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Article

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Structure-activity relationship studies of Retro-1 analogs against Shiga toxin

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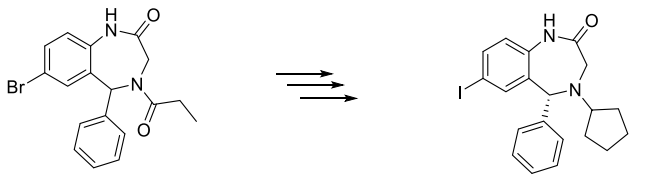
ABSTRACT

High-throughput screening has shown that Retro-1 inhibits ricin and Shiga toxins by diminishing their intracellular trafficking via the retrograde route, from early endosomes to the Golgi apparatus. In order to improve the activity of Retro-1, a SAR study was undertaken and yielded an analog with a roughly 70-fold better EC_{50} against Shiga toxin cytotoxicity measured in a cell protein synthesis assay.



INTRODUCTION

Shiga toxins (Stx) are a family of structurally and functionally similar protein toxins produced by *Shigella dysenteriae* and some serogroups of *Escherichia coli*.[1] Gram-negative bacteria producing Stx are pathogenic and responsible for a number of human foodborne diseases such as bloody diarrhea and hemolytic uremic syndrome (HUS), the most feared complication of the infection defined by acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia.[2]

Stx belong to the group of AB_5 toxins and consist of a catalytically active A-subunit (StxA) and a binding pentameric B-subunit (StxB). After binding of StxB to the globotriaosylceramide (Gb3) receptor at the cell surface, Stx is endocytosed and traffics through endosomes to the endoplasmic reticulum (ER) by the retrograde pathway.[3] Once in the ER, StxA translocates to the cytosol where it arrests protein biosynthesis of host cells by enzymatically inactivating ribosomes.

Hitherto, no specific therapies are available to treat Stx intoxication. At the hospital, management of HUS is based on general supportive care, and early dialysis for acute renal failure. Plasma exchange has been explored without indication of its efficacy.[4,5] Yet, the massive outbreak of Stx-producing *E. coli* in Germany in 2011 allowed assessment of new drugs, such as the humanized monoclonal antibody eculizumab directed against the complement protein C5 and approved for the treatment of the genetic related disorder atypical HUS (aHUS). However, a retrospective study concluded that there was no benefit for eculizumab-treated HUS patients.[5]

Therapeutics that hamper Stx binding, uptake, trafficking, translocation or enzymatic activity are highly relevant for the treatment of STEC infections (for a review see [6]). Thus, blocking of the intracellular retrograde trafficking of Stx can be one viable strategy to arrest the intracellular action of the toxin, as demonstrated either by the activity of chemical compounds (Figure 1), or by manganese ions.[7–16]

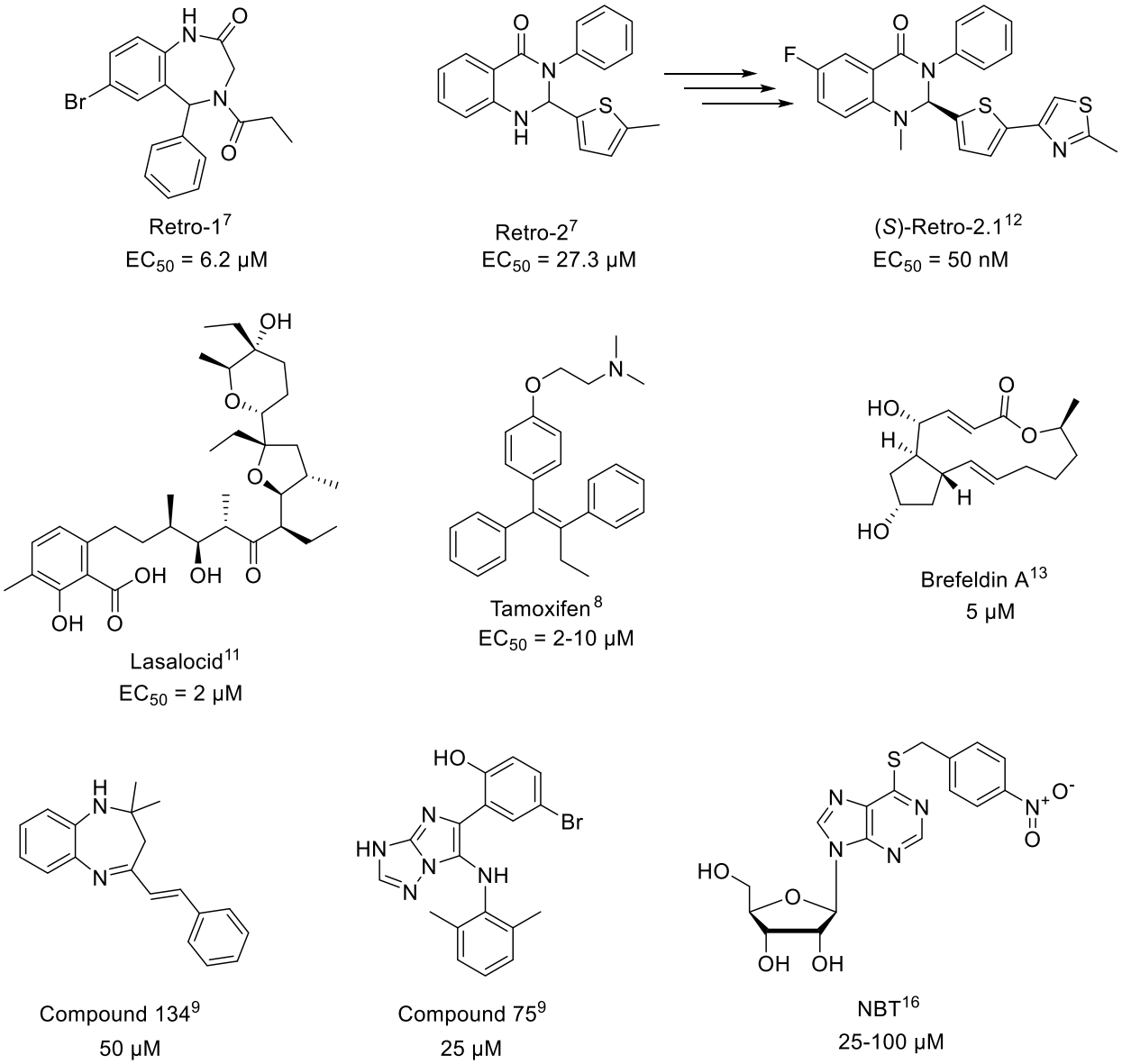


Figure 1. Chemical structures of some known cellular inhibitors of Shigatoxins. EC_{50} values or active concentrations are indicated. NBT: Nitrobenzylthioinosine.

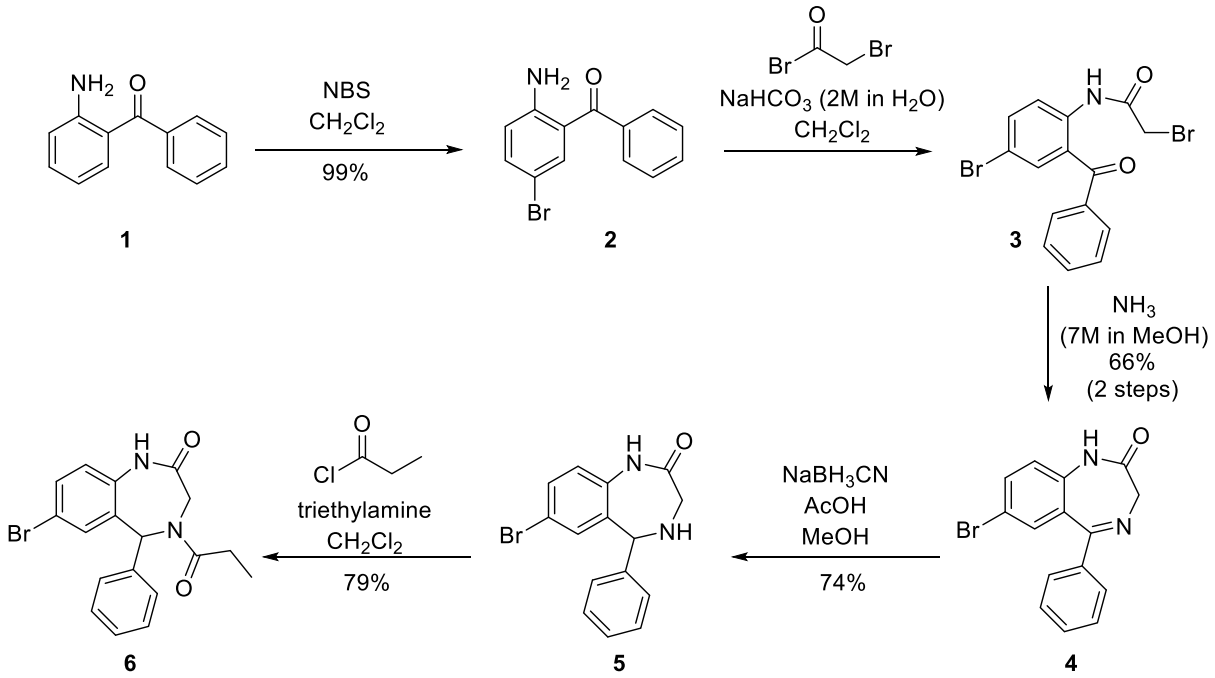
Identified by high-throughput screening, Retro-1 selectively blocked Stx retrograde trafficking at the early endosome/trans-Golgi network (TGN) interface, thus protecting exposed cells from the cytotoxic action of Stx.[7] Unlike various small molecules that inhibit intracellular Stx transport,

Retro-1 did not perturb cellular morphology nor did it affect other trafficking pathways. Here, we report on the development of a related compound Retro-1.1 with a similar mode of action and conferring improved protective efficacy against Stx on human cells.

RESULTS AND DISCUSSION

Synthesis of Retro-1. The synthesis of compound **6**, Retro-1, was achieved starting from the commercially available 2-amino, 5-bromo benzophenone **1** (Scheme 1) in 39% yield over 4 steps. First, regioselective bromination was performed with NBS with complete conversion. Acetylation with bromoacetyl bromide was immediately followed by cyclization with ammonia to yield the benzodiazepine **4** in 66% yield over two steps. Then, reduction of the imino moiety with $NaBH_3CN$ yielded the two enantiomers of benzodiazepine **5** which were treated with propionyl chloride to obtain Retro-1 as a mixture of two enantiomers. In addition, racemic Retro-1 was obtained as a 1:1 mixture of conformers as detected by ^{1}H and ^{13}C NMR.[17] High-temperature NMR allowed us to obtain coalescence of the two conformer signals (see supplementary materials).

Scheme 1. Synthetic route to Retro-1



Evaluation of benzodiazepine drugs and derivatives. We first started to evaluate the biological activities of a few commercially available (some are marketed drugs) related analogs of Retro-1 along with other structural analogs that we synthesized (Figure 2). The latter were obtained via the synthetic route depicted in Scheme 1 starting either from commercially available aminobenzophenones or were synthesized in house (by a Friedel-Craft reaction between the desired aniline and the corresponding benzonitrile). All tested molecules are depicted in Figure 2.

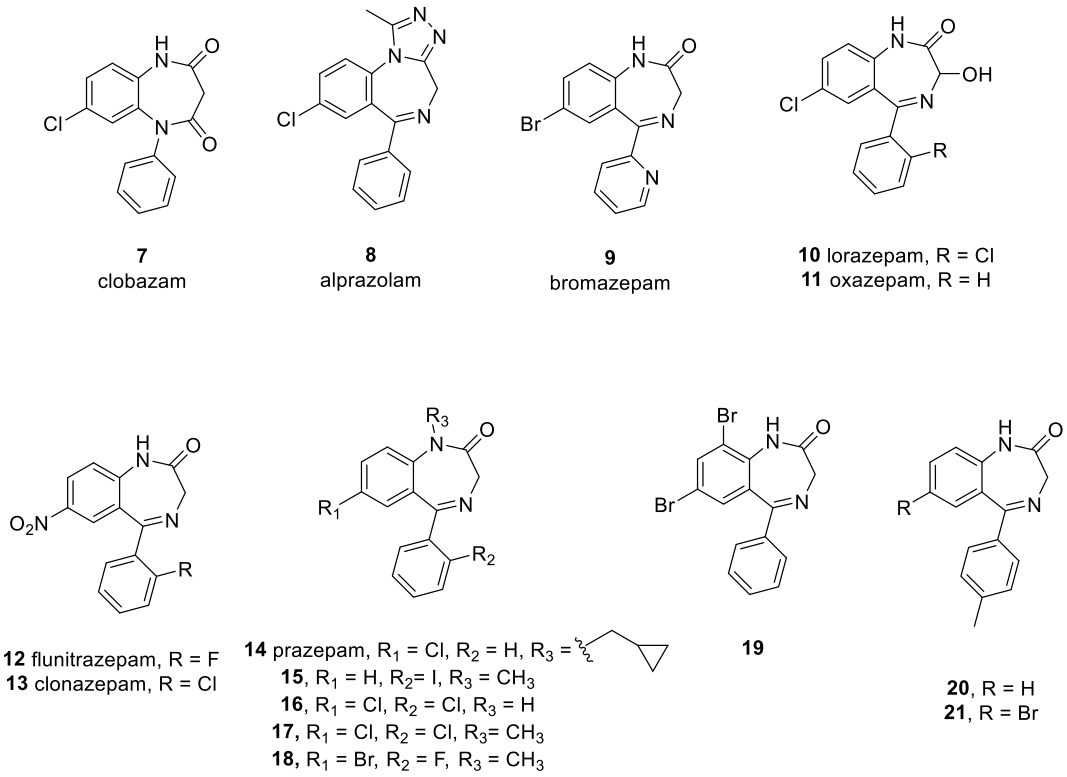
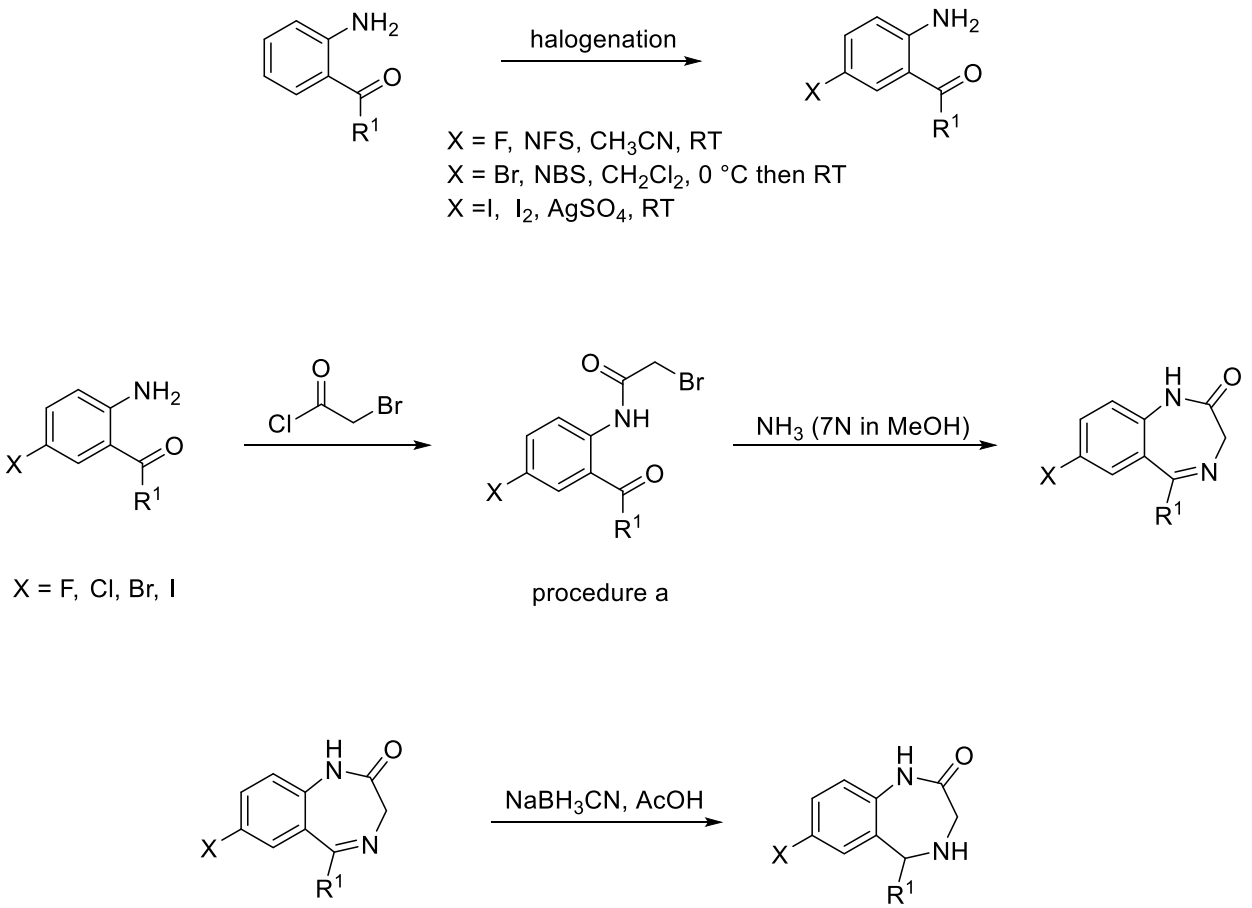


