.56 (m, 1H), 8.09 (d, *J* 7.9 Hz, 1H), 7.92 (s, 1H), 7.83 – 7.76 (m, 2H), 7.57 (d, *J* 2.2 Hz, 1H), 7.48 (d, *J* 8.6 Hz, 1H), 7.39-7.35 (m, *J* 7.5, 4.8, 1.1 Hz, 1H), 6.07 (d, *J* 12.2 Hz, 1H), 4.47 – 4.43 (m, 2H), 4.18 (d, *J* 12.2 Hz, 1H), 4.11 (t, *J* 19.0, 6.0 Hz, 2H). ^{13}C NMR (126 MHz, CDCl$_3$): δ_C 167.0 (s), 160.1 (s), 156.3 (s), 148.6 (s), 136.9 (s), 135.5 (s), 135.3 (s), 134.9 (s), 134.8 (s), 134.6 (s), 128.5 (s), 127.2 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.3 (s), 67.3 (s), 54.7 (s), 45.0 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{19}$H$_{14}$N$_5$OBr 435.0695; found 435.0691.

(*S*)-2-(8-Bromo-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)-4-methyl-4,5-dihydrooxazole 5a. The oxazoline **5a** was prepared from **2a** by following the same procedure described for **5** from **2**. The chloroalkane intermediate was purified by crystallization (15% DCM and hexane). The chloroalkane obtained was a light yellow colored solid. The ethyl oxazoline **5a**, so obtained, was purified by crystallization using 5% MeOH in EtOAc to afford compound **5a** as white colored needle shaped crystals (230 mg, 82%).

The characterization of chloroalkane intermediate: ^{1}H NMR (300 MHz, CDCl$_3$): δ_H 8.56 (d, *J* 4.5 Hz, 1H), 8.15 (d, *J* 7.9 Hz, 1H), 7.83 (dd, *J* 7.7, 1.7 Hz, 1H), 7.81 (s, 1H), 7.78 (dd, *J* 8.5, 2.2 Hz, 1H), 7.60 (d, *J* 2.2 Hz, 1H), 7.44 (d, *J*

8.6 Hz, 1H), 7.37 (dd, *J* 7.6, 4.9 Hz, 1H), 7.27 (d, *J* 10.2 Hz, 1H), 6.27 (d, *J* 12.4 Hz, 1H), 4.56 – 4.48 (m, 1H), 4.15 (d, *J* 12.5 Hz, 1H), 3.68 (dd, *J* 10.8, 3.9 Hz, 2H), 1.37 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ_C 166.8 (s), 162.1 (s), 156.3 (s), 148.6 (s), 136.9 (s), 136.0 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.3 (s), 128.6 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.4 (s), 49.2 (s), 45.1 (s), 44.8 (s), 18.1 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{17}$BrClN$_5$O 458.0383; found 458.0363.

The characterization of oxazoline **5a**: mp 199-200 °C (crystallized from 5% MeOH in EtOAc). [α]$_D^{25}$ -40 (*c* 1.0, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ_H ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.58 (d, *J* 4.9 Hz, 1H), 8.08 (d, *J* 7.9 Hz, 1H), 7.91 (d, *J* 1.0 Hz, 1H), 7.83 (d, *J* 7.6 Hz, 1H), 7.78 (ddd, *J* 8.5, 2.3, 1.0 Hz, 1H), 7.57 (dd, *J* 2.2, 1.0 Hz, 1H), 7.51 – 7.44 (m, 1H), 7.41 – 7.33 (m, 1H), 6.05 (d, *J* 12.6 Hz, 1H), 4.53 (s, 1H), 4.40 (h, *J* 6.9 Hz, 1H), 4.18 (d, *J* 12.7 Hz, 1H), 3.97 (t, *J* 7.7 Hz, 1H), 1.38 (d, *J* 6.5 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ_C 167.1 (s), 159.0 (s), 156.4 (s), 148.8 (s), 136.9 (s), 135.3 (s), 135.2 (s), 135.0 (s), 134.9 (s), 134.6 (s), 128.5 (s), 127.4 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.3 (s), 73.9 (s), 61.9 (s), 45.1 (s), 21.5 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{16}$BrN$_5$O 422.0616; found 422.0608.

