The First Structure–Activity Relationship Studies for Designer Receptors Exclusively Activated by Designer Drugs

Xin Chen,†,∥ Hyunah Choo,‡,§,∥ Xi-Ping Huang,‡ Xiaobao Yang,† Orrin Stone,‡ Bryan L. Roth,* ‡ and Jian Jin*†

†Departments of Structural and Chemical Biology, Oncological Sciences, and Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, New York 10029, United States
‡National Institute of Mental Health - Psychoactive Drug Screening Program, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States
§Center for Neuro-Medicine, Korea Institute of Science and Technology, Seongbuk-gu, Seoul 136-791, Republic of Korea

ABSTRACT: Over the past decade, two independent technologies have emerged and been widely adopted by the neuroscience community for remotely controlling neuronal activity: optogenetics which utilize engineered channelrhodopsin and other opsins, and chemogenetics which utilize engineered G protein-coupled receptors (Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)) and other orthologous ligand–receptor pairs. Using directed molecular evolution, two types of DREADDs derived from human muscarinic acetylcholine receptors have been developed: hM3Dq which activates neuronal firing, and hM4Di which inhibits neuronal firing. Importantly, these DREADDs were not activated by the native ligand acetylcholine (ACh), but selectively activated by clozapine N-oxide (CNO), a pharmacologically inert ligand. CNO has been used extensively in rodent models to activate DREADDs, and although CNO is not subject to significant metabolic transformation in mice, a small fraction of CNO is apparently metabolized to clozapine in humans and guinea pigs, lessening the translational potential of DREADDs. To effectively translate the DREADD technology, the next generation of DREADD agonists are needed and a thorough understanding of structure–activity relationships (SARs) of DREADDs is required for developing such ligands. We therefore conducted the first SAR studies of hM3Dq. We explored multiple regions of the scaffold represented by CNO, identified interesting SAR trends, and discovered several compounds that are very potent hM3Dq agonists but do not activate the native human M3 receptor (hM3). We also discovered that the approved drug perlapine is a novel hM3Dq agonist with >10,000-fold selectivity for hM3Dq over hM3.

KEYWORDS: DREADD, CNO, hM3Dq, SAR, neuronal activation, perlapine

To elucidate how neuronal ensembles interactively encode higher brain processes, new and improved methods for both recording and manipulating neuronal activity will be required.1,12 The ability to selectively modulate the activity of defined neuronal populations and to elucidate the behavioral consequences of this selective neuronal modulation affords powerful approaches for studying mammalian brain function in health and disease. Historically, important methods include Wilder Penfield’s pioneering studies of focal electrical stimulation of the human cortex.3 The development of the optogenetics technology pioneered by Diesseroth and colleagues to visualize and activate neuronal activity with exquisite temporal resolution using engineered channelrhodopsin1,12 and other opsins6 has provided an expanding toolbox for decoding the neuronal correlates of brain function.5,6—10 More recently, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) have been developed as a powerful chemogenetics technology for remotely controlling neuronal activity11,12 and have been widely adopted by the neuroscience and greater biological communities.13—17

DREADDs, first revealed in 2005,5 were developed using directed molecular evolution of human muscarinic acetylcholine receptors.11,12 After multiple rounds of random mutagenesis, DREADDs derived from the human muscarinic acetylcholine M3 receptors (hM3Dq) to be insensitive to the endogenous ligand acetylcholine (ACh) but potently and selectively activated by the pharmacologically inert clozapine N-oxide (CNO) were discovered. Importantly, CNO lacks appreciable affinity (Kᵢ > 1 μM) for all relevant native CNS (central nervous system) targets.11,18 The DREADDs have no detectable constitutive activity in vitro11 and, thus, provide an attractive orthologous receptor-effector chemogenetic platform for modifying neuronal activity remotely with minimal invasiveness. In addition to hM3Dq, which activates neuronal firing upon the CNO stimulation in part by depolarization and elevation of intracellular calcium levels,12 hM4Di was
developed from human muscarinic acetylcholine M4 receptors for inhibiting neuronal firing via activation of G-protein inwardly rectifying potassium (GIRK) channels. Since the introduction of the DREADD technology, a large number of papers have independently validated the utility of excitatory and inhibitory DREADDs. In addition, no effect related to the ectopic expression of hM3Dq or hM4Di has been observed.