Figure 2. Initial screening of commercially available benzodiazepinones and miscellaneous derivatives

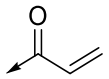
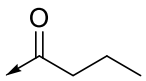
All of these compounds were inactive which strongly suggests that a benzodiazepine scaffold and not a benzodiazepinone one is mandatory. All benzodiazepines were synthesized according to the scheme 2:

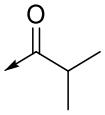
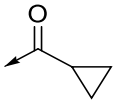
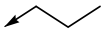
Scheme 2. General procedure for the synthesis of 1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one.



Modification of N4. We then checked the impact of the N4 amide substitution by introducing small variations around the propionyl group.

Table 1. Preliminary evaluation of the substitution at N4

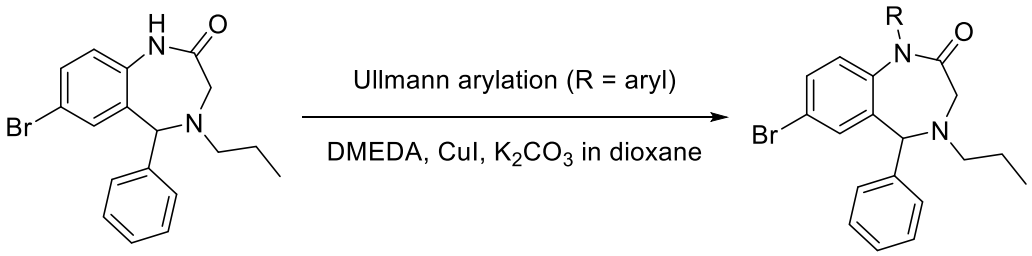
Compound	R_1	Yield (%)	Protection (%)	EC_{50} (µM)
22		50	inactive	n.d.
23		78	80.2	10.93±0.15

24		84	inactive	n.d.
25		83	inactive	n.d.
26		91	>100	3.98±0.63

Unfortunately, the four amide analogs we synthesized proved inactive (Table 1, compounds **22**, **24**, **25**) or less active than the parent Retro-1 (Table 1, compound **23**) in Stx protection assays. It should be reiterated that Retro-1 (see below), but also the analogs presented in Table 1 (compounds **22-25**), give rise to conformers, presumably due to the presence of the tertiary amide. We therefore decided to synthesize a reduced analog of Retro-1, i.e. compound **26**. Compound **26** was indeed the best analog and showed slightly better protection compared to Retro-1 (6.2 µM). This reduced analog not only yielded an active compound, but also got rid of conformers, thereby simplifying the analysis. This propylated compound was therefore a good starting point for more extensive SAR studies. Based on this N-propyl analog, we then investigated the impact of the N1 amide substitution (Table 2).

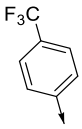
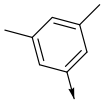
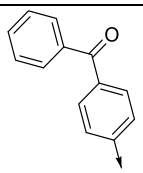
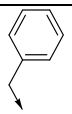
Substitution at N1. The synthesis of N1-substituted benzophenones were achieved via either classical alkylation reaction from diazepinones (see experimental part for conditions) or Ullmann arylation according to scheme 3:

Scheme 3. Synthesis of N1-substituted diazepin-2-one from 7-bromo-5-phenyl-4-propyl, 1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one.



None of the analogs we synthesized in this series proved active against Shiga toxin, with aliphatic (Table 2, compound **27**), allylic or benzylic (compounds **32** and **33**) or aromatic/heteroaromatic (compounds **28**-**31**) substituents. These results suggest that the NH bond is crucial for bioactivity and may be involved in H bonding with the target(s) or may point to a small hydrophilic cavity.

Table 2. Modification on the N1 amide

Compound	R	Yield (%)	Protection (%)	EC_{50} (µM)
27		48	inactive	n.d.
28		50	inactive	n.d.
29		41	inactive	n.d.
30		52	inactive	n.d.
31		37	inactive	n.d.
32		81	inactive	n.d.
33		70	inactive	n.d.

Modification of the aromatic rings. Faced with these rather disappointing results, we decided to evaluate the impact of the BZD phenyl ring substitution. The latter was functionalized

either by palladium-catalyzed cross-coupling, taking advantage of the bromine at C7, or by the use of initially functionalized 2-aminobenzophenones according to scheme 4.

Scheme 4. Late stage functionalization of 7-bromo-5-phenyl-4-propyl, 1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one via palladium-catalyzed cross-coupling or via early stage functionalization.

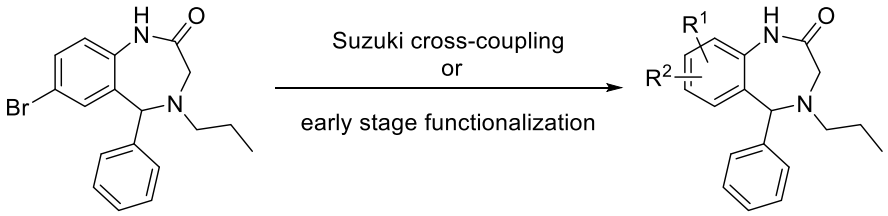
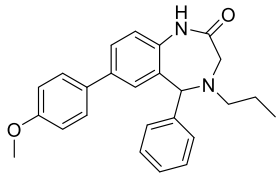
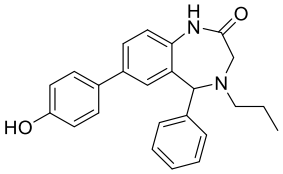
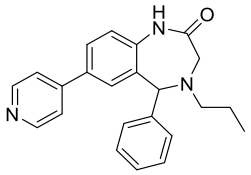
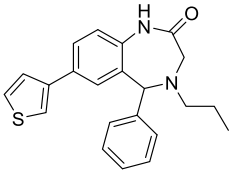
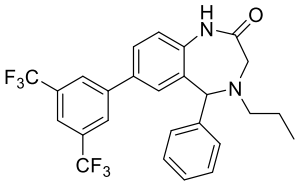
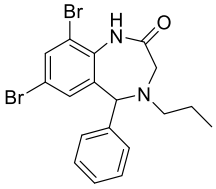
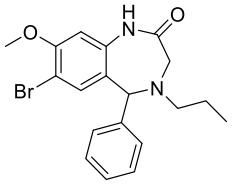
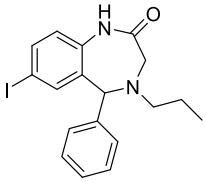
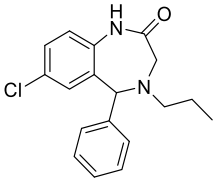
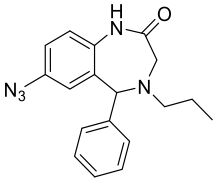


Table 3. Evaluation of the substitution at the phenyl benzodiazepine ring

Compound	Structure	Yield (%)	Protection (%)	EC_{50} (µM)
34		93	inactive	n.d.
35		20	inactive	n.d.
36		56	inactive	n.d.

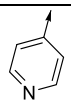
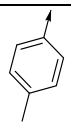
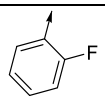
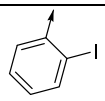
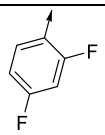
37		70	inactive	n.d.
38		55	inactive	n.d.
39		72	inactive	n.d.
40		44	30.7	n.d.
41		54	>100	1.98±1.12
42		99	>100	12.82±1.41
43		56	>100	11.05±1.62

Based on palladium coupling reactions, various aromatic (compounds **34**, **35**, **38**) and heteroaromatic (compounds **36**, **37**) compounds were synthesized, but were completely inactive.

9-Bromo or 8-methoxy derivatives of compound **29** were also obtained (compounds **39**, **40**), but did not protect cells against Stx. Substitution of the bromide at C7 by a chloride, iodide or azide maintained the protection of the cells against Stx (compounds **41**, **42**, **43**).

Substitution at C5. Because of the difficulty of late-stage modifications, we decided to obtain some benzodiazepines modified at C5 from different 2-amino, 5-bromobenzophenone analogs. The latter were either commercially available (Table 4, compounds **45**, **46**, **48**) or obtained by Friedel-Craft reaction between 5-bromoaniline and the corresponding benzonitrile (compound **44**).

Table 4. Evaluation of substitution at C5

Compound	R	Yield (%)	Protection (%)	EC_{50} (µM)
44		66	14.4	n.d.
45		56	inactive	n.d.
46		62	>100	3.87±0.01
47		71	59.6	n.d.
48		21	>100	12.82±0.21

Compound **47** was obtained via a directed palladium iodination reaction previously developed in our group.[15] Except for a 2-monofluorinated analog (compound **46**) and a 2,4-difluorinated analog

(compound **48**), all compounds tested proved less potent than compound **29**. Despite the fact that the fluoride atom is slightly larger than the hydrogen atom, little change to the steric bulk of the molecule is usually seen. The biggest changes in bioactivity are usually due to a large electronegativity difference between these two atoms (besides the higher lipophilicity of fluoride), but here there seems to be no influence of this parameter on bioactivity against Stx, though better cell membrane permeation cannot be ruled out.

Modification at the N4 secondary amine. In a final round of the SAR study, based on previous experience with the reduction of the propionyl substituent (see Table 2), we examined more extensively the effect of substitution on the secondary amine. The substituents were introduced by either reductive amination or nucleophilic substitution from compound **5** according to scheme 5:

Scheme 5. Introduction of substituents at N4.

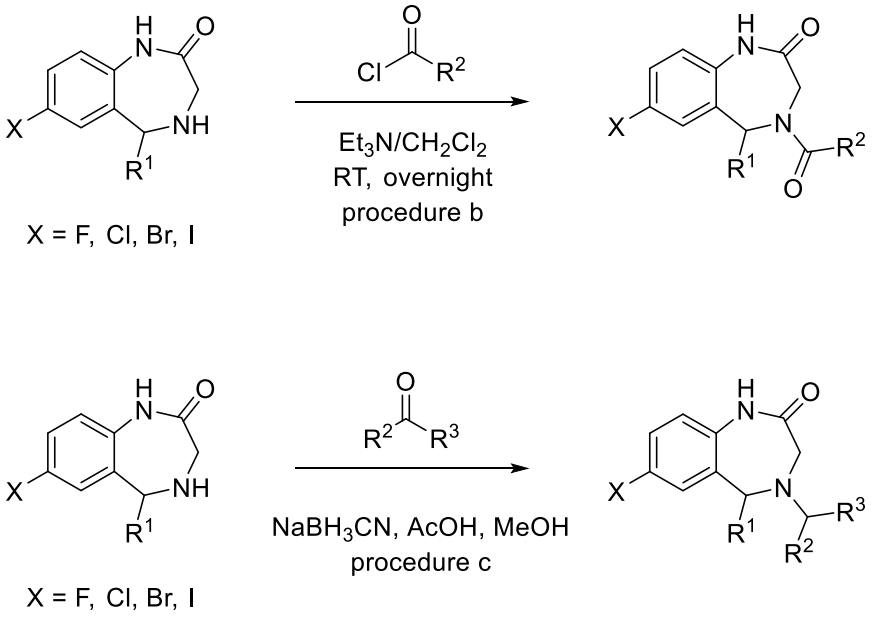
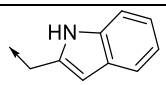
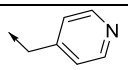
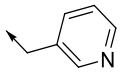
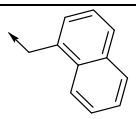
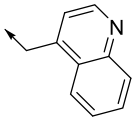
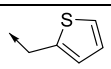
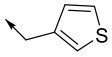
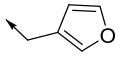
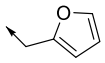
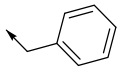
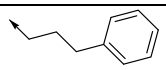
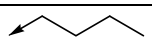
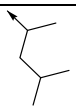
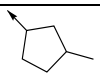
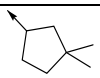
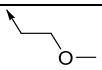


Table 5. Structure-activity relationship of compounds modified at N4

Compound	R	Yield (%)	Protection (%)	EC_{50} (μM)
49		50	20.6	n.d.
50		79	5.3	n.d.
51		91	38.8	n.d.
52		85	inactive	n.d.
53		74	29.9	n.d.
54		86	58.2	n.d.
55		88	12.2	n.d.
56		92	2.9	n.d.
57		88	Inactive	n.d.
58		95	Inactive	n.d.
59		78	Inactive	n.d.
5	H	74	Inactive	n.d.
60		90	Inactive	n.d.
61		23	85.8	8.81±2.70
62		85	29.5	n.d.
63		62	Inactive	n.d.
64		24	>100	4.70±0.01

65		27	>100	5.16±0.64
66		64	>100	0.60±0.04
67		46	Inactive	n.d.
68		57	>100	1.29±0.26
69		63	>100	3.91±0.63
70		72	Inactive	n.d.
71		47	Inactive	n.d.
72		100	Inactive	n.d.
73		55	Inactive	n.d.
74		38	>100	6.50±0.14

Numerous analogs were synthesized and tested. First, compounds bearing an indole ring were obtained, but were less active than Retro-1 (compound **49**). Then, pyridine substituents were introduced (4-substituted, compound **50** or 3-substituted pyridine, compound **51**), but neither compound showed improved efficiency. The naphthyl derivative **52** was completely inactive. A closely related structure, the quinoline moiety **53**, showed lower activity than Retro-1. A few small heterocyclic thiophenyl (compounds **54, 55**) and furyl (compounds **56**, **57**) substituents were synthesized and screened, but without any improvement. A phenyl substituent was introduced with either a one-methylene (compound **58**) or three-methylene (compound **59**) linker, but with complete loss of activity. Many aliphatic substituents (compounds **60-70**) were introduced and we obtained more potent inhibitors, especially with acyclic or cyclic derivatives (compounds **66, 68, 69**). It should be noted that more sterically demanding ramified aliphatic chains (compounds 6**3,**

67) and substituted aliphatic rings (compound **70**) gave lower bioactivity. The most promising candidates in this series were cyclopentyl derivatives (compounds **66, 68, 69**). Finally, functionalized aliphatic chains were inactive (compounds **71-73)**, or less active (compound **74**).