(R)-8-Bromo-N-(1-chloropropan-2-yl)-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxamide 5b. The oxazoline **5b** was prepared from **2b** by following the same procedure described for **5** from **2**. The chloroalkane intermediate was purified by crystallization (15% DCM and hexane). The chloroalkane obtained was a light yellow colored solid. The ethyl oxazoline **5b**, so obtained, was purified by crystallization using 5% MeOH in EtOAc to afford compound **5b** as white colored needle shaped crystals (224 mg, 80%).

The characterization of chloroalkane intermediate: ^{1}H NMR (300 MHz, CDCl$_3$): δ_H 8.56 (d, *J* 4.5 Hz, 1H), 8.15 (d, *J* 7.9 Hz, 1H), 7.83 (dd, *J* 7.7, 1.7 Hz, 1H), 7.81 (s, 1H), 7.76 (dd, *J* 8.5, 2.2 Hz, 1H), 7.60 (d, *J* 2.2 Hz, 1H), 7.44 (d, *J* 8.5 Hz, 1H), 7.37 (ddd, *J* 7.7, 4.8, 1.2 Hz, 1H), 7.27 (d, J 10.2 Hz, 1H), 6.28 (d, *J* 12.4 Hz, 1H), 4.52 (s, 1H), 4.15 (d, *J* 13.0 Hz, 1H), 3.68 (dd, *J* 10.8, 3.9 Hz, 2H), 1.37 (d, *J* 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ_C 166.8 (s), 162.1 (s), 156.3 (s), 148.6 (s), 136.9 (s), 136.0 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.3 (s), 128.6 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.5 (s), 49.2 (s), 45.1 (s), 44.8 (s), 18.0 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{17}$BrClN$_5$O 458.0383; found 458.0367.

The characterization of oxazoline **5b**: mp 201-202 °C (crystallized from 5% MeOH in EtOAc). [α]$_D^{25}$ +44 (*c* 1.0, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ_H 8.58 (d, *J* 4.0 Hz, 1H), 8.07 (d, *J* 7.9 Hz, 1H), 7.91 (s, 1H), 7.81 (td, *J* 7.8, 1.8 Hz, 1H), 7.77 (dd, *J* 8.6, 2.2 Hz, 1H), 7.56 (d, *J* 2.3 Hz, 1H), 7.47 (d, *J* 8.6 Hz, 1H), 7.37 (ddd, *J* 7.6, 4.8, 1.2 Hz, 1H), 6.05 (d, *J* 11.1 Hz, 1H), 4.53 (dd, *J* 7.2, 2.1 Hz, 1H), 4.45 – 4.35 (m, 1H), 4.17 (d, *J* 12.7 Hz, 1H), 4.00 – 3.91 (m, 1H), 1.37 (d, *J* 6.6 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ_C 167.1 (s), 159.0 (s), 156.4 (s), 148.8 (s), 136.9 (s), 135.3 (s), 135.2 (s), 135.0 (s), 134.9 (s), 134.7 (s), 128.5 (s), 127.4 (s), 124.8 (s), 124.3 (s), 124.0 (s), 120.3 (s), 73.9 (s), 61.9 (s), 45.1 (s), 21.5 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{20}$H$_{16}$BrN$_5$O 422.0616; found 422.0594.

(S)-2-(8-Bromo-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepin-3-yl)-4-ethyl-4,5-dihydrooxazole (KPP-III-96B, 5c). The oxazoline **5c** was prepared from **2c** by following the same procedure described for **5** from **2**. The chloroalkane intermediate was purified by crystallization (15% DCM and hexane). The chloroalkane obtained was a light yellow colored solid. The ethyl oxazoline **5c**, so obtained, was purified by crystallization using 5% MeOH in EtOAc to afford compound **5c** as white colored needle shaped crystals (235 mg, 78%).