In addition to being pharmacologically inert, CNO, the “chemical switch” of this chemogenetic approach, is orally bioavailable and CNS penetrant and is not subject to significant metabolic transformation in mice and rats. However, a small fraction of CNO is apparently metabolized to clozapine in humans, nonhuman primates and guinea pigs. Because clozapine modulates the activity of many native CNS receptors, thus interfering with the selective activation of the DREADDs in defined neuronal populations, the “back-metabolism” issue presents a hurdle for translating the DREADD technology forward. To ultimately develop the next generation of DREADD ligands that can selectively activate defined neuronal populations in primates including human, a thorough understanding of structure–activity relationships (SARs) of DREADDs is needed. To date, no SAR studies have been reported for any DREADDs including hM3Dq and hM4Di.

Here, we report the first SAR studies of hM3Dq. We extensively explored multiple regions of the scaffold represented by CNO, which resulted in the discovery of compounds 13 and 21 that are very potent hM3Dq agonists but do not activate the native human M3 receptor (hM3). We describe the design, synthesis, and pharmacological evaluation of new CNO analogues and discuss the interesting SAR trends revealed from the studies. We also report the discovery that perlapine, a hypnotic agent, has a permanent positive charge. As illustrated in Scheme 2, compound 4a (clozapine) was converted to the quaternary ammonium iodide 6 by stirring overnight with CH3I in acetone at room temperature. Compound 3 was treated with piperazin-2-one at 99 °C overnight in the 1:1 mixture of 1,4-dioxane and ethanol to give compound 7. Similarly, compound 9 was produced by treating compound 3 with commercially available 1,3,8-triazaspiro[4.5]decane-2,4-dione (8) in the 2:1 mixture of 1,4-dioxane and N,N-dimethylformamide (DMF) at 130 °C for 24 h. Hydrolysis of compound 9 using 0.5 N aqueous NaOH solution in 1,2-dimethoxyethane under microwave irradiation afforded compound 10. Likewise, compound 11 was prepared from compound 3 and piperazine in toluene at 120 °C for 2 h.

To determine whether the 8-Cl group on the tricyclic core is required to activate hM3Dq, we prepared compounds 21–23 according to the synthetic route outlined in Scheme 3. The commercially available 2-aminobenzoic acid (15) and 2-nitrophenyl iodide (16) were subjected to Ullmann coupling conditions to afford the aniline 17. Reduction of the nitro moiety of compound 17 yielded compound 18, which was refluxed in xylene to generate the benzodiazepine 19. Treatment of compound 19 with POCl3 provided the chloride 20, which was then displaced with piperazine to afford compound 21. Similarly, compound 22 was prepared by the displacement reaction of the chloride 20 with 1-ethylpiperazine in toluene under reflux conditions. In addition, the oxidation of compound 22 by mCPBA in CH2Cl2 afforded the N-oxide 23.

Biological Evaluation. The newly synthesized compounds were evaluated in the hM3Dq and hM3 Ca2+ mobilization fluorometric imaging plate reader (FLIPR TETRA) assays according to the protocols reported previously. Agonist activities of these compounds in the hM3Dq and hM3 functional assays are summarized in Table 1.

For the size of the N-alkyl group in compounds 4a–4d and 5a–5d, we observed a clear trend showing that the longer and/or bulkier the N-alkyl group, the weaker the compounds’ potency for hM3Dq. The replacement of the methyl group in compounds 4a and 5a with the n-propyl group in compounds 4d and 5d resulted in a potency decrease of approximately 70- and 100-fold, respectively. In addition to the loss in potency, the compounds with a longer or bulkier N-alkyl group (e.g., compounds 4b–4d and 5b–5d) in general displayed lower selectivity for inhibiting neuronal activity.