Halide effect. Having the best analog in hand (compound **66**), we focused our attention on the effect of bromide, since we already experienced a change in bioactivity with different halogens at C7 (see Table 4, compounds **41**, **42**). Therefore, we synthesized analogs containing respectively no halide (**75**), fluoride (**76**), chloride (**77**) and iodide (**78**). The iodinated analog (compound **78**) afforded an improved EC_{50} value of around 300 nM, suggesting that a putative X-bond with electron-rich groups present in the target(s) might account for this ranking of halogenated derivatives (Figure 3).[18,19]

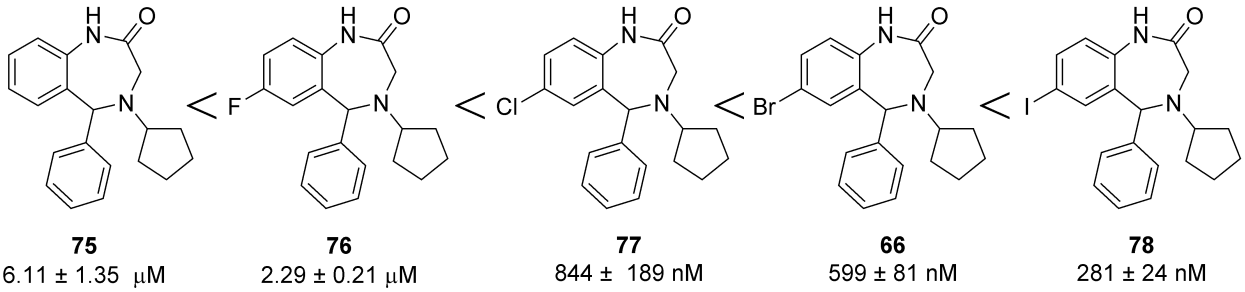


Figure 3. Effect of halogen at C7 on the bioactivity against Shiga toxin

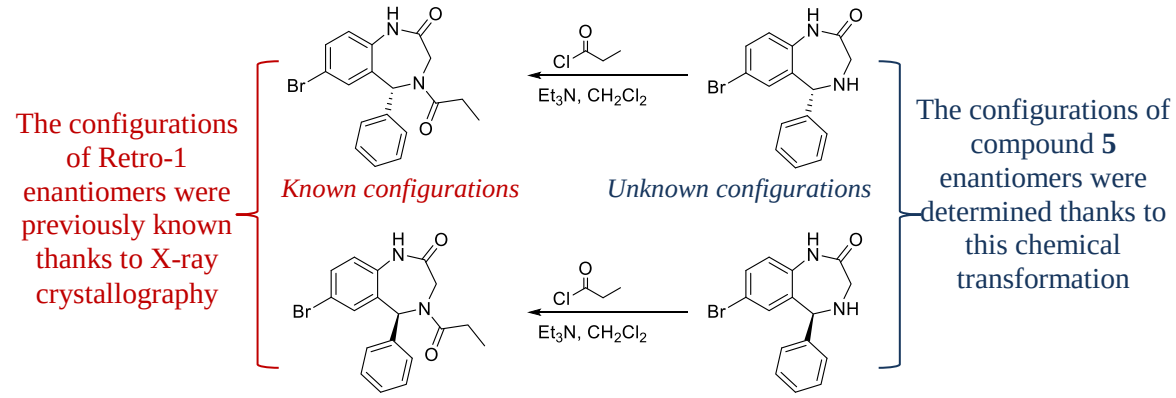
Since compound **78** was the best analog of Retro-1 synthesized during this SAR study, we decided to name it Retro-1.1.

Separation of enantiomers and attribution of configuration and biological activities. A chiral phase separation of the enantiomers of Retro-1.1 was carried out on a ChiralPak IA HPLC, which allowed us to obtain two enantiomers, Retro-1.1.a and Retro-1.1.b (Figure S1).

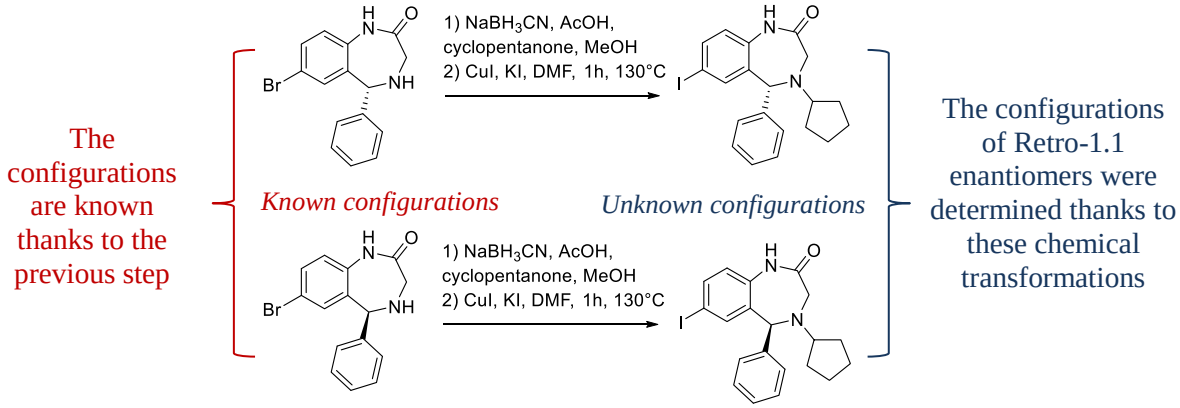
In order to determine the absolute configuration of each enantiomer, we decided to take advantage of the enantiomers of Retro-1 (scheme 6), an assignment that was previously achieved by X-ray crystallography.[17]

Scheme 6. Strategy to determine the absolute configurations of compounds 5 and 78 (Retro-1.1)

Step 1: We synthesized the two enantiomers of Retro-1 from the separated enantiomers of **5** which allowed us retrospectively to identify (*S*)-**5** and (*R*)-**5**.



Step 2: With the assignment of compound **5** enantiomers in hand, we decided to perform a two-step chemical modification, knowing that no inversion of configuration could occur during these transformations, which allowed us to obtain an HPLC profile of (*S*)-Retro-1.1 and (*R*)-Retro-1.1.



HeLa cells were then challenged against Stx in the presence of each enantiomer of Retro-1.1 (Figure 4). (*S*)-enantiomer of Retro-1.1 proved to be the eutomer, as already experienced with Retro-1.[17]

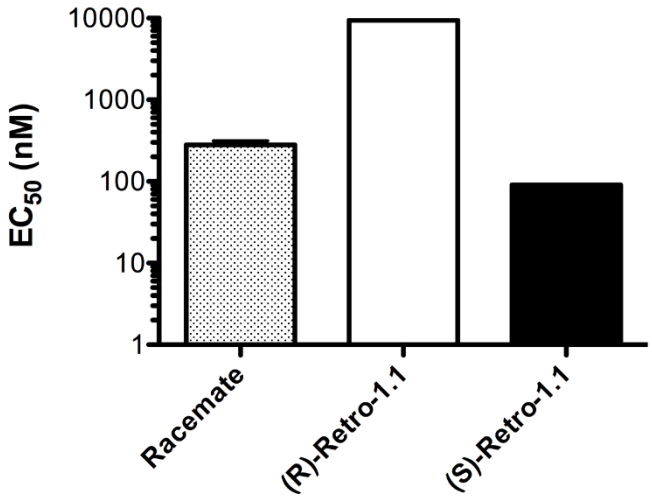


Figure 4. Evaluation of protective activity towards Stx cytotoxicity of each enantiomer of Retro-1.1. HeLa cells were incubated for 4 hours with racemic Retro-1.1 (grey), (*R*)-Retro-1.1 (white)**,** or (*S*)-Retro-1.1 (black) before the addition of Stx for 20 hours. Medium was removed and replaced by DMEM containing [^{14}C]-leucine at 0.5 µCi/mL for 7 hours before counting. Each data point represents the mean of duplicate ± SD of two independent experiments.

To prove that the mode of action of Retro-1.1 obtained herein prevents the deleterious effect of Stx by blocking its intracellular trafficking through the retrograde pathway, as reported for Retro-1, we examined the subcellular distribution of fluorescently labeled Stx. These experiments

showed that Shiga toxin is not able to reach the Golgi apparatus in the presence of (*S*)-Retro-1.1, whereas (*R*)-Retro-1.1 appeared unable to block Stx trafficking inside cells (Figure 5, Figure S2).

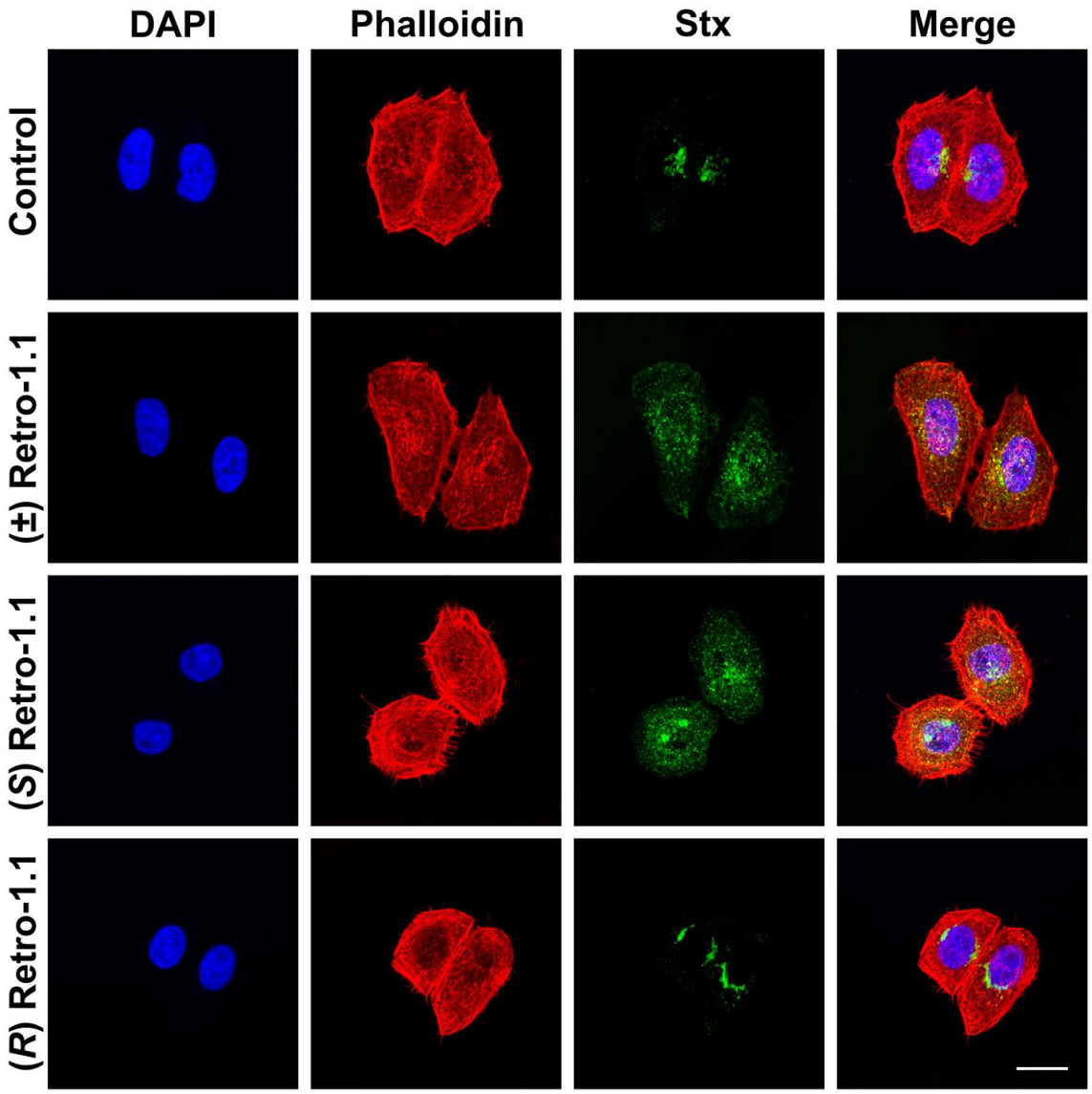


Figure 5. (±)-Retro-1.1 and (*S*)-Retro-1.1 block the retrograde transport of Shiga toxin. Cells were pretreated for 1 hour with (±)-Retro-1.1 (upper panel), (*S*)-Retro-1.1 (middle panel) or (*R*)-Retro-1.1 (lower panel) at 1 µM before addition of Alexa488-labeled StxB (0.1 µg/mL, green). Cells

were fixed with 4% PFA, and labeled with phalloidin-Atto-550 (red) and DAPI (blue) for actin and nuclei staining, respectively. Scale bar, 20 µm.

CONCLUSION

Based on our SAR study, we were able to obtain benzodiazepinones that afforded cells greater protection against Shiga toxin than the parent molecule Retro-1. This SAR study shows that a halogen atom at C7 is mandatory. The most active compound, (*S*)-Retro-1.1, provides an EC_{50} of 90 nM corresponding to a 70-fold improvement compared to the parent Retro-1. We also show that this compound blocks the retrograde trafficking of Shiga toxin. Experiments are in progress to decipher the mode of action of this compound and to identify its cellular target(s). As Retro-1 blocks retrograde trafficking similarly to Retro-2, it would be worth testing optimized Retro-1 compounds against various pathogens, in particular viruses such as poxvirus, cytomegalovirus and enterovirus 71, for which Retro-2 derivatives proved efficient.[20–22]

EXPERIMENTAL SECTION

Synthesis

All chemicals and solvents used in the syntheses were reagent grade and were used without additional purification. THF and CH_2Cl_2 were distilled respectively from sodium/benzophenone ketyl and calcium hydride before use. Glassware was flame-dried under vacuum and cooled under nitrogen to room temperature. All reactions were performed under dry nitrogen gas and monitored by thin-layer chromatography (TLC). TLC was performed with precoated TLC silica gel 60 F254,

and organic compounds were visualized by UV light (254 nm), iodine vapor, or phosphomolybdic acid [10% (w/v) in ethanol] staining with heating.

Large-scale purification was performed on a CombiFlash with a UV-visible detector with RediSep columns. The samples were adsorbed on Celite or silica and loaded into solid load cartridges. An ethyl acetate/cyclohexane or methanol/methylene chloride gradient was employed. Fractions were collected based on UV detection at 254 nm.

HPLC-MS analysis and purification were performed using a Waters system (2525 binary gradient module, in-line degasser, 2767 sample manager, 2996 Photodiode Array Detector) with a binary gradient solvent delivery system. This system was coupled with a Waters Micromass ZQ system with a ZQ2000 quadrupole analyzer. The ionization was performed by electrospray and the other parameters were as follows: source temperature 120 °C, cone voltage 20 V, and continuous sample injection at 0.3 mL/min flow rate. Mass spectra were recorded in both positive and negative ion mode in the m/z 100-2,000 range and treated with the Mass Lynx 4.1 software.