The characterization of chloroalkane intermediate: ^{1}H NMR (500 MHz, CDCl$_3$): δ_H 8.55 (dd, *J* 4.8, 1.8, 1H), 8.14 (dt, *J* 8.0, 1.1 Hz, 1H), 7.92 – 7.73 (m, 3H), 7.59 (d, *J* 2.2 Hz, 1H), 7.37-7.32 (m, 2H), 7.31 – 7.25 (m, 1H), 6.27 (d, *J* 12.2 Hz, 1H), 4.47 – 3.98 (m, 2H), 3.78 – 3.69 (m, 2H), 1.97 – 1.51 (m, 2H), 1.00 (d, *J* 9.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ_C 166.8 (s), 162.4 (s), 156.2 (s), 148.5 (s), 137.0 (s), 136.0 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.2

(s), 131.2 (s), 128.5 (s), 124.9 (s), 124.23 (s) 124.0 (s), 120.5 (s), 50.5, 47.7 (s), 44.8 (s), 24.9 (s), 10.45 (s). HRMS (ESI/IT-TOF) *m/z*: [M+H] $^+$ Calcd for $C_{21}H_{19}N_5OClBr$ 472.0540; found 472.0531.

The characterization of oxazoline **5c:** mp 247-248 °C (crystallized from 5% MeOH in EtOAc). $[\alpha]_D^{25}$ -13.6 (*c* 1.0, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.55 (d, *J* 4.1 Hz, 1H), 8.04 (d, *J* 6.4 Hz, 1H), 7.89 (s, 1H), 7.79 (td, *J* 7.7, 1.2 Hz, 1H), 7.74 (dd, *J* 8.6, 2.0 Hz, 1H), 7.53 (d, *J* 1.9 Hz, 1H), 7.44 (d, *J* 8.6 Hz, 1H), 7.34 (dd, *J* 6.8, 5.1 Hz, 1H), 6.03 (s, 1H), 4.47 (s, 1H), 4.22 (dd, *J* 15.5, 7.4 Hz, 1H), 4.15 (d, *J* 11.6 Hz, 1H), 4.03 (t, *J* 7.9 Hz, 1H), 1.82 − 1.73 (m, 1H), 1.59 (dt, *J* 13.8, 7.1 Hz, 1H), 1.01 (s, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 167.0 (s), 158.9 (s), 156.4 (s), 148.7 (s), 136.9 (s), 135.3 (s), 135.2 (s), 134.9 (s), 134.8 (s), 134.6 (s), 128.5 (s), 127.4 (s), 124.8 (s), 124.2 (s), 124.0 (s), 120.2 (s), 72.0 (s), 68.0 (s), 45.1 (s), 28.7 (s), 10.3 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for $C_{21}H_{18}BrN_5O$ 436.0773; found 436.0756.

(*R*)-2-(8-Bromo-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)-4-ethyl-4,5-dihydrooxazole (KPP-IV-09, 5d).

The oxazoline **5d** was prepared from **2d** by following the same procedure described for **5** from **2**. The chloroalkane intermediate was purified by crystallization (15% DCM and hexane). The chloroalkane obtained was a light yellow colored solid. The ethyl oxazoline **5d**, so obtained, was purified by crystallization using 5% MeOH in EtOAc to afford compound **5d** as white colored needle shaped crystals (213 mg, 75%).

The characterization of chloroalkane intermediate: ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.57 (dd, *J* 4.9, 1.7 Hz, 1H), 8.16 (d, *J* 7.9 Hz, 1H), 7.93 − 7.74 (m, 3H), 7.60 (d, *J* 2.2 Hz, 1H), 7.44 (d, *J* 8.6 Hz, 1H), 7.37 (dd, *J* 7.5, 4.8 Hz, 1H), 7.27 (d, *J* 13.5 Hz, 1H), 6.29 (d, *J* 12.5 Hz, 1H), 4.32 (m, 1H), 4.16 (d, *J* 12.1 Hz, 1H), 3.79 − 3.70 (m, 2H), 2.05 − 1.37 (m, 2H), 1.02 (t, *J* 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 166.7 (s), 162.4 (s), 156.2 (s), 148.5 (s), 137.0 (s), 136.0 (s), 135.4 (s), 134.9 (s), 134.6 (s), 133.2 (s), 131.4 (s), 128.8 (s), 124.6 (s), 124.2 (s), 124.0 (s), 120.5 (s), 50.5 (s), 47.7 (s), 44.81(s), 24.1 (s), 10.5 (s). HRMS (ESI/IT-TOF) *m/z*: [M+H] $^+$ Calcd for $C_{21}H_{19}N_5OClBr$ 472.0540; found 472.0538.