Figure 1. SAR studies of the CNO scaffold.
agonist efficacy for hM3Dq and became partial agonists of hM3Dq rather than full agonists as seen for compounds 4a and 5a. Interestingly, compounds 4c and 5c, which contain an i-propyl group, were more potent than compounds 4d and 5d,

Reagents and conditions: (a) xylene, reflux, 48 h, 95% yield; (b) POCl₃, N,N-dimethylaniline, toluene, 95 °C, 2 h, 67% yield; (c) N-alkylpiperazines, toluene, 120 °C, 2 h, 69–80% yield; and (d) mCPBA, CH₂Cl₂, rt, 10 min, 65–75% yield.

Reagents and conditions: (a) CH₃I, acetone, rt, overnight, 55% yield; (b) 2-oxypiperazine, 1,4-dioxane/ethanol 1:1, 99 °C, overnight, 65% yield; (c) 1,3,8-triazaspiro[4.5]decan-2,4-dione (8), 1,4-dioxane/DMF (2:1), 130 °C, 24 h, 66% yield; (d) 1,2-dimethoxyethane, 0.5 N NaOH, microwave, 150 °C, 10 min, 16% yield; (e) piperazine, toluene, 120 °C, 2 h, 69% yield; (f) AcCl, TEA, CH₂Cl₂, 0 °C, 1 h, 86% yield; (g) (1) LiAlD₄, THF, N₂, reflux, 2h, (2) CD₃OD, 0 °C, (3) NH₄OH, 0 °C, 84% yield; (h) MsCl, DIPEA, CH₂Cl₂, 0 °C, 1 h, 93% yield.
were also pleased to find that compounds 4b–4d and 5b–5d did not display any agonist activity (EC\textsubscript{50} > 30 000 nM) for the native human M3 receptor (hM3), in contrast to compound 4a (clozapine), which was a hM3 agonist with sub-\(\mu\)M potency. In addition, compounds 4a–4d were in general more potent than their corresponding N-oxides 5a–5d at activating hM3Dq, suggesting that the negative charge on the N-oxides is not only not required for activating hM3Dq, but also reduces agonist potency.

The quaternary ammonium salt 6 was an extremely potent full agonist of hM3Dq with an EC\textsubscript{50} value of 69 pM and about 15-fold more potent than compound 4a (clozapine). However, compound 6 was also a potent full agonist of hM3 (EC\textsubscript{50} = 9.5 nM, E\textsubscript{max} = 92) even though it achieves >100-fold higher potency for hM3Dq over hM3. On the other hand, compounds 7, 12, and 14, which do not contain a basic amino group or a group with permanent positive charge, did not display any agonist activity for hM3Dq. As expected, these compounds did not activate hM3 either. Taken together, these results suggest that either a basic amino group as in compounds 4a and 4b or a group with permanent positive charge as in compounds 5a and 6 is required to retain hM3Dq agonist activity. In addition, compound 9 that contains a hydantoin moiety and compound 10 that contains an amino acid moiety in this region did not activate hM3Dq and hM3. On the other hand, compound 11, which is the des-methyl clozapine, showed similar potency and efficacy for hM3Dq and hM3 as clozapine, suggesting that the N-methyl group is not required for activating hM3Dq. Interestingly, compound 13, which is a deuterated analogue of compound 4b, exhibited similar potency and efficacy (EC\textsubscript{50} = 9.6 nM, E\textsubscript{max} = 86%) for hM3Dq as compounds 4b and 5a (CNO) (Figure 2). Importantly, compound 13 did not display any agonist activity for hM3. Because compound 13 contains an \(\alpha,\beta\)-dideuterio ethyl group, it is likely that the N-dealkylation, the major metabolic pathway that converts clozapine to des-methyl clozapine,\cite{34,36} will be significantly reduced on the basis of the well-documented primary kinetic isotope effect\cite{48} in similar systems.\cite{49,50}