The eluent was a gradient of (99.9% water / 0.1% HCOOH) and (99.9% MeCN / 0.1% HCOOH) or (99.9% water / 0.1% HCOOH) and (99.9% MeOH / 0.1% HCOOH). Each compound was applied to a 100 x 4.6 mm (5 µm) WATERS XBridge C18 column equilibrated with H_2O/MeCN or H_2O/MeOH 95:5.

Gradient A: Samples were eluted by increasing MeOH to 100% (25 min) then 100% (5 min).

Gradient B: Samples were eluted by increasing MeOH to 90% (24 min), then 100% (1 min) and then staying at 100% (5 min).

Gradient C: Samples were eluted by increasing MeOH to 80% (24 min), then 100% (1 min) and then staying at 100% (5 min).

Gradient D: Samples were eluted by increasing MeCN to 100% (25 min) then 100% (1 min).

Gradient E: Samples were eluted by increasing MeCN to 90% (24 min), then 100% (1 min) and then staying at 100% (5 min).

Gradient F: Samples were eluted by increasing MeCN to 80% (24 min), then 100% (1 min) and then staying at 100% (5 min).

Gradient G: Samples were eluted by increasing MeCN to 60% (24 min), then 100% (1 min) and then staying at 100% (5 min).

HPLC (chiral) analyses were performed on a system equipped with a binary gradient solvent delivery system (LC-20AB, Shimadzu), an SIL-20A autosampler (Shimadzu), and a photodiode array detector (SPD-20A, Shimadzu).

The purity of the compounds was assessed by UPLC/UV (DAD 210 – 400 nm) using a Waters Acquity system equipped with a BEH XBridge C18 column (1.7 µM, 2.1*50 at 40 °C) and purity was ≥ 95% from the analysis detection mode (compounds 65, 67, 68, 69 were mixtures of diastereomers). Elution conditions were as follow:

Solvent:	A: H_2O+1/1000 HCO_2H, B: ACN+1/1000 HCO_2H		
T0	0.4 mL/min	95%A	5%B
T3min	0.4 mL/min	0%A	100%B
T3.1min	0.6 mL/min	0%A	100%B
T4min	0.6 mL/min	0%A	100%B

NMR experiments were performed on a Bruker Avance 400 Ultrashield spectrometer. ^{1}H-NMR and ^{13}C spectra were recorded at room temperature at 400 MHz and 100 MHz, respectively, with samples dissolved in DMSO-D6 at a concentration of approximately 5 mM. The DMSO singlet signal was set up at 2.50 ppm. Chemical shifts are given in ppm and the coupling constants in Hz. Spectral data are consistent with assigned structures.

High-resolution mass spectrometry (HRMS) was performed on an ESI/TOF LCP premier XE mass spectrometer (Waters) using flow injection analysis mode.

Physicochemical properties were calculated using MarvinSketch 5.4.1.1 software (ChemAxon)

High-resolution mass spectrometry (HRMS) was performed using the imagif platform (CNRS, Gif-sur-Yvette, France), and recorded on an ESI/TOF LCP premier XE mass spectrometer (Waters) using flow injection analysis mode.

For procedure (a): *see the synthesis of Compound **4***

For procedure (b): *see the synthesis of Compound **6***

For procedure (c): *see the synthesis of Compound **26***

For procedure (d): *see the synthesis of Compound **28***

For procedure (e): *see the synthesis of HA467*

For procedure (f): *see the synthesis of Compound **34***

For procedure (g): *see the synthesis of HA244*

7-Bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (4)

To a solution of 2-aminobenzophenone (10.14 mmol, 2 g) in dichloromethane (100 mL) was added N-bromosuccinimide (10.14 mmol, 1.8 g) at 0 °C. The mixture was stirred for 1 hour at this

temperature and for 2 hours at room temperature. The organic layer was washed with water (20 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was used in the next step without purification.

Procedure (a): To a solution of 5-bromo-2-aminobenzophenone **2** (10.14 mmol) in dichloromethane (100 mL) was added bromoacetyl bromide (12.16 mmol, 1.27 mL) followed by a 2M aqueous solution of Na_2CO_3 at 0 °C. The mixture was stirred for 2 hours at this temperature. The organic layer was separated and washed with water, dried over Na_2SO_4, filtered and concentrated under vacuum to give 5-bromo-2-bromoacetamidebenzophenone **3** as a yellowish solid. At 0 °C, 5-bromo-2-bromoacetamidebenzophenone **3** (10.14 mmol) was dissolved in a solution of NH_3 (7M in MeOH, 130 mL) and the mixture was stirred for 1 hour at this temperature and then allowed to warm up to room temperature overnight. The crude mixture was dried under vacuum, diluted in ethyl acetate and washed with water. The organic layer was concentrated under vacuum, dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was purified by flash chromatography (cyclohexane-ethyl acetate, 1-1). The desired compound **4** was obtained as a white solid (2.11 g, 66%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 10.66 (s, 1H), 7.76 (dd, *J* = 8.7 Hz, *J* = 2.3 Hz, 1H), 7.61-7.30 (m, 5H), 7.33 (d, *J* = 2.3 Hz, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 4.16 (s, 2H)

^{13}C-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 170.6, 168.8, 139.5, 139.1, 133.08, 129.6, 128.6, 123.8, 114.8, 57.5

I.R. (neat, cm^{-1}) 3430, 2951, 1680, 1605, 1476, 1381, 1355, 1319, 1285, 1259, 1233, 1193, 1082, 1012, 946, 895

HRMS *m/z* [(M+H)$^+$] calcd for $C_{15}H_{11}BrN_2O$ 315.0133, found 315.0134

7-Bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (5)

To a solution of **4** (1.58 mmol, 500 mg) in methanol (15 mL) was added $NaBH_3CN$ (2.37 mmol, 150 mg) followed by acetic acid (7.9 mmol, 440 µL) dropwise. The mixture was stirred at room temperature until complete conversion of the starting material. The mixture was than evaporated to dryness, diluted in ethyl acetate and washed with a saturated solution of $NaHCO_3$, then water. The organic layer was concentrated under vacuum, dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was purified by flash chromatography (cyclohexane-ethyl acetate, 1-1 to 1-2). The desired compound **5** was obtained as a white solid (370 mg, 74%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 9.96 (s, 1H), 7.44-7.26 (m, 6H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 2.14 Hz, 1H), 5.23 (d, *J* = 4.9 Hz, 1H), 3.68 (s br, 1H), 3.39 (dd, *J* = 15.7 Hz, *J* = 5.4 Hz, 1H), 3.26 (dd, *J* = 15.7 Hz, *J* = 8.0 Hz, 1H)

^{13}C-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 173.6, 141.9, 136.6, 128.8, 127.8, 122.9, 115.5, 61.7, 50.8

I.R. (neat, cm^{-1}) 3441, 3309, 3258, 3208, 3150, 3095, 3064, 2947, 2825, 1675, 1578, 1482, 1380, 1284, 1248, 1227, 1173, 119, 1076, 1052, 1027, 948, 913, 880, 855

HRMS *m/z* [$(M+H)^+$] calcd for $C_{15}H_{14}BrN_2O$ 317.0290, found 317.0291

Procedure (b): 7-Bromo-5-phenyl-4-propionyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (6)

To a solution of compound **5** (0.315 mmol, 100 mg) in dichloromethane (3 mL) was added propionyl chloride (0.410 mmol, 36 µL) followed by triethylamine (0.315 mmol, 44 µL). The mixture was allowed to stir at room temperature overnight. A solution of saturated $NaHCO_3$ was added and the organic layer was extracted with dichloromethane, dried over Na_2SO_4, filtered, then

concentrated under vacuum. The crude mixture was washed with diethyl ether. The crude mixture was purified by flash chromatography (cyclohexane-ethyl acetate, 5-1 to 1-1) furnishing the desired compound **6** as a white solid (374 mg, 79%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm 100 °C: 9.65 (s, 1H), 7.65 (s, 1H), 7.47 (dd, J = 8.7 Hz, *J* = 1.9 Hz, 1H), 7.23-7.32 (m, 3H), 7.04 (dd, *J* = 8.5 Hz, *J* = 14 Hz, 3H), 6.57 (s, 1H), 4.09 (q, *J* = 15.7 Hz, 2H), 1.05 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 172.9-172.3, 168.8-168.3, 139.3-139.1, 136.7- 136.5, 133.8-133.4, 132.0-131.7, 131.7-131.6, 128.7-128-4, 127.5-127.2, 126.6-126.4, 123.5- 123.5, 115.9-115.8, 61.4-59.2, 49.2-46.1, 26.2-25.7, 9.3-9.2

I.R. (neat, cm^{-1}) 3198, 3131, 3055, 2973, 2935, 1684, 1628, 1491, 1449, 1421, 1382, 1331, 1315, 1244,

1201, 1129, 1082, 1023, 964, 921, 892, 827

HRMS m/z [(M+H)+] calcd for $C_{18}H_{18}BrN_2O_2$ 373.0552, found 373.0554

7,9-Dibromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (19)

To a solution of 2-amino-5-bromobenzophenone **2** (1.014 mmol, 200 mg) in dichloromethane (10 mL) was added N-bromosuccinimide (1.014 mmol, 189 mg) at 0 °C. The mixture was stirred for 1 hour at this temperature and for 2 hours at room temperature. The organic layer was washed with water (20 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was used in the next step without purification. Then, according to procedure (a), 120 mg of the desired compound was obtained (0.304 mg, 30% over 2 steps).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.98 (s, 1H), 8.19 (d, *J* = 2.2 Hz, 1H), 7.57-7.41 (m, 5H), 7.37 (d, *J* = 2.2 Hz, 1H), 5.23 (s, 1H), 4.49 (m, 1H), 3.89 (m, 1H)

^{13}C-NMR (150 MHz, $CDCl_3$) δ (ppm) = 169.3, 167.5, 138.1, 137.0, 136.6, 130.6, 129.1, 128.4, 117.8, 115.5, 56.7, 26.3

I.R. (neat, cm^{-1}) 3367, 3204, 3073, 1688, 1607, 1579, 1461, 1446, 1379, 1317, 1231, 1175, 1151, 1011, 858, 736

HRMS *m/z* [$(M+H)^+$] calcd for $C_{15}H_{11}Br_2N_2O$ 329.9238 found 329.9233

5-(2-Iodophenyl)-1-methyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (15)

To a solution of 5-phenyl-1-methyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (30 mg, 0.12 mmol) in CH_3CN (1.2 mL) were added $Pd(OAc)_2$ (2.7 mg, 0.012 mmol) and N-iodosuccinimide (54 mg, 0.24 mmol). The mixture was stirred at 100 °C for 15 minutes. The crude mixture was evaporated, diluted in ethyl acetate (10 mL), and washed with a 2M aqueous solution of NaOH (5 mL). The residue was purified by flash chromatography (cyclohexane/ethyl acetate 1:1), affording 30 mg (69%) of the desired compound.

^{1}H-NMR (400 MHz, CD_3CN) δ (ppm) = 7.83 (d, *J* = 8.0 Hz, 1H), 7.56 (dt', *J* = 1.5Hz, *J* = 8.7 Hz, 1H), 7.51-7.45 (m, 2H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.15 (ddd, *J* = 9.2 Hz, *J* = 6.4 Hz, *J* = 2.8 Hz, 1H), 7.09 (dt', *J* = 7.9Hz, *J* = 0.9Hz, 1H), 6.94 (dd, *J* = 7.8 Hz, *J* = 1.4 Hz, 1H), 4.58 (d, *J* = 10.6 Hz, 1H), 3.76 (d, *J* = 10.6 Hz, 1H), 3.39 (s, 3H)

^{13}C-NMR (75 MHz, CD_3CN) δ (ppm) = 173.5, 170.1, 145.5, 140.3, 132.5, 131.6, 130.1, 129.6, 129.3, 124.9, 122.6 (C-9), 118.3 (C-9a), 96.8 (C-2'), 57.7 (C-3), 35.1 (1-$\underline{C}H_3$)

I.R. (neat, cm^{-1}) 3057, 2988, 2850, 1676, 1611, 1573, 1489, 1449, 1361, 1324, 1280, 1201, 1167, 1128, 1076, 1046, 1014, 984, 939, 915

HRMS *m/z* [(M+H)$^+$] calcd for $C_{16}H_{14}IN_2O$ 377.0151 found 377.0145

5-(p-Tolyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (20)

According to procedure (a): From 2-amino-4'-methylbenzophenone (0.946 mmol, 200 mg), 124 mg of the desired product was obtained (0.870 mmol, 92% over 2 steps)

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.52 (s, 1H), 7.56 (dt, *J* = 8.4 Hz, *J* = 1.4 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 6.9 Hz, 4H), 7.17 (t, *J* = 7.4 Hz, 1H), 5.23 (s, 1H), 4.14-4.01 (m, 2H), 2.35 (s, 3H)

^{13}C-NMR (150 MHz, $CDCl_3$) δ (ppm) = 172.1, 171.5, 141.1, 139.1, 136.2, 132.1, 131.7, 130.0, 129.0, 127.0, 123.4, 121.4, 56.2, 21.5

I.R. (neat, cm^{-1}) 3182, 3104, 3059, 2973, 2923, 2843, 1675, 1603, 1577, 1484, 1442, 1428, 1322, 1299, 1181, 1020, 1006, 924

HRMS *m/z* [(M+H)$^+$] calcd for $C_{30}H_{27}N_3O_2$ 251.1184 found 251.1172

7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (16)

According to procedure (a): From 2-amino-2',5-dichlorobenzophenone (1.87 mmol, 500 mg), 220 mg of the desired product was obtained (0.729 mmol, 39% over 2 steps)

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.79 (s, 1H), 7.62-7.49 (m, 5H), 7.25 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 4.21 (s, 2H)

I.R. (neat, cm^{-1}) 3210, 3123, 3072, 2928, 2851, 1688, 1616, 1591, 1569, 1482, 1434, 1387, 1325, 1230, 1195, 1059, 951

HRMS *m/z* [(M+H)$^+$] calcd for $C_{15}H_{11}Cl_2N_2O$ 305.0248 found 305.0240

7-Bromo-5-(p-tolyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (21)

To a solution of 2-amino-4'-methylbenzophenone (0.946 mmol, 200 mg) in dichloromethane (10 mL) was added N-bromosuccinimide (0.946 mmol, 168 mg) at 0 °C. The mixture was stirred for 1 hour at this temperature and 2 hours at room temperature. The organic layer was washed with water (20 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was used in the next step without purification. Then, according to procedure (a), 209 mg of the desired product was obtained (0.633 mmol, 67%)

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 10.63(s, 1H), 7.75 (dd, *J* = 8.7 Hz, *J* = 2.3 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 2.2 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 4.18-4.04 (s, 2H), 2.36 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$) δ ppm: 172.3, 169.8, 141.1, 138.0, 136.0, 134.7, 129.7, 129.2, 128.9, 123.1, 116.2, 56.5, 21.2

IR (neat, cm^{-1}) 3209, 3117, 3049, 2922, 2853, 1682, 1604, 1567, 1478, 1381, 1346, 1320, 1230, 1182, 1021, 1011, 945

HRMS *m/z* [(M+H)$^+$] calcd for $C_{16}H_{14}BrN_2O$ 329.0290 found 329.0282

7-Chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (17)

To a suspension of NaH (17 mg, 0.708 mmol) in THF (4 mL) was added at 0 °C 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (100 mg, 0.329 mmol). After 30

minutes of stirring at room temperature, MeI (20 µL, 0.329 mmol) was added and the mixture was stirred for an additional hour at room temperature. A saturated solution of NH_4Cl (10 mL) was added and the mixture was extracted with ethyl acetate (2 × 20 mL). The residue was concentrated under vacuum and purified by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1), affording 60 mg (57%) of the desired compound.