The characterization of oxazoline **5d**: mp 248-249 °C (crystallized from 5% MeOH in EtOAc). $[\alpha]_D^{25}$ -10.5 (*c* 1.0, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.54 (d, *J* 4.7 Hz, 1H), 8.03 (d, *J* 7.8 Hz, 1H), 7.89 (s, 1H), 7.78 (td, *J* 7.8, 1.8 Hz, 1H), 7.73 (dd, *J* 8.7, 2.3 Hz, 1H), 7.53 (d, *J* 2.2 Hz, 1H), 7.44 (d, *J* 8.6 Hz, 1H), 7.33 (dd, *J* 7.6, 4.8 Hz, 1H), 6.03 (t, *J* 11.4 Hz, 1H), 4.46 (d, *J* 10.4 Hz, 1H), 4.25 − 4.12 (m, 2H), 4.01 (t, *J* 8.0 Hz, 1H), 1.76 (dt, *J* 14.4, 7.0 Hz, 1H), 1.59 (dt, *J* 13.9, 7.2 Hz, 1H), 1.00 (t, *J* 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 167.0 (s), 158.9 (s), 156.4 (s), 148.1 (s), 136.9 (s), 135.2 (s), 135.2 (s), 134.9 (s), 134.8 (s), 134.6 (s), 128.5 (s), 127.4 (s), 124.8 (s), 124.3 (s), 124.0 (s), 120.2 (s), 72.0 (s), 68.0 (s), 45.1 (s), 28.5 (s), 10.4 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for $C_{21}H_{18}BrN_5O$ 436.0773; found 436.0757.

2-(8-Ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)oxazole (LKG-I-70, 6).

A mixture of palladium (II) acetate (27.6 mg, 0.12 mmol), tri-*o*-tolylphosphine (75.2 mg, 0.25 mmol) and anhydrous acetonitrile (15 mL) was allowed to stir at rt for 15 min. The bromide **4** (1 g, 2.47 mmol), triisopropylsilylacetylene (541 mg, 1.97 mmol), triethylamine (724 mg, 7.15 mmol), and acetonitrile (10 mL) were added to the mixture. The reaction mixture was monitored by TLC (silica gel, EtOAc/hexane/methanol 2:1:0.5 and a few drops of triethylamine). The solvent was removed under reduced pressure and the residue was dissolved in CH$_2$Cl$_2$ (40 mL) and filtered through a bed of celite. The filtrate was washed with brine (20 mL) and dried (Na$_2$SO$_4$). The solvent was removed under reduced pressure and the residue was passed through a short pad of silica gel (silica gel, EtOAc/hexanes 3:1 with 3% triethylamine) to afford a light yellow colored solid (1.3 g, 93%), which was used for the next step without further purification. To the mixture of this solid (1.3 g, 2.9 mmol), THF (25 mL) and water (0.15 mL), TBAF xH$_2$O (0.9 g, 3.4 mmol, 1M in THF) was added dropwise at -10 °C and the mixture was allowed to stir at rt for 1 h. Analysis by TLC (Silica gel, EtOAc/hexane/MeOH 2:1:0.5 and a few drops of 14% aq. NH$_4$OH) indicated the disappearance of starting material. The reaction mixture was then quenched with water and diluted with EtOAc (30 mL). The organic

layer was separated and washed with brine (20mL) and dried (Na$_2$SO$_4$). The solvents were removed under reduced pressure and the residue was purified using flash column chromatography (silica gel, EtOAc/hexane 2:1 and 3% triethylamine) to yield a light yellow colored solid **LKG-I-70** or **6** (650 mg, 75%). Small amount of oxazole **6** was crystalize from EtoAc for melting point (white needle shape crystals). mp 215-216 °C (crystallized from EtOAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.57 (dd, *J* 5.0, 1.7 Hz, 1H), 8.08 (d, *J* 7.9 Hz, 1H), 8.01 (s, 1H), 7.81 (td, *J* 7.8, 1.8 Hz, 1H), 7.75 (dd, *J* 8.4, 1.9 Hz, 1H), 7.70 (s, 1H), 7.59 (d, *J* 8.4 Hz, 1H), 7.54 (d, *J* 1.8 Hz, 1H), 7.40-7.32 (m, 1H), 7.24 (s, 1H), 6.16 (s, 1H), 4.27 (s, 1H), 3.15 (s, 1H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 167.6 (s), 157.2 (s), 156.3 (s), 148.6 (s), 137.9 (s), 137.1 (s), 136.3 (s), 135.6 (s), 135.4 (s), 135.0 (s), 133.1 (s), 128.1 (s), 127.3 (s), 127.0 (s), 124.9 (s), 124.2 (s), 122.9 (s), 121.1 (s), 81.7 (s), 79.5 (s), 44.9 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{21}$H$_{13}$N$_5$O 352.1198; found 352.1169.