We were also pleased to find that the 8-chloro group was not required to maintain high agonist potency and efficacy for hM3Dq. In particular, compound 21 was a potent full agonist (EC\textsubscript{50} = 1.7 nM, E\textsubscript{max} = 100%) of hM3Dq (Figure 2). In contrast to compound 11, a full hM3 agonist with sub-micromolar potency, compound 21 displayed little agonist

### Table 1. Agonist Activities of New Compounds in hM3Dq and hM3 FLIPR Assays\textsuperscript{a}

| compd | EC\textsubscript{50} (nM) | E\textsubscript{max} (relative to CNO) | hM3Dq | hM3 |
|-------|-----------------|-------------------------------|-------|-----|
| 4a    | 1.1             | 95                           | 360   | 88  |
| 4b    | 7.0             | 91                           | >30 000 | NA |
| 4c    | 13              | 45                           | >30 000 | NA |
| 4d    | 71              | 50                           | >30 000 | NA |
| 5a    | 6.0             | 100                          | >30 000 | NA |
| 5b    | 19              | 50                           | >30 000 | NA |
| 5c    | 190             | 45                           | >30 000 | NA |
| 5d    | 740             | 79                           | >30 000 | NA |
| 6     | 0.069           | 100                          | 9.5   | 92  |
| 7     | >30 000         | NA                           | >30 000 | NA |
| 9     | >30 000         | NA                           | >30 000 | NA |
| 10    | >30 000         | NA                           | >30 000 | NA |
| 11    | 2.1             | 95                           | 490   | 86  |
| 12    | >30 000         | NA                           | >30 000 | NA |
| 13    | 9.6             | 86                           | >30 000 | NA |
| 14    | >30 000         | NA                           | >30 000 | NA |
| 21    | 1.7             | 100                          | NA    | ~20 |
| 22    | 1.3             | 81                           | >30 000 | NA |
| 23    | 220             | 59                           | >30 000 | NA |

\textsuperscript{a}EC\textsubscript{50} values are the average of at least two duplicate experiments with standard deviation (SD) values that are 3-fold less than the average. NA: not applicable.

*which contain a \(n\)-propyl group, suggesting that the length of the \(N\)-alkyl group plays a more significant role than the bulkiness of the \(N\)-alkyl group in reducing agonist potency. We were also pleased to find that compounds 4b–4d and 5b–5d did not display any agonist activity (EC\textsubscript{50} > 30 000 nM) for the native human M3 receptor (hM3), in contrast to compound 4a (clozapine), which was a hM3 agonist with sub-\(\mu\)M potency. In addition, compounds 4a–4d were in general more potent than their corresponding N-oxides 5a–5d at activating hM3Dq,
activity for hM3 ($E_{\text{max}} \approx 20\%$). In addition, compound 22 was found to be a potent hM3Dq agonist ($EC_{50} = 1.3$ nM, $E_{\text{max}} = 81\%$), which was more potent than the corresponding chloro analogue, compound 4b ($EC_{50} = 7.0$ nM, $E_{\text{max}} = 91\%$). On the other hand, the N-oxide 23 ($EC_{50} = 220$ nM, $E_{\text{max}} = 59\%$) was about 10-fold less potent for hM3Dq than compound 5b, the corresponding chloro analog ($EC_{50} = 19$ nM, $E_{\text{max}} = 50\%$). Similar to compound 21, both compounds 22 and 23 did not exhibit any agonist activity for hM3.

We next selected a subset of the above hM3Dq agonists that are inactive against hM3 and assessed their binding affinities to other aminergic GPCRs. Because compound 4a (clozapine) showed high binding affinities to SHT$_{2A}$ and SHT$_{2C}$ serotonin, $\alpha_{1A}$ adrenergic, and H$_1$ histamine receptors with $K_i$ values of 5.4, 9.4, 1.6, and 1.1 nM, respectively (Table 2), we tested compounds 4b, 4c, 5b, 5c, 13, and 21 in SHT$_{2A}$, SHT$_{2C}$, $\alpha_{1A}$, and H$_1$ radioligand binding assays. The assay results are summarized in Table 2.