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 10.63 (s, 1H), 7.75 (dd, *J* = 8.7 Hz, *J* = 2.3 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 2.2 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 4.18-4.04 (s, 2H), 2.36 (s, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$) δ ppm: 169.2, 168.6, 141.7, 137.7, 132.9, 131.4, 131.1, 131.0, 130.9, 129.5, 127.9, 127.1, 122.7, 56.7, 34.8

IR (neat, cm^{-1}) 2986, 2923, 2854, 1678, 1484, 1345, 1323, 1196, 1129

HRMS *m/z* [(M+H)$^+$] calcd for $C_{16}H_{13}Cl_2N_2O$ 319.0405 found 319.0398

4-Acryloyl-7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (22)

According to procedure (b): From 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (100 mg, 0.419 mmol) and acroyl chloride (44 µL, 0.545 mmol), 78 mg of the desired product was obtained (0.210 mmol, 50%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 2 rotamers 10.11 (s, 1H), 10.07 (s, 1H), 7.92 (d, *J* = 2.1 Hz, 1H), 7.85 (dd, *J* = 16.6 Hz, *J* = 10.5 Hz, 1H), 7.74 (d, *J* = 2.1 Hz, 1H), 7.50-7.57 (m, 2H), 7.20-7.35 (m, 5H), 6.94-7.01 (m, 5H), 6.68-6.77 (m, 2H), 6.62 (s, 1H), 6.14-6.26 (m, 2H), 5.71-

5.83 (m, 2H), 4.37 (d, *J* = 14.9 Hz, 1H), 4.22 (d, *J* = 15.6 Hz, 1H), 4.12 (d, *J* = 15.6 Hz, 1H), 3.92 (d, *J* = 14.9 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.4, 166.6, 166.6, 166.0, 138.4, 137.5, 135.5, 134.7, 134.2, 133.1, 132.7, 132.1, 130.8, 130.6, 130.4, 129.9, 129.0, 128.4, 128.2, 127.9, 127.0, 126.8, 126.6, 123.7, 122.9, 117.5, 117.4, 63.4, 48.7, 46.2,

I.R. (neat, cm^{-1}) 3211, 3129, 2989, 1662, 1367, 791, 699

HRMS *m/z* [(M+H)$^+$] calcd for $C_{18}H_{16}BrN_2O_2$ 371.0395, found 371.0405

7-Bromo-4-butyryl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one as mixture 1:1 of 2 conformers (23)

According to procedure (b): From 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (200 mg, 0.633 mmol) and butyryl chloride (66 µL, 0.633 mmol), 192 mg of the desired product was obtained (0.495 mmol, 78%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.11 (s, 1H), 10.05 (s, 1H), 7.96 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 2.1 Hz, 1H), 7.54 (dt, *J* = 9.7 Hz, *J* = 2.2 Hz, 2H), 7.39-7.20 (m, 6H), 7.05-6.92 (m, 6H), 6.71 (s, 1H), 6.44 (s, 1H), 4.27 (d, *J* = 15.1 Hz, 1H), 4.13 (d, *J* = 15.5 Hz, 1H), 4.02 (d, *J* = 15.5 Hz, 1H), 3.89 (d, *J* = 15.1 Hz, 1H), 2.55-2.12 (m, 4H), 1.61-1.48 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.82 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.1, 171.4, 168.7, 168.3, 139.3, 139.2, 136.7, 136.5, 133.8, 133.4, 132.0, 131.8, 131.7, 131.6, 128.6, 128.4, 127.5, 127.2, 126.6, 126.3, 123.6, 123.4, 115.9, 115.8, 61.5, 59.1, 49.3, 46.0, 34.7, 34.0, 18.14, 18.05, 13.7, 13.6

I.R. (neat, cm^{-1}) 3213, 3134, 3000, 2966, 2875, 1662, 1617, 1492, 1438, 1401, 1339, 1321, 1302, 1232, 1213, 1193, 1157, 1140, 1031, 962, 835

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{20}BrN_2O_2$ 387.0708 found 387.0724

7-Bromo-4-isobutyryl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one as mixture 5:6 of 2 conformers (24)

According to procedure (b): From 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (200 mg, 0.633 mmol) and isobutyryl chloride (66 μL, 0.633 mmol), 205 mg of the desired product was obtained (0.532 mmol, 84%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.13 (s, 1H), 10.06 (s, 1H), 7.96 (d, *J* = 1.7 Hz, 1H), 7.79 (d, *J* = 1.8 Hz, 1H), 7.54 (dt, *J* = 1.9 Hz, *J* = 8.5 Hz, 2H), 7.39-7.18 (m, 6H), 7.05-6.89 (m, 6H), 6.64 (s, 1H), 6.51 (s, 1H), 4.22 (d, *J* = 15.2 Hz, 1H), 4.11 (s, 1H), 3.92 (d, *J* = 15.2 Hz, 1H), 3.06-2.97 (m, 1H), 2.94-2.84 (m, 1H), 1.17 (d, *J* = 6.7 Hz, 3H), 1.05-0.94 (m, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 176.7, 170.4, 138.6, 134.7, 134.6, 132.2, 130.7, 129.3, 129.1, 128.2, 127.7, 126.9, 122.7, 117.7, 59.5, 48.6, 31.1, 19.9, 19.1

I.R. (neat, cm^{-1}) 3218, 3137, 2972, 2933, 2876, 1676, 1489, 1410, 1222, 1203, 1160, 1087, 908

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{20}BrN_2O_2$ 387,0708 found 387.0692

7-Bromo-4-(cyclopropanecarbonyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one as mixture 5:6 of 2 conformers (25)

According to procedure (b): From 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (200 mg, 0.633 mmol) and cyclopropanecarbonyl chloride (57 µL, 0.633 mmol), 202 mg of the desired product was obtained (0.526 mmol, 83%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.13 (s, 1H), 10.07 (s, 1H), 7.97 (d, *J* = 1.8 Hz, 1H), 7.69 (d, *J* = 1.5 Hz, 1H), 7.56 (dd, *J* = 8.5 Hz, *J* = 1.9 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.37-7.19 (m, 6H), 7.07-6.92 (m, 6H), 6.78 (s, 1H), 6.71 (s, 1H), 4.35-4.21 (m, 2H), 3.90 (d, *J* = 15.0 Hz, 1H), 3.34 (s, 1H), 2.06 (m, 1H), 1.93 (m, 1H), 0.85-0.63 (m, 8H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.8, 171.9, 168.6, 139.4, 139.3, 136.7, 136.4, 133.8, 133.4, 132.0, 131.9, 131.7, 131.3, 128.6, 128.4, 127.4, 127.2, 126.7, 126.4, 123.6, 123.4, 115.9, 115.8, 61.6, 59.5, 49.1, 46.4, 11.2, 8.3, 8.02, 7.7, 7.4

I.R. (neat, cm^{-1}) 3216, 3126, 3084, 3101, 3059, 3030, 2939, 2918, 1677, 1609, 11583, 1492, 1448, 1425, 1381, 1298, 1245, 1217, 1191, 1171, 1084, 1058, 1033, 887

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{18}BrN_2O_2$ 385.0552 found 385.0538

Procedure (c): 7-Bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (26)

Procedure (c): To a solution of 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (100 mg, 0.315 mmol) in methanol (3 mL, 0.01 M) was added $NaBH_3CN$ (30 mg, 0.473 mmol) and acetic acid (88 µL, 1.58 mmol). The solution was stirred at room temperature for 4 hours and then propionaldehyde (21 µL, 0.378 mmol) was added and the solution was stirred at

room temperature until complete consumption of starting materials. The crude mixture was evaporated, diluted in ethyl acetate (10 mL) and washed with a saturated solution of $NaHCO_3$ (3 mL). The residue was concentrated under vacuum and purified by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1), affording 103 mg (91%) of the desired compound.

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.00 (s, 1H), 7.42-7.19 (m, 6H), 7.02 (m, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 4.97 (s, 1H), 3.52 (d, *J* = 16.1 Hz, 1H), 3.40 (d, *J* = 16.1 Hz, 1H), 2.70-2.58 (m, 2H), 1.66-1.53 (m, 2H), 0.9 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.9, 140.5, 136.1, 133.8, 133.0, 131.3, 128.6, 128.5, 127.8, 122.0, 117.1, 68.5, 55.3, 52.8, 36.7, 20.8, 11.5

I.R. (neat, cm^{-1}) 3202, 3084, 2960, 2932, 2872, 1662, 1486, 1400, 1375, 732, 699

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{20}BrN_2O$ 359.0759 found 359.0756

Procedure (d): 7-Bromo-5-phenyl-4-propyl-1-(pyridin-2-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (28)

To a solution of 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (50 mg, 0.14 mmol) in dioxane (500 µL, 0.2 M) was added CuI (2.6 mg, 0.014 mmol), K_2CO_3 (38 mg, 0.28 mmol, N,N'-dimethylethylenediamine (3.4 µL, 0.028 mmol) and 2-bromopyridine (13.3 µL, 0.14 mmol). The mixture was heated at 110 °C in a sealed tube overnight than purified by flash chromatography (cyclohexane/ethyl acetate 10:1 to 3:1), affording 30 mg (50%) of the desired compound.

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 8.44 (d, *J* = 3.5 Hz, 1H), 7.85 (dt, *J* = 7.8 Hz, *J* = 1.8 Hz, 1H), 7.53-7.25 (m, 6H), 7.16 (d, *J* = 8.1 Hz, 1H), 7.00 (s, 1H), 6.87 (d, *J* = 8.6 Hz, 1H), 4.93 (s, 1H), 3.49 (d, *J* = 12.8 Hz, 1H), 3.42-3.26 (m, 2H), 3.19 (d, *J* = 12.9 Hz, 1H), 1.62-1.41 (m, 2H), 0.81 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 167.6, 153.1, 149.0, 141.3, 140.1, 137.8, 137.5, 133.1, 131.4, 128.6, 127.9, 126.1, 122.1, 121.8, 120.1, 67.8, 55.9, 53.5, 20.8, 11.6

I.R. (neat, cm^{-1}) 3060, 3028, 2959, 2927, 2871, 2851, 1681, 1586, 1571, 1477, 1465, 1432, 1338, 1303, 1284, 1235, 1174, 1113, 1062, 979, 862

HRMS *m/z* [$(M+H)^+$] calcd for $C_{23}H_{23}N_3OBr$ 436.1024 found 436.1022

7-Bromo-5-phenyl-4-propyl-1-(4-(trifluoromethyl)phenyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (29)

According to procedure (d): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (50 mg, 0.139 mmol) and 2-bromobenzotrifluoride (20 µL, 0.139 mmol), 29 mg of the desired product was obtained (0.057 mmol, 41%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 7.52-7.17 (m, 10H), 6.91 (s br, 1H), 6.69 (d, *J* = 8.5 Hz, 1H), 5.15 (s, 1H), 3.36 (d, *J* = 11.8 Hz, 1H), 3.15 (d, *J* = 11.8 Hz, 1H), 1.59-1.43 (m, 2H), 1.29-1.15 (m, 2H), 0.86 (t, *J* = 7.2 Hz, 3H)

I.R. (neat, cm^{-1}) 3063, 3026, 2961, 2933, 2873, 2819, 1682, 1613, 1596, 1490, 1477, 1449, 1326, 1278, 1267, 1166, 1125, 1069, 1029, 907

HRMS *m/z* [$(M+H)^+$] calcd for $C_{25}H_{23}N_2OBr\,F_3$ 503.0946 found 503.0932

7-Bromo-1-(3,5-dimethylphenyl)-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (30)

According to procedure (d): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (50 mg, 0.139 mmol) and 1-bromo-3,5-dimethylbenzene (19 µL, 0.139 mmol), 34 mg of the desired product was obtained (0.072 mmol, 52%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.53-7.25 (m, 6H), 7.04 (s, 1H), 6.90 (s, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 6.61-6.52 (m, 2H), 4.88 (s, 1H), 3.57 (d, *J* = 12.5 Hz, 1H), 3.33 (d, *J* = 12.5 Hz, 1H), 2.67-2.61 (m, 2H), 1.63-1.54 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 167.6, 142.5, 140.9, 140.2, 138.9, 136.6, 133.2, 131.5, 129.2, 128.7, 128.3, 127.8, 125.8, 125.3, 119.3, 68.4, 67.2, 57.0, 21.3, 20.9, 11.7

I.R. (neat, cm^{-1}) 3060, 3025, 2960, 2931, 2871, 2824, 1678, 1610, 1596, 1476, 1452, 1403, 1317, 1082, 986, 845

HRMS *m/z* [$(M+H)^+$] calcd for $C_{26}H_{28}N_2OBr$ 463.1385 found 463.1380

1-(4-Benzoylphenyl)-7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (31)

According to procedure (d): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (50 mg, 0.139 mmol) and 4-bromobenzophenone (36 mg, 0.139 mmol), 28 mg of the desired product was obtained (0.051 mmol, 37%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.75-7.65 (m, 5H), 7.57 (t, *J* = 7.6 Hz, 2H), 7.53-7.46 (m, 5H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.34-7.25 (m, 2H), 7.12-7.05 (m, 2H), 6.78 (d, *J* = 8.5 Hz, 1H),

3.49 (d, *J* = 12.3 Hz, 1H), 3.19 (d, *J* = 12.3 Hz, 1H), 1.61-1.43 (m, 2H), 1.26-1.17 (m, 2H), 0.89-0.80 (m, 3H)

I.R. (neat, cm^{-1}) 3059, 2960, 2930, 2876, 1685, 1659, 1599, 1476, 1447, 1306, 1277, 1175, 1079, 1028, 938

HRMS *m/z* [(M+H)$^+$] calcd for $C_{31}H_{28}N_2O_2Br$ 539.1334 found 539.1326

1-Benzyl-7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (HA253)

According to procedure (e): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (200 mg, 0.636 mmol) and benzyl bromide (76 µL, 0.636 mmol), 200 mg of the desired product was obtained (0.496 mmol, 78%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.63 (dd, *J* = 8.8 Hz, *J* = 2.3 Hz, 1H), 7.52-7.32 (m, 6H), 7.27 (d, *J* = 2.3 Hz, 1H), 7.17-7.12 (m, 3H), 7.03-6.96 (m, 2H), 5.54 (d, *J* = 15.6 Hz, 1H), 4.75 (d, *J* = 15.6 Hz, 1H), 4.66 (d, *J* = 10.5 Hz, 1H), 3.87 (d, *J* = 10.5 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 169.6, 168.9, 141.2, 137.7, 136.3, 134.4, 132.8, 132.0, 131.0, 129.6, 128.8, 128.6, 128.5, 127.6, 127.5, 124.2, 117.6, 56.6, 49.7

I.R. (neat, cm^{-1}) 3062, 3029, 2962, 2925, 2853, 1672, 1606, 1478, 1402, 1320, 1262, 1182, 1089, 1067, 1028, 908

HRMS *m/z* [(M+H)$^+$] calcd for $C_{22}H_{18}BrN_2O$ 405.0603 found 405.0583

1-Benzyl-7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (32)

According to procedure (c): From 1-benzyl-7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (30 mg, 0.074 mmol) and propionaldehyde (5 µL, 0.088 mmol), 27 mg of the desired product was obtained (0.059 mmol, 81%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.33-7.01 (m, 12H), 6.70 (s, 1H), 5.35 (d, *J* = 14.7 Hz, 1H), 4.42 (d, *J* = 14.7 Hz, 1H), 4.30 (s, 1H), 3.37 (d, *J* = 13.2 Hz, 1H), 3.22 (d, *J* = 13.2 Hz, 1H), 2.53-2.33 (m, 2H), 1.53-1.22 (m, 2H), 0.80 (t, *J* = 7.3 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 168.1, 141.5, 140.2, 137.6, 137.5, 133.1, 131.5, 128.7, 128.6, 128.4, 127.9, 127.8, 123.6, 119.7, 67.6, 56.0, 52.7, 50.3, 20.9, 11.7

I.R. (neat, cm^{-1}) 3062, 3028, 2961, 2931, 2873, 2826, 1666, 1479, 1454, 1412, 1376, 1317, 1080, 956, 865

HRMS *m/z* [$(M+H)^+$] calcd for $C_{25}H_{26}N_2OBr$ 449.1228 found 449.1228

7-Bromo-1-methyl-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (27)

To a suspension of NaH (8 mg, 0.333 mmol) in THF (2 mL) was added at 0 °C 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (60 mg, 0.167 mmol). After 30 minutes of stirring at room temperature, MeI (10 µL, 0.250 mmol) was added and the mixture was stirred for an additional hour at room temperature. A saturated solution of NH_4Cl (10 mL) was added and the mixture was extracted with ethyl acetate (2 × 20 mL). The residue was concentrated under vacuum and purified by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1), affording 30 mg (48%) of the desired compound.