4-Ethyl-2-(8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepin-3-yl)oxazole (KPP-III-51, 6c).
The ethinyl **6c** was prepared from **4c** by following the same procedure described for the synthesis of **4** from **3**. The ethinyl **6c**, so obtained was purified using flash column chromatography (silica gel, EtOAc/hexane 2:1 and 3% triethylamine) to yield a light yellow colored solid **6c** (382 mg, 72%). A small amount of oxazole **6c** was crystallized from EtOAc for a melting point (white needle shaped crystals).
The characterization of the TIPS protected **6c**: ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.60 − 8.56 (m, 1H), 8.07 − 8.03 (m, 1H), 7.80 (td, *J* 7.7, 1.8 Hz, 2H), 7.72 (dd, *J* 8.3, 1.9 Hz, 1H), 7.53 (d, *J* 8.3 Hz, 1H), 7.47 (d, *J* 1.8 Hz, 1H), 7.40 (d, *J* 1.4 Hz, 1H), 7.36 (ddd, *J* 7.5, 4.8, 1.3 Hz, 1H), 6.21 − 6.04 (m, 1H), 4.25 (s, 1H), 2.62 (dd, *J* 7.5, 1.3 Hz, 2H), 1.27 (t, *J* 7.5 Hz, 3H), 1.10 (d, *J* 3.5 Hz, 21H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 167.8 (s), 156.7 (s), 156.5 (s), 148.7 (s), 143.7 (s), 136.9 (s), 135.8 (s), 135.5 (s), 135 (s), 134.9 (s), 132.9 (s), 132.7 (s), 127.6 (s), 126.9 (s), 124.8 (s), 124.1 (s), 122.7 (s), 122.5 (s), 104.9 (s), 93.7 (s), 45.0 (s), 19.9 (s), 18.6 (s), 12.7 (s), 11.24 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{32}$H$_{37}$N$_5$OSi 536.2846; found 536.2855.
The characterization of 8-ethynyl imidazodiazepine **6c**: mp 240-241 °C (crystallized from EtOAc). ^{1}H NMR (500 MHz, CDCl$_3$): δ$_H$ 8.60 (d, *J* 4.8, 1.3 Hz, 1H), 8.08 (d, 1H), 8.01 (s, 1H), 7.83 (td, *J* 7.7, 1.8 Hz, 1H), 7.77 (dd, *J* 8.4, 1.9 Hz, 1H), 7.61 (d, 1H), 7.78 − 7.76 (dd, 1H), 7.43 (s, 1H), 7.4-7.32 (m, 1H), 6.18 (br, 1H), 4.29 (br, 1H), 3.17 (s, 1H), 2.65 (m, *J* 7.5, 1.2 Hz, 2H), 1.42 − 1.26 (t, 3H). ^{13}C NMR (126 MHz, CDCl$_3$): δ$_C$ 167.0 (s), 156.0 (s), 156.4 (s), 148.0 (s), 143.1 (s), 137.7 (s), 136.2 (s), 135.7 (s), 135.4 (s), 134.9 (s), 132.8 (s), 132.7 (s), 127.5 (s), 126.9 (s), 124.8 (s), 123.1 (s), 122.8 (s), 121 (s), 81.1 (s), 78.8 (s), 45.0 (s), 19.9 (s), 12.6 (s). HRMS (ESI/IT-TOF) *m/z*: [M + H]$^+$ Calcd for C$_{23}$H$_{17}$N$_5$O 380.1506; found 380.1478.

Acknowledgements

Supplementary Material

Figure S1, Chiral column data of oxazolines **5c** and **5d**, ^{1}H NMR and ^{13}C NMR spectra for all new compounds are available in the Supplementary material file accompanying this paper.

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# These authors contributed equally to this research

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