### Table 2. Binding Affinities of Selected hM3Dq Agonists to Other GPCRs

| compd | SHT$_{2A}$ (nM) | SHT$_{2C}$ (nM) | $\alpha_{1A}$ (nM) | H$_1$ (nM) |
|-------|----------------|----------------|-------------------|------------|
| 4a    | 5.4            | 9.4            | 1.6               | 1.1        |
| 4b    | 29             | 24             | 46                | 1.9        |
| 4c    | 16             | 17             | 37                | 4.6        |
| 5b    | 1900           | 5100           | >10000            | 160        |
| 5c    | 5200           | 6700           | 320               | 6200       |
| 13    | 71             | 280            | 67                | 5.0        |
| 21    | 66             | 170            | 280               | 6.0        |

$K_i$ values are the average of at least 2 duplicate experiments with standard deviation (SD) values that are 3-fold less than the average.

Compounds 4b and 4c had reduced binding affinities to SHT$_{2A}$, SHT$_{2C}$, and $\alpha_{1A}$ ($K_i = 16\text{–}46$ nM) compared with compound 4a (clozapine), but retained high binding affinities to H$_1$ ($K_i < 5.0$ nM). On the other hand, the N-oxide 5b displayed weak binding affinities for SHT$_{2A}$, SHT$_{2C}$, and $\alpha_{1A}$ ($K_i > 1000$ nM) and was about 8-fold selective for hM3Dq over H$_1$, while the N-oxide 5c displayed poor binding affinities to SHT$_{2A}$, SHT$_{2C}$, and H$_1$ ($K_i > 5000$ nM) but was only about 2-fold selective for hM3Dq over $\alpha_{1A}$. Interestingly, compound 13, a deuterated analogue of compound 4b, exhibited reduced binding affinities to all four receptors compared with compound 4b. Compound 13 was selective for hM3Dq over SHT$_{2A}$ (7-fold), SHT$_{2C}$ (29-fold), and $\alpha_{1A}$ (7-fold), but was not selective over H$_1$. We were pleased to find that compound 21 displayed much improved selectivity compared with compound 4a (clozapine). In addition to being inactive at hM3, compound 21, a potent full agonist of hM3Dq ($EC_{50} = 1.7$ nM), was 40-fold selective over SHT$_{2A}$, 100-fold selective over SHT$_{2C}$, and 165-fold selective over $\alpha_{1A}$. Although it was only 3.5-fold selective for hM3Dq over H$_1$, the overall selectivity profile of compound 21 is significantly better than compound 4a (clozapine).

Lastly, to identify another compound that might activate hM3Dq, we conducted a screen of the commercially available Library Of Pharmaceutically Active Compounds (LOPAC; $N = 1280$ compounds) and Prestwick Chemical Library ($N = 1280$ compounds) using the hM3Dq FLIPR assay. From this screen, we discovered perlazine as a novel, potent agonist of hM3Dq (Figure 3). Importantly, perlazine was >10 000-fold selective for hM3Dq over hM3. Interestingly, perlazine contains a different tricyclic core in comparison with CNO. The high hM3Dq potency of perlazine suggests that the benzodiazepine tricyclic core of the CNO (compound 5a) scaffold is not required for maintaining high hM3Dq agonist activity.

## CONCLUSION

In summary, we conducted the first SAR studies for hM3Dq, a chemogenetic platform for activating neuronal firing, by the design, synthesis, and pharmacological evaluation of new CNO analogues. We explored multiple regions of the CNO scaffold and observed the following interesting SAR trends: (1) a longer or bulkier N-alkyl group as in compounds 4c, 4d, 5c, and 5d reduces both potency and efficacy for hM3Dq; (2) a basic amino group as in compounds 4a and 4b or a permanent positive charge group as in compounds 5a and 6 is required to retain hM3Dq agonist activity; (3) the negative change on the N-oxides such as 5a–5d reduces hM3Dq agonist potency; (4) the 8-chloro group is not required to maintain high agonist potency and efficacy for hM3Dq; and (5) modifications to the benzodiazepine tricyclic core of CNO is tolerated. From these SAR studies, we discovered several compounds such as 13 and 21, which are very potent full agonists of hM3Dq but do not activate the native human M3 receptor (hM3). In addition, the selectivity of compound 21 against a number of aminergic GPCRs is significantly improved compared with clozapine.
Furthermore, we discovered perlapine as a novel, potent hM3Dq agonist, which is >10 000-fold selective for hM3Dq over hM3. These SAR studies lay the foundation for developing the next generation of DREADD ligands that can selectively activate defined neuronal populations in primates.