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 7.58 (dd, *J* = 2.3 Hz, *J* = 8.5 Hz, 1H), 7.37-7.22 (m, 6H), 7.15 (s, 1H), 3.23 (d, *J* = 12.2 Hz, 1H), 4.78 (s, 1H), 2.88 (d, *J* = 12.3 Hz, 1H), 2.86 (s, 3H), 2.51-2.42 (m, 2H), 1.51-1.43 (m, 2H), 0.82 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 168.7, 141.9, 140.5, 135.9, 133.1, 131.5, 128.3, 127.5, 123.2, 120.1, 119.3, 67.6, 56.5, 53.3, 34.0, 28.4, 20.6, 11.4

HRMS *m/z* $[(M+H)^+]$ calcd for $C_{19}H_{22}BrN_2O$ 373.0916 found 373.0914

Procedure (e): 1-Allyl-7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (HA467)

To a solution of 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (200 mg, 0.636 mmol), in THF (6.4 mL, 1M) was added a 1M solution of NaHMDS (636 µL, 0.636 mmol). The mixture was stirred for 1 hour at 0 °C, then allyl iodide (58 µL, 0.636 mmol) was added and the mixture was stirred for 6 hours at room temperature. A saturated solution of NH_4Cl (10 mL) was added and the mixture was extracted with ethyl acetate (2 × 20 mL). The residue was concentrated under vacuum and purified by flash chromatography (cyclohexane/ethyl acetate 5:1 to 1:1), affording 180 mg (80%) of the desired compound.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.49-7.13 (m, 8H), 5.72-5.59 (m, 1H), 5.14-4.93 (m, 2H), 4.69 (d, *J* = 10.5 Hz, 1H), 4.46 (dd, *J* = 16.1 Hz, *J* = 4.2 Hz, 1H), 4.27 (dd, *J* = 16.0 Hz, *J* = 5.1 Hz, 1H), 3.67 (d, *J* = 10.5 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 169.0, 168.5, 141.9, 138.1, 134.3, 132.7, 132.7, 131.1, 130.7, 129.4, 128.4, 123.6, 117.4, 117.2, 56.9, 49.7

I.R. (neat, cm^{-1}) 3347, 3063, 3025, 2986, 2924, 1672, 1645, 1606, 1587, 1575, 1478, 1423, 1400, 1356, 1319, 1264, 1222, 1187, 1073, 1013, 986, 938, 916

HRMS *m/z* [(M+H)$^+$] calcd for $C_{18}H_{16}BrN_2O$ 355.0446 found 355.0441

1-Allyl-7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (33)

According to procedure (c): From 1-allyl-7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (30 mg, 0.084 mmol) and propionaldehyde (5 µL, 0.100 mmol), 28 mg of the desired product was obtained (0.058 mmol, 70%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.33-7.12 (m, 6H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.81 (s, 1H), 5.72-5.57 (m, 1H), 5.08-5.1 (m, 2H), 4.50 (s, 1H), 4.25 (dd, *J* = 15.4 Hz, *J* = 4.6 Hz, 1H), 3.97 (dd, *J* = 15.4 Hz, *J* = 5.6 Hz, 1H), 3.29 (d, *J* = 12.7 Hz, 1H), 3.06 (d, *J* = 12.7 Hz, 1H), 2.48-2.39 (m, 2H), 1.53-1.35 (m, 2H), 0.75 (t, *J* = 7.3 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 167.5, 141.7, 140.7, 136.7, 133.4, 133.3, 131.5, 128.6, 128.1, 127.8, 123.4, 119.4, 117.8, 68.1, 56.6, 53.5, 49.8, 20.9, 11.7

I.R. (neat, cm^{-1}) 3062, 3025, 2960, 2931, 2872, 2823, 1668, 1480, 1452, 1411, 1372, 1305, 1239, 1227, 1177, 1127, 1089, 1068, 926

HRMS *m/z* [(M+H)$^+$] calcd for $C_{21}H_{24}N_2OBr$ 399.1072 found 399.1057

Procedure (f) 7-(4-Methoxyphenyl)-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (34)

To a solution of **26** (0.028 mmol, 10 mg) in a mixture DME-H_2O (250 µL -25 µL) were added 4-methoxyphenylboronic acid (0.030 mmol, 4.6 mg), $Pd(PPh_3)_4$ (0.002 mmol, 2.6 mg) and K_2CO_3

(0.055 mmol, 7.7 mg). The mixture was stirred at 110 °C under microwave irradiation for 1 hour. The mixture was washed with water (2 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layer was concentrated under vacuum, dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was purified by flash chromatography (cyclohexane-diethyl ether-ethyl acetate, 6-5.5-0.5), furnishing the desired compound (10 mg, 93%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 10.02 (s, 1H), 7.52 (dd, *J* = 8.3 Hz, *J* = 2.0 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.37-7.31 (m, 2H), 7.30-7.24 (m, 3H), 7.19-7.13 (m, 2H), 6.96 (d, *J* = 8.7 Hz, 1H), 5.04 (s, 1H), 3.76 (s, 3H), 3.33-3.27 (m, 1H), 3.18 (d, *J* = 14.9 Hz, 1H), 2.58-2.40 (m, 2H), 1.63-42 (m, 2H), 0.85 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (400 MHz, $(CDCl_3)$) δ ppm: 174.5, 159.2, 141.5, 136.7, 135.8, 135.5, 132.5, 132.2, 131.1, 128.8, 128.6, 128.2, 127.8, 127.6, 126.6, 121.1, 114.3, 69.0, 55.5, 55.4, 53.2, 21.0, 11.7

I.R. (neat, cm^{-1}) 3186, 3062, 3034, 2960, 2935, 2818, 1670, 1606, 1489, 1465, 1378, 1244, 1178, 1064, 1026, 817

HRMS *m/z* [(M+H)$^+$] calcd for $C_{25}H_{27}N_2O_2$ 387,2073, found 387.2082

7-(4-Hydroxyphenyl)-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (35)

According to procedure (f): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (20 mg, 0.055 mmol) and 4-hydroxyphenylboronic acid (8.5 mg, 0.061 mmol), 4 mg of the desired product was obtained (0.011 mmol, 20%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 9.97 (s, 1H), 9.49 (s, 1H), 7.47 (dd, *J* = 8.2 Hz, *J* = 1.9 Hz, 1H), 7.40-7.09 (m, 9H), 6.78 (d, *J* = 8.5 Hz, 1H), 5.03 (s, 1H), 3.33-3.27 (m, 1H), 3.18 (d, *J* = 14.9 Hz, 1H), 2.58-2.40 (m, 2H), 1.62-1.43 (m, 2H), 0.85 (t, *J* = 7.5 Hz, 3H)

I.R. (neat, cm^{-1}) 3585, 3183, 3064, 2963, 1878, 1651, 1609, 1491, 1436, 1393, 1267, 1222, 1177, 1078, 1065, 844

HRMS *m/z* [(M+H)$^+$] calcd for $C_{24}H_{25}N_2O_2$ 373.1916, found 373.1903

5-Phenyl-4-propyl-7-(pyridin-4-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (36)

According to procedure (f): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (20 mg, 0.055 mmol) and pyridineboronic acid (12.5 mg, 0.061 mmol), 11 mg of the desired product was obtained (0.031 mmol, 56%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.63 (d, *J* = 4.6 Hz, 2H), 8.34 (s, 1H), 7.69 (dd, *J* = 7.4 Hz, *J* = 12 Hz, 1H), 7.73-7.22 (m, 7H), 7.14 (d, *J* = 8.3 Hz, 1H), 5.15 (s, 1H), 3.76 (s, 3H), 3.58 (d, *J* = 16.3 Hz, 1H), 3.48 (d, *J* = 16.3 Hz, 1H), 2.74.269 (m, 2H), 1.67-1.61 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (400 MHz, $CDCl_3$) δ ppm: 173.9, 149.2, 148.2, 140.9, 138.2, 133.3, 132.3, 132.1, 131.6, 130.3, 128.8, 128.7, 128.6, 128.0, 127.1, 121.5, 121.2, 69.1, 55.5, 53.0, 21.0, 11.7

I.R. (neat, cm^{-1}) 3209, 3059, 2961, 1669, 1596, 1514, 1485, 1359, 1260, 1094, 1029, 908, 814

HRMS *m/z* [(M+H)$^+$] calcd for $C_{23}H_{24}N_3O$ 358.1919, found 358.1919

5-Phenyl-4-propyl-7-(thiophen-3-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (37)

According to procedure (f): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (20 mg, 0.055 mmol) and 3-thienylboronic acid (0.061 mmol, 7.8 mg), 14 mg of the desired product was obtained (0.038 mmol, 70%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 9.99 (s, 1H), 7.69 (m, 1H), 7.62 (dd, *J* = 1.9 Hz, *J* = 8.2 Hz, 1H), 7.40 (dd, J = 2.9 Hz, J = 5.0 Hz, 1H), 7.38-7.21 (m, 6H), 7.15 (d, J = 8.3 Hz, 2H), 5.07

(s, 1H), 3.33-3.27 (m, 1H), 3.19 (d, *J* = 15.2 Hz, 1H), 2.62-2.40 (m, 2H), 1.63-1.42 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 172.0, 142.3, 141.1, 137.2, 131.0, 130.8, 129.1, 128.8, 128.5, 127.7, 126.3, 121.3, 120.6, 68.4, 55.9, 54.2, 20.7, 11.9

I.R. (neat, cm^{-1}) 3203, 3054, 2961, 2926, 2873, 2850, 1663, 1586, 1493, 1421, 1264

HRMS *m/z* [(M+H)$^+$] calcd for $C_{22}H_{23}N_2OS$ 363.1531, found 363.1534

7-(3,5-Bis(trifluoromethyl)phenyl)-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (38)

According to procedure (f): From 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (20 mg, 0.055 mmol) and 3,5-bis(trifluoromethyl)phenylboronic acid (0.061 mmol, 16 mg) (0.061 mmol, 7.8 mg), 15 mg of the desired product was obtained (0.030 mmol, 55%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ ppm: 10.12 (s, 1H), 8.26 (s, 2H), 8.02 (s, 1H), 7.83 (s, *J* = 2.06 Hz, *J* = 8.3 Hz, 1H), 7.66 (d, *J* = 1.8 Hz, 1H), 7.36-7.17 (m, 6H), 5.27 (s, 1H), 3.42-3.2 (m, 2H), 2.62-2.40 (m, 2H), 1.63-1.42 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H)

I.R. (neat, cm^{-1}) 3207, 3083, 2962, 2933, 2875, 1666, 1610, 1379, 1276, 1179, 1131, 1070, 1002, 893, 844

HRMS *m/z* [(M+H)$^+$] calcd for $C_{26}H_{23}F_6N_2O$ 493.1715, found 493.1697

7,9-Dibromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (39)

According to procedure (c): From 7,9-dibromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (100 mg, 0.255 mmol) and propionaldehyde (17 µL, 0.306 mmol), 80 mg of the desired product was obtained (0.183 mmol, 72%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.65 (s, 1H), 7.88 (d, *J* = 2.1 Hz, 1H), 7.42-7.25 (m, 4H), 6.97 (d, *J* = 1.9 Hz, 1H), 5.23 (s, 1H), 3.27 (d, *J* = 13 Hz, 1H), 2.99 (d, *J* = 13 Hz, 1H), 2.52-2.35 (m, 2H), 1.67-1.39 (m, 2H), 0.81 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (150 MHz, $CDCl_3$) δ (ppm) = 168.4, 141.2, 137.3, 137.2, 134.5, 132.8, 128.9, 128.1, 118.0, 117.9, 68.1, 56.6, 53.2, 20.5, 11.9

I.R. (neat, cm^{-1}) 3378, 3188, 3066, 3028, 2960, 2932, 2872, 2825, 1674, 1581, 1556, 1462, 1351, 1268, 1239, 1207, 1149, 1081, 1063, 1029, 939, 863

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{19}Br_2N_2O$ 436.9864 found 436.9855.

Procedure (g) + procedure (a) 7-Bromo-8-methoxy-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (HA244)

Procedure (g) To a 1M solution of BCl_3 (2.7 mL, 2.72 mmol) in 1,2-dichlorethane (24 mL, 0.1 M) were added at 0 °C 4-bromo-3-methoxyaniline (500 mg, 2.47 mmol), benzonitrile (383 µL, 3.70 mmol) and $AlCl_3$ (362 mg, 2.72 mmol). The reaction was stirred at room temperature for 30 minutes and then refluxed for 16 hours. The reaction was cooled to 0 °C and a 1M solution of HCl (3 mL) was added. The mixture was stirred for 2 hours at 80 °C and then extracted with CH_2Cl_2 (3 × 30 mL) after the addition of H_2O (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The crude mixture was purified by flash chromatography (cyclohexane-ethyl acetate, 10-1 to 1-1), affording the desired compound (250 mg, 33%). Then,

according to procedure (a), 160 mg of the desired compound was obtained (0.442 mg, 54% over 2 steps).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.58 (s, 1H), 7.52-7.42 (m, 5H), 7.33 (s, 1H), 6.92 (s, 1H), 3.91 (s, 3H), 3.48-3.22 (m, 2H)

^{13}C-NMR (100 MHz, $(CDCl_3)$) δ (ppm) = 171.7, 170.2, 158.2, 139.9, 138.8, 135.5, 130.7, 129.7, 128.4, 120.9, 106.5, 103.9, 56.7, 56.6

7-Bromo-8-methoxy-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (40)

According to procedure (c): From 7-bromo-8-methoxy-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (60 mg, 0.174 mmol) and propionaldehyde (17 µL, 0.306 mmol), 30 mg of the desired product was obtained (0.076 mmol, 44%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.99 (s, 1H), 7.38-7.19 (m, 5H), 7.05 (s, 1H), 6.86 (s, 1H), 4.90 (s, 1H), 3.28 (d, *J* = 15.0 Hz, 1H), 3.15 (d, *J* = 15.0 Hz, 1H), 2.65-2.31 (m, 2H), 1.64-1.42 (m, 2H), 0.83 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, ($CDCl_3$**)** δ (ppm) = 174.2, 155.7, 141.1, 135.4, 127.8, 124.7, 106.7, 104.4, 68.0, 56.5, 55.5, 53.0, 20.9, 11.7

I.R. (neat, cm^{-1}) 3205, 3083, 2961, 2932, 2873, 1667, 1603, 1575, 1493, 1451, 1364, 1246, 1203, 1053, 850

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{22}N_2O_2Br$ 389.0865 found 389.0866

7-Iodo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (41)

To a solution of 2-aminobenzophenone (500 mg, 2.53 mmol) in EtOH (25 mL) were added I_2 (966 mg, 7.59 mmol) and $AgSO_4$ (3.16 g, 9.36 mmol). The mixture was stirred overnight at room temperature. Flash chromatography (cyclohexane-ethyl acetate, 9-1 to 7-1) afforded 289 mg of the monoiodinated compound (0.885 mmol, 35%) along with some diiodinated compound (ortho and para positions).

Then procedure (a): From (2-amino-5-iodophenyl)(phenyl)methanone (289 mg, 0.894 mmol) and bromoacetyl bromide (93 µL, 1.072 mmol), 215 mg of the desired compound was obtained (0.596 mmol, 67%).

Then procedure (c): From 7-iodo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (0.298 mmol) and propylaldehyde (20 µL, 0.357 mmol), 65 mg of the desired product was obtained (0.160 mmol, 54%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.01 (s, 1H), 7.59 (dd, *J* = 8.3 Hz, *J* = 1.9 Hz, 1H), 7.35 (d, J = 7.1 Hz, 2H), 7.32-7.26 (m, 2H), 7.20 (d, *J* = 7.2 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 1H), 4.97 (s, 1H), 3.35-3.25 (m, 1H), 3.16 (d, *J* = 15.1 Hz, 1H), 2.58-2.41 (m, 2H), 1.54-1.42 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 174.8, 140.8, 139.9, 137.4, 136.9, 133.2, 128.7, 128.4, 127.8, 122.5, 87.7, 68.5, 55.4, 53.1, 21.9, 20.9, 11.7

I.R. (neat, cm^{-1}) 3213, 3099, 2957, 2934, 2869, 1667, 1475, 1450, 1375, 1329, 1255, 1116, 1064, 1030, 931, 845

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{20}N_2OI$ 407.0620 found 407.0615

7-Chloro-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (42)

According to procedure (c): From 7-chloro-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (171 mg, 0.627 mmol) and propionaldehyde (43 µL, 0.752 mmol), 197 mg of the desired product was obtained (0.622 mmol, 99%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.08 (s, 1H), 7.38-7.33 (m, 2H), 7.32 (d, *J* = 2.4 Hz, 1H), 7.31-7.26 (m, 1H), 7.25-7.20 (m, 2H), 7.11 (d, *J* = 8.6 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 4.96 (s, 1H), 3.28 (d, *J* = 15.0 Hz, 1H), 3.16 (d, *J* = 15.0 Hz, 1H), 2.58-2.52 (m, 2H), 1.40-1.56 (m, 2H), 0.81 (t, *J* = 7.4 Hz, 3H),

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 174.2, 140.6, 135.8, 132.8, 130.9, 129.4, 128.6, 128.4, 127.7, 121.8, 68.5, 55.3, 52.8, 20.8, 11.5

I.R. (neat, cm^{-1}) 3067, 2926, 1652, 1491, 1402,700

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{20}ClN_2O$ 315.1264 found 315.1267

7-Azido-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (43)

To a solution of 7-bromo-5-phenyl-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (50 mg, 0.139 mmol) in a mixture of dioxane-H_2O (1.4 mL, 0.4 mL) were added NaN_3 (18 mg, 0.278 mmol), CuI (5 mg, 0.027 mmol), sodium ascorbate (3 mg, 0.014 mmol) and N, N'-dimethylethylenediamine (7 µL, 0.041 mmol). The mixture was stirred at 80 °C for 2 hours under microwave irradiation. Flash chromatography (cyclohexane-ethyl acetate, 5-1 to 1-1) afforded 25 mg of the desired compound (0.077 mmol, 56%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.97 (s, 1H), 7.38-7.21 (m, 5H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.06 (dd, *J* = 2.5 Hz, *J* = 8.5 Hz, 1H), 6.62 (d, *J* = 2.4 Hz, 1H), 4.95 (s, 1H), 3.40-3.22 (m, 1H), 3.14 (d, *J* = 14.8 Hz, 1H), 2.67-2.32 (m, 2H), 1.54-1.38 (m, 2H), 0.83 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (150 MHz, $CDCl_3$) δ (ppm) = 173.4, 140.6, 136.1, 134.1, 133.0, 128.6, 127.8, 121.9, 121.6, 118.9, 68.6, 55.5, 52.7, 20.7, 11.4

I.R. (neat, cm^{-1}) 3203, 3062, 2961, 2873, 2112, 1664, 1496, 1451, 1304, 1080

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{20}N_5O$ 322.1668 found 322.1670

7-Bromo-4-propyl-5-(pyridin-4-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (44)

According to procedure (g): From 4-bromoaniline (500 mg, 2.9 mmol) and 4-cyanopyridine (454 mg, 4.36 mmol), 70 mg of the desired product was obtained (0.261 mmol, 9%).

Then procedure (a): From (2-amino-5-bromophenyl)(pyridin-3-yl)methanone (70 mg, 0.254 mmol) and bromoacetyl bromide (26 µL, 0.30 mmol), 8 mg of the desired product was obtained (0.025 mmol, 8%).

Then procedure (c): From 7-bromo-5-(pyridin-4-yl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (8 mg, 0.025 mmol) and propionaldehyde (2 µL, 0.030 mmol), 6 mg of the desired product was obtained (0.016 mmol, 66%).

^{1}H-NMR (400 MHz, CD_3CN) δ (ppm) = 8.46 (d, *J* = 6.02 Hz, 2H), 8.00 (s, 1H), 7.44 (dd, *J* = 2.3 Hz, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 5.5 Hz, 2H), 7.14 (d, *J* = 2.2 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H),

4.88 (s, 1H), 3.29 (d, *J* = 13.7 Hz, 1H), 3.11 (d, *J* = 13.7 Hz, 1H), 2.61-2.42 (m, 2H), 1.53-1.46 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (150 MHz, $CDCl_3$) δ (ppm) = 169.9, 144.0, 136.4, 134.0, 133.3, 130.9, 124.8, 124.4, 118.9, 68.1, 58.2, 54.8, 21.0, 11.8

I.R. (neat, cm^{-1}) 3075, 2962, 2932, 1678, 1596, 1485, 1391, 1259, 1185, 1085, 1017, 908

HRMS *m/z* [(M+H)$^+$] calcd for $C_{17}H_{19}BrN_3O$ 360.0711 found 360.0708

7-Bromo-4-propyl-5-(p-tolyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (45)

According to procedure (c): From 7-bromo-5-(p-tolyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (50 mg, 0.152 mmol) and propionaldehyde (9 µL, 0.182 mmol), 32 mg of the desired product was obtained (0.085 mmol, 56%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.06 (s, 1H), 7.44 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.19-7.01 (m, 4H), 4.92 (s, 1H), 3.27 (d, *J* = 15.2 Hz, 1H), 3.15 (d, *J* = 15.2 Hz, 1H), 1.52-1.39 (m, 2H), 0.91-0.75 (m, 3H)

^{13}C-NMR (150 MHz, $CDCl_3$) δ (ppm) = 171.2, 138.1, 137.6, 133.3, 133.0, 130.8, 129.1, 128.0, 122.4, 115.4, 67.2, 55.3, 53.2, 20.7, 20.1, 11.5

I.R. (neat, cm^{-1}) 3204, 3084, 2961, 2930, 2872, 1662, 1487, 1375, 1397, 1071, 905

HRMS *m/z* [(M+H)$^+$] calcd for $C_{19}H_{22}BrN_2O$ 373.0916 found 373.0902

7-Bromo-5-(2-fluorophenyl)-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (46)

According to procedure (a): From 2-amino-5-bromo-2'-fluorobenzophenone (100 mg, 0.429 mmol), 68 mg of the desired product was obtained (0.205 mmol, 48%).

Then procedure (c): From 7-bromo-5-(2-fluorophenyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (68 mg, 0.205 mmol) and propionaldehyde (12 µL, 0.245 mmol), 48 mg of the desired product was obtained (0.265 mmol, 62%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.19 (s, 1H), 7.47 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.46-7.39 (m, 2H), 7.30-7.18 (m, 2H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 5.11 (s, 1H), 3.45-3.29 (m, 1H), 3.20 (d, *J* = 15.2 Hz, 1H), 2.67-2.32 (m, 2H), 1.56-1.42 (m, 2H), 0.81 (t, *J* = 7.3 Hz, 3H)

I.R. (neat, cm^{-1}) 3215, 3089, 2960, 2872, 1665, 1580, 1481, 1373, 1229, 1097

HRMS *m/z* $[(M+H)^+]$ calcd for $C_{18}H_{19}BrFN_2O$ 377.0665 found 377.0655

7-Bromo-2'-iodo-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepine-2-one (HA211)

To a solution of 7-bromo-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepine-2-one (30 mg, 0.095 mmol) in CH_3CN (950 µL) were added $Pd(OAc)_2$ (2.2 mg, 0.0095 mmol) and N-iodosuccinimide (43 mg, 0.19 mmol). The mixture was stirred at 100 °C for 15 minutes. The crude mixture was evaporated, diluted in ethyl acetate (10 mL), and washed with a 2M aqueous solution of NaOH (5 mL). The residue was purified by flash chromatography (cyclohexane/ethyl acetate 1:1), affording the desired product (17 mg, 41%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ= 10.82 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.71 (dd, *J* = 8.7 Hz, *J* = 2.3 Hz, 1H), 7.52 (dt', *J* = 7.4 Hz, *J* = 0.8 Hz, 1H), 7.45 (dd, *J* = 7.5 Hz, *J* = 1.4 Hz, 1H), 7.22 (d appt, *J* = 7.8 Hz, *J* = 1.4 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 2.2 Hz, 1H), 4.19 (s, 2H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$): δ= 170.7, 169.1, 143.7, 139.2, 139.1, 134.3, 131.4, 130.8, 128.1, 123.1, 114.3, 96.9, 56.8

I.R. (neat, cm^{-1}) 3207, 3117, 2927, 2852, 1689, 1617, 1479, 1429, 1382, 1322, 1291, 1255, 1230, 1195, 1164, 1134, 1088, 1047, 1011, 945 cm^{-1}

HR-MS (ESI+): *m/z* calcd for $C_{15}H_{11}BrIN_2O$ ($M+H^+$): 440.9099, found: 440.9090

7-Bromo-5-(2-iodophenyl)-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (47)

According to procedure (c): From 7-bromo-5-(2-iodophenyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (58 mg, 0.131 mmol) and propionaldehyde (9 µL, 0.156 mmol), 45 mg of the desired product was obtained (0.093 mmol, 71%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.28 (s, 1H), 7.92 (d, *J* = 7.1 Hz, 1H), 7.58 (dd, *J* = 1.6 Hz, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.1 Hz, 1H), 7.48 (dd, *J* = 8.4 Hz, *J* = 2.2 Hz, 1H), 7.15 (dt, *J* = 7.3 Hz, *J* = 1.7 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 4.81 (s, 1H), 3.37-3.27 (m, 1H), 3.20 (d, *J* = 15.6 Hz, 1H), 2.32-2.68 (m, 2H), 1.64-1.42 (m, 2H), 0.78 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 169.8, 141.6, 139.8, 139.0, 132.9, 131.6, 131.2, 130.2, 130.0, 128.5, 122.6, 115.9, 101.7, 71.9, 55.0, 51.7, 19.8, 11.5

I.R. (neat, cm^{-1}) 3165, 3041, 2955, 2926, 2869, 1682, 1666, 1597, 1478, 1457, 1435, 1379, 1323, 1262, 1171, 1115, 1067, 937

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{19}BrIN_2O$ 484.9725 found 484.9726

7-Bromo-5-(2,4-difluorophenyl)-4-propyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (48)

Procedure (g): From 4-bromoaniline (500 mg, 2.9 mmol), 2,4-difluorobenzonitrile (606 mg, 4.36 mmol), 35 mg of the desired product was obtained (0.116 mmol, 4%).

Then procedure (a): From (2-amino-5-bromophenyl)(2,4-difluorophenyl)methanone (35 mg, 0.116 mmol) and bromoacetyl bromide (12 µL, 0.13 mmol), 8 mg of the desired product was obtained (0.025 mmol, 21%).

Then procedure (c): From 7-bromo-5-(2,4-difluorophenyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (8 mg, 0.025 mmol) and propionaldehyde (9 µL, 0.03 mmol), 19 mg of the desired product was obtained (0.010 mmol, 43%).

^{1}H-NMR (400 MHz, CD_3CN) δ (ppm) = 10.19 (s, 1H), 7.52-7.36 (m, 2H), 7.25 (dt, *J* = 9.9 Hz, *J* = 2.3 Hz, 1H), 7.15 (dt, *J* = 8.4 Hz, *J* = 2.3 Hz, 2H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 1.8 Hz, 1H), 5.10 (s, 1H), 3.31 (d, *J* = 15.2 Hz, 1H), 3.19 (d, *J* = 15.1 Hz, 1H), 2.63-2.35 (m, 2H), 1.59-1.39 (m, 2H), 0.81 (t, *J* = 7.3 Hz, 3H)

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{18}N_2OBrF_2$ 395.0571 found 395.0563

4-((1H-Indol-2-yl)methyl)-7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (49)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and 1H-indole-3-carbaldehyde (55 mg, 0.378 mmol), 70 mg of the desired product was obtained (0.157 mmol, 50%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.96 (s, 1H), 10.09 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.47 (dd, *J* = 8.5 Hz, *J* = 2.2 Hz, 1H), 7.41-7.27 (m, 6H), 7.21 (d, *J* = 2.2 Hz, 1H), 6.95-7.10 (m, 4H), 5.02 (s, 1H), 3.88 (d, *J* = 13.2 Hz, 1H), 3.78 (d, *J* = 13.2 Hz, 1H), 3.18 (d, *J* = 14.8 Hz, 1H), 3.13 (d, *J* = 14.8 Hz, 1H),

I.R. (neat, cm^{-1}) 3450, 3058, 1666, 1486, 1456, 1398, 1226, 736

HRMS *m/z* $[(M+H)^+]$ calcd for $C_{24}H_{21}BrN_3O$ 446.0868 found 446.0882

7-Bromo-5-phenyl-4-(pyridin-4-ylmethyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (50)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and isonicotinaldehyde (36 µL, 0.378 mmol), 102 mg of the desired product was obtained (0.248 mmol, 79%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.18 (s, 1H), 8.53-8.50 (m, 2H), 7.48 (dd, *J* = 8.5 Hz, *J* = 2.1 Hz, 1H), 7.42-7.28 (m, 7H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 2.1 Hz, 1H), 4.99 (s, 1H), 3.78 (d, *J* = 14.7 Hz, 1H), 3.70 (d, *J* = 14.7 Hz, 1H), 3.29 (d, *J* = 14.9 Hz, 1H), 2.93 (d, *J* = 14.9 Hz, 1H),

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 172.0, 149.7, 147.4, 139.4, 136.6, 133.7, 133.0, 131.7, 128.9, 128.6, 128.3, 123.7, 122.3, 117.8, 68.1, 56.7, 52.6,

I.R. (neat, cm^{-1}) 3207, 3069, 1676, 1476, 1375, 1069, 826, 794, 697, 493

HRMS *m/z* $[(M+H)^+]$ calcd for $C_{21}H_{19}BrN_3O$ 408.0711 found 408.0720

7-Bromo-5-phenyl-4-(pyridin-3-ylmethyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (51)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and nicotinaldehyde (36 µL, 0.378 mmol), 117 mg of the desired product was obtained (0.286 mmol, 91%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.18 (s, 1H), 8.49 (d, *J* = 1.7 Hz, 1H), 8.47 (dd, *J* = 4.8 Hz, *J* = 1.7 Hz, 1H), 7.48 (td, *J* = 7.8 Hz, *J* = 1.7 Hz, 1H), 7.48 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.42-7.28 (m, 6H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 4.98 (s, 1H), 3.78 (d, *J* = 13.8 Hz, 1H), 3.67 (d, *J* = 13.8 Hz, 1H), 3.25 (d, *J* = 14.7 Hz, 1H), 2.94 (d, *J* = 14.7 Hz, 1H),

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 171.9, 149.9, 148.5, 139.6, 137.0, 136.6, 133.7, 133.6, 133.1, 131.7, 128.9, 128.6, 128.3, 123.5, 122.3, 117.8, 68.1, 55.1, 52.2

I.R. (neat, cm^{-1}) 3057, 2844, 1677, 1477, 1356, 1356, 1078, 788, 697

HRMS *m/z* [$(M+H)^+$] calcd for $C_{21}H_{19}BrN_3O$ 408.0711 found 408.0707

7-Bromo-4-(naphthalen-1-ylmethyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (52)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and 2-naphthaldehyde (51 µL, 0.378 mmol), 123 mg of the desired product was obtained (0.267 mmol, 85%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.16 (s, 1H), 8.18 (d, *J* = 7.4 Hz, 1H), 7.95-7.90 (m, 1H), 7.86 (dd, *J* = 7.4 Hz, *J* = 1.9 Hz, 1H), 7.58-7.49 (m, 3H), 7.48-7.40 (m, 2H), 7.36-7.30 (m, 2H), 7.28-7.17 (m, 4H), 7.12 (d, *J* = 8.6 Hz, 1H), 5.18 (s, 1H), 4.23 (d, *J* = 13.3 Hz, 1H), 4.09 (d, *J* = 13.3 Hz, 1H), 3.21 (d, *J* = 15.2 Hz, 1H), 3.06 (d, *J* = 15.2 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 174.7, 140.3, 136.1, 134.1, 133.9, 132.3, 131.8, 131.6, 128.7, 128.6, 128.1, 127.9, 126.0, 125.8, 125.2, 124.5, 122.3, 116.9, 67.8, 56.2, 53.1,

I.R. (neat, cm^{-1}) 3105, 3061, 1658, 1487, 1378, 905, 726, 693

HRMS *m/z* $[(M+H)^+]$ calcd for $C_{26}H_{22}BrN_2O$ 457.0916 found 457.0932

7-Bromo-5-phenyl-4-(quinolin-4-ylmethyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (53)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and quinoline-2-carbaldehyde (60 mg, 0.378 mmol), 107 mg of the desired product was obtained (0.233 mmol, 74%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.15 (s, 1H), 8.35 (d, *J* = 8.5 Hz, 1H), 7.97-7.92 (m, 2H), 7.75-7.70 (m, 2H), 7.59-7.54 (m, 1H), 7.48 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.40-7.26 (m, 5H), 7.10 (s, 1H), 7.09 (d, *J* = 6.3 Hz, 1H), 5.17 (s, 1H), 4.06 (d, *J* = 14.4 Hz, 1H), 4.01 (d, *J* = 14.4 Hz, 1H), 3.33 (d, *J* = 15.1 Hz, 1H), 3.12 (d, *J* = 15.1 Hz, 1H),

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.6, 158.8, 147.5, 140.0, 136.8, 136.0, 133.9, 132.2, 131.6, 129.6, 129.0, 128.8, 128.6, 128.5, 128.0, 127.5, 127.4, 126.4, 122.4, 120.5, 117.2, 68.5, 60.3, 53.7,

I.R. (neat, cm^{-1}) 3057, 1666, 1478, 1379, 725

HRMS *m/z* [$(M+H)^+$] calcd for $C_{25}H_{21}BrN_3O$ 458.0868 found 458.0846

7-Bromo-5-phenyl-4-(thiophen-2-ylmethyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (54)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and thiophene-2-carbaldehyde (35 µL, 0.378 mmol), 112 mg of the desired product was obtained (0.271 mmol, 86%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.14 (s, 1H), 7.48-7.43 (m, 2H), 7.41-7.28 (m, 5H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.98-6.93 (m, 3H), 5.00 (s, 1H), 3.94 (d, *J* = 14.2 Hz, 1H), 3.86 (d, *J* = 14.2 Hz, 1H), 3.25 (d, *J* = 14.7 Hz, 1H), 3.09 (d, *J* = 14.7 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.3, 141.7, 139.9, 136.3, 133.8, 132.7, 131.6, 128.8, 128.5, 128.0, 126.6, 126.4, 125.5, 122.3, 117.5, 67.3, 52.8, 52.6

I.R. (neat, cm^{-1}) 3237, 3044, 2929, 1671, 1477, 1374, 692

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{18}BrN_2OS$ 413.0323 found 413.0317

7-Bromo-5-phenyl-4-(thiophen-3-ylmethyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (55)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and thiophene-3-carbaldehyde (33 µL, 0.378 mmol), 115 mg of the desired product was obtained (0.277 mmol, 88%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.11 (s, 1H), 7.51-7.44(m, 2H), 7.41-7.28 (m, 5H), 7.05 (dd, *J* = 4.9 Hz, *J* = 1.1 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 4.94 (s, 1H), 3.70 (s, 2H), 3.22 (d, *J* = 14.5 Hz, 1H), 3.01 (d, *J* = 14.5 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.2, 140.0, 138.8, 136.4, 133.8, 133.0, 131.5, 128.8, 128.5, 128.1, 128.0, 125.9, 123.4, 122.3, 117.5, 67.7, 52.9, 52.6

I.R. (neat, cm^{-1}) 3237, 3057, 2931, 1671, 1477, 1376, 777, 698

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{18}BrN_2OS$ 413.0323 found 413.0328

7-Bromo-4-(furan-3-ylmethyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (56)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and furan-3-carbaldehyde (32 µL, 0.378 mmol), 115 mg of the desired product was obtained (0.289 mmol, 92%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.09 (s, 1H), 7.62-7.60 (m, 1H), 7.56 (brs, 1H), 7.46 (dd, *J* = 8.5 Hz, *J* = 2.2 Hz, 1H), 7.39-7.26 (m, 5H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 2.2 Hz, 1H), 6.95 (d, *J* = 1.0 Hz, 1H), 4.94 (s, 1H), 3.54 (s, 2H), 3.23 (d, *J* = 14.5 Hz, 1H), 3.06 (d, *J* = 14.5 Hz, 1H),

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 172.6, 143.3, 141.1, 140.0, 136.3, 133.8, 133.2, 131.5, 128.8, 128.5, 128.0, 122.1, 121.7, 117.6, 110.8, 67.5, 52.4, 48.3,

I.R. (neat, cm^{-1}) 3057, 1666, 1477, 1377, 1066, 821, 701

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{18}BrN_2O_2$ 397.0552 found 397.0551

7-Bromo-4-(furan-2-ylmethyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (57)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and furan-2-carbaldehyde (32 µL, 0.378 mmol), 110 mg of the desired product was obtained (0.277 mmol, 88%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.09 (s,1H), 7.61-7.57 (m, 1H), 7.46 (dd, *J* = 8.6 Hz, *J* = 2.4 Hz, 1H), 7.38-7.38 (m, 2H), 7.31-7.25 (m, 3H), 7.02 (d, *J* = 8.6 Hz, 1H), 6.98 (d, *J* = 2.1 Hz, 1H), 6.39 (dd, *J* = 3.1 Hz, *J* = 1.8 Hz, 1H), 6.30 (d, *J* = 3.1 Hz, 1H), 4.98 (s, 1H), 3.74 (d, *J* = 14.5 Hz, 1H), 3.69 (d, *J* = 14.5 Hz, 1H), 3.23 (d, *J* = 14.5 Hz, 1H), 3.09 (d, *J* = 14.5 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.3, 151.3, 142.4, 139.8, 136.3, 133.8, 132.8, 131.5, 128.7, 128.5, 127.9, 122.3, 117.4, 110.2, 109.3, 67.6, 53.0, 50.6

I.R. (neat, cm^{-1}) 3187, 3097, 2931, 1671, 1477, 816, 725, 700

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{18}BrN_2O_2$ 397.0552 found 397.0545

4-Benzyl-7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (58)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and benzaldehyde (38 µL, 0.378 mmol), 122 mg of the desired product was obtained (0.299 mmol, 95%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.16 (s, 1H), 8.53-8.50 (m, 11H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.92 (d, *J* = 2.2 Hz, 1H), 4.95 (s, 1H), 3.76 (d, *J* = 13.4 Hz, 1H), 3.61 (d, *J* = 13.4 Hz, 1H), 3.22 (d, *J* = 14.7 Hz, 1H), 2.95 (d, *J* = 14.7 Hz, 1H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.4, 140.2, 137.9, 133.7, 133.0, 131.5, 128.9, 128.8, 128.6, 128.4, 128.0, 127.4, 122.3, 117.4, 67.8, 57.8, 52.6

I.R. (neat, cm^{-1}) 3031, 2928, 1669, 1475, 1378, 1066, 815, 750, 695

HRMS *m/z* [$(M+H)^+$] calcd for $C_{22}H_{20}BrN_2O$ 407.0759 found 407.0744

7-Bromo-5-phenyl-4-(3-phenylpropyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (59)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and hydrocinnamaldehyde (49 µL, 0.378 mmol), 107 mg of the desired product was obtained (0.245 mmol, 78%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.04 (s, 1H), 7.44 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.37-7.10 (m, 11H), 7.06 (d, *J* = 8.5 Hz, 1H), 5.01 (s, 1H), 3.28 (d, *J* = 15.0 Hz, 1H), 3.16 (d, *J* = 15.0 Hz, 1H), 2.60-2.54 (m, 4H), 1.85-1.72 (m, 2H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 174.4, 141.8, 140.5, 136.1, 133.8, 132.6, 131.4, 128.7, 128.6, 128.3, 127.8, 125.8, 122.1, 117.0, 68.4, 53.0, 52.7, 33.0, 29.2

I.R. (neat, cm^{-1}) 3057, 2934, 1671, 1477, 1378, 719, 693

HRMS *m/z* [$(M+H)^+$] calcd for $C_{24}H_{24}BrN_2O$ 435.1072 found 435.1061

7-Bromo-4-methyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (60)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and paraformaldehyde (11 mg, 0.378 mmol), 94 mg of the desired product was obtained (0.283 mmol, 90%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.12 (s, 1H), 7.48 (dd, *J* = 8.5 Hz, *J* = 2.1 Hz, 1H), 7.40-7.25 (m, 5H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 4.69 (s, 1H), 3.39 (d, *J* = 14.7 Hz, 1H), 3.02 (d, *J* = 14.7 Hz, 1H), 2.36 (s, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 172.1, 139.7, 136.5, 134.0, 133.5, 131.4, 128.7, 128.5, 128.0, 122.1, 117.8, 69.6, 56.4, 43.1

I.R. (neat, cm^{-1}) 3241, 3043, 2989, 1652, 1486, 1416, 1378, 1354, 1227, 749, 699, 561, 502

HRMS *m/z* [$(M+H)^+$] calcd for $C_{16}H_{16}BrN_2O$ 331.0446 found 331.0446

7-Bromo-4-ethyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (61)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (100 mg, 0.315 mmol) and acetaldehyde (21 µL, 0.378 mmol), 25 mg of the desired product was obtained (0.072 mmol, 23%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.06 (s, 1H), 7.44 (dd, *J* = 8.5 Hz, *J* = 2.1 Hz, 1H), 7.37-7.20 (m, 5H), 7.04 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 2.4 Hz, 1H), 4.96 (s, 1H), 3.26 (d, *J* = 14.9 Hz, 1H), 3.16 (d, *J* = 14.9 Hz, 1H), 2.58 (q, *J* = 7.1 Hz, 2H), 1.03 (t, *J* = 7.1 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.4, 140.4, 136.3, 133.8, 133.3, 131.3, 128.7, 128.5, 127.8, 122.0, 117.3, 68.0, 52.5, 47.8, 13.1

I.R. (neat, cm^{-1}) 3202, 3084, 2970, 2931, 1662, 1484, 1389, 700

HRMS *m/z* [$(M+H)^+$] calcd for $C_{17}H_{18}BrN_2O$ 345.0603 found 345.0598

7-Bromo-4-butyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (62)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (50 mg, 0.158 mmol) and butyraldehyde (17 µL, 0.189 mmol), 50 mg of the desired product was obtained (0.134 mmol, 85%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.06 (s, 1H), 7.46 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.39-7.20 (m, 5H), 7.09 (d, *J* = 2.2 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 4.97 (s, 1H), 3.28 (d, *J* = 15.0 Hz, 1H), 3.16 (d, *J* = 15.0 Hz, 1H), 2.58-2.45 (m, 2H), 1.50-1.40 (m, 2H), 1.30-1.22 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 175.5, 140.8, 136.4, 133.9, 133.1, 131.4, 130.4, 130.1, 128.8, 128.7, 127.9, 122.3, 117.2, 68.7, 53.3, 53.03, 29.9, 20.3, 14.1

I.R. (neat, cm^{-1}) 3203, 3084, 2955, 2928, 2869, 1659, 1579, 1485, 1450, 1399, 1374, 1256, 1132, 1099, 1029, 972, 89

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{22}BrN_2O$ 373.0916 found 373.0902

7-Bromo-4-neopentyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (63)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (20 mg, 0.063 mmol) and trimethylacetaldehyde (16 µL, 0.151 mmol), 15 mg of the desired product was obtained (0.039 mmol, 62%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.03 (s, 1H), 7.47-7.42 (m, 2H), 7.36-7.22 (m, 3H), 7.15-7.06 (m, 3H), 5.16 (s, 1H), 3.28 (d, *J* = 15.0 Hz, 1H), 3.28 (s, 2H), 2.68-2.31 (m, 2H), 0.91 (s, 9H)

I.R. (neat, cm^{-1}) 3207, 3085, 2952, 2865, 1660, 1580, 1489, 1400, 1379, 1227, 1029

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{24}BrN_2O$ 387.1072 found 387.1064

7-Bromo-4-cyclohexyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (64)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (20 mg, 0.063 mmol) and cyclohexanone (8 µL, 0.076 mmol), 6 mg of the desired product was obtained (0.015 mmol, 24%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ= 9.82 (s, 1H), 7.59 (d, *J* = 2.2 Hz, 1H), 7.44 (dd, *J* = 8.5 Hz, *J* = 2.3 Hz, 1H), 7.32-7.22 (m, 2H), 7.22-7.16 (m, 3H), 6.95 (d, *J* = 8.5 Hz, 1H), 5.34 (s, 1H), 3.34-3.25 (m, 1H), 3.17 (d, J = 13.5 Hz, 1H), 2.52-1.13 (m, 11H)

^{13}C-NMR (100 MHz, $(CDCl_3)$): δ= 176.1, 142.2, 136.1, 133.7, 133.4, 131.2, 128.4, 128.1, 127.3, 122.5, 116.7, 65.7, 60.3, 50.7, 35