**METHODS**

**Chemistry. General Methods.** HPLC spectra of all compounds were acquired from an Agilent 6100 Series system with UV detector set to at 220 nm. Samples were injected (5 μL) onto an Agilent Eclipse 5 μm, C18 column at room temperature. After 10 min, the reaction was completed. The resulting mixture was concentrated and purified by flash column chromatography with 5–15% C (2% NH4OH in MeOH) in CH2Cl2 to give the desired N-oxide compound 5b (0.073 g,) in 70% yield: 1H NMR (400 MHz, MeOH-d4) δ 7.59–7.34 (m, 1H), 7.32 (d, J = 7.8, 1H), 7.05 (t, J = 7.5 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 6.87 (d, J = 8.4, 2.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 3.88 (br s, 2H), 3.74 (t, J = 12.1 Hz, 2H), 3.55–3.43 (m, 2H), 3.367 (q, J = 7.2 Hz, 2H), 3.19–3.09 (m, 2H), 1.38 (t, J = 7.2 Hz, 3H).

13C NMR (101 MHz, CDCl3) δ 162.02, 153.15, 141.64, 140.61, 132.57, 132.50, 130.55, 129.24, 126.94, 123.69, 123.18, 123.13, 120.25, 120.15, 54.80 (2C), 48.95 (2C), 47.77, 18.80 (2C). HPLC purity 100%, RT 4.099 min. MS (ESI) 355.2 [M + H]+. HRMS (ESI) calcd for C20H24ClN4O+: 371.1639. Found: 371.1642.

4-Chloro-5H-dibenzo[b,e][1,4]diazepin-1-yl)-1-ethylpiperazine N-oxide (5c). Compound 4c (0.068 g, 0.293 mmol) was used as a positive control in the hM3Dq FLIPR assay.

**Figure 3.** Perlapine is a potent full agonist of hM3Dq and does not activate hM3. CNO (compound 5a) was used as a positive control in the hM3Dq FLIPR assay.

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4-Chloro-5H-dibenzo[b,e][1,4]diazepin-11-yl)-1-propylpiperazine N-oxide (5d). Compound 5d (0.075 g, 72% yield) was prepared using the same procedure as preparing 5b from compound 4d (0.100 g, 0.282 mmol) and mCPBA (0.063 g, 0.365 mmol) in CH₂Cl₂ (5 mL). ¹H NMR (400 MHz, MeOH-d₄) δ 7.59–7.34 (m, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 19.4 Hz, 1H), 7.25 (d, J = 7.9 Hz, 3H), 7.08 (d, J = 8.6 Hz, 1H), 4.36–3.59 (m, 4H), 2.70–2.40 (m, 2H), 2.40–2.01 (m, 2H). HPLC purity 100%, RT 3.585 min. MS (ESI) 371.2 [M + H⁺]. HRMS (ESI) calc'd for C₁₅H₁₅ClN₂O₂⁺ [M + H⁺]: 371.1269. Found: 371.1264.

Compound 11 was prepared according to the previously published procedures.¹²

1-(4-(8-Chloro-5H-dibenzo[b,e][1,4]diazepin-11-yl)-1-propylpiperazin-1-yl)ethanone (12). To the solution of compound 10 (0.400 g, 1.28 mmol) and TEA (0.27 mL, 2.0 mmol) in CH₂Cl₂, was added A9CI (0.10 mL, 1.4 mmol) at 0°C. The resulting mixture was then stirred at 0°C for 1 h. After removing the solvents, the residue was purified by flash column chromatography with 0–5% MeOH in CH₂Cl₂, to give the desired product 12 (0.390 g, 1.10 mmol) in 86% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.30 (td, J = 7.7, 1.5 Hz, 1H), 7.26–7.22 (m, 2H), 7.04 (d, J = 2.4 Hz, 1H), 7.02 (td, J = 7.6, 1.0 Hz, 1H), 6.86–6.77 (m, J = 2.4 Hz, 2H), 6.60 (d, J = 8.3 Hz, 1H), 4.89 (s, 1H), 3.93–3.46 (m, 4H), 3.34 (br, s, 2H), 2.11 (s, J = 11.5 Hz, 3H). ¹C NMR (101 MHz, CDCl₃) δ 169.41, 162.90, 153.15, 151.47, 140.65, 132.40, 132.09, 129.25, 129.73, 127.75, 123.39, 123.32, 120.42, 120.34, 47.80, 47.26, 46.13, 41.50, 21.62. HPLC purity 100%, RT 4.919 min. MS (ESI) 355.2 [M + H⁺]. HRMS (ESI) calc'd for C₁₇H₁₇ClN₂O₂ [M + H⁺]: 355.1320. Found: 355.1315.

5-Chloro-11-(4-(1-dimethylaminoethoxy)piperazin-1-yl)-5H-dibenzo[b,e][1,4]diazepine (13). To the solution of compound 12 (0.100 g, 0.282 mmol) in 15 mL of anhydrous THF was added LiAlD₄ (0.024 g, 0.572 mmol) at room temperature under N₂ atmosphere. The reaction mixture was heated under reflux conditions for 2 h. The reaction was quenched with 0.1 mL of CDOD at 0°C. The resulting mixture was treated with 0.5 mL of NH₄OH at 0°C and filtered through Celite and the filtrate was concentrated. The residue was purified by flash column chromatography with 0–10% MeOH in CH₂Cl₂ to give the desired product 13 (0.106 g, 0.246 mmol) in 24% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.30–7.20 (m, 3H), 7.00 (d, J = 2.4 Hz, 1H), 7.01 (td, J = 7.6, 1.0 Hz, 1H), 6.81 (d, J = 8.3, 2.4 Hz, 2H), 6.60 (d, J = 8.3 Hz, 1H), 4.89 (s, 1H), 3.93–3.46 (m, 4H), 3.34 (br, s, 2H), 2.11 (s, J = 11.5 Hz, 3H). ¹C NMR (101 MHz, CDCl₃) δ 169.41, 162.90, 153.15, 151.47, 140.65, 132.40, 132.09, 129.25, 129.73, 127.75, 123.39, 123.32, 120.42, 120.34, 47.80, 47.26, 46.13, 41.50, 21.62. HPLC purity 100%, RT 4.919 min. MS (ESI) 355.2 [M + H⁺]. HRMS (ESI) calc'd for C₁₇H₁₇ClN₂O₂ [M + H⁺]: 355.1320. Found: 355.1315.

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11-[(4-Ethylpiperazin-1-yl)-5H-dibenzo[b,e][1,4]diazepine (22). A solution of compound 20 (0.420 g, 1.84 mmol) and 1-ethylpiperazine (1.5 mL, 11.81 mmol) in toluene (20 mL) was heated under reflux conditions for 2 h. After cooling down to room temperature and concentration, the residue was purified by flash column chromatography with 0–10% MeOH in CH2Cl2 to give the desired product 22 (0.409 g) in 72% yield. 1H NMR (400 MHz, CDCl3) δ 7.30–7.18 (m, 3H), 6.97 (m, 2H), 3.84 (br s, 2H), 3.79 (br s, 2H), 3.70 (s, 2H). 13C NMR (101 MHz, CDCl3) δ 163.66, 155.93, 144.50, 141.44, 133.64, 131.23, 127.98, 125.69, 125.05, 124.16, 124.13, 114.44, 102.49. MS (ESI) 323.2 [M + H]+. HRMS (ESI) calcd for C19H23N4O+ [M + H]+: 323.1866. Found: 323.1863.

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The authors declare no competing financial interest.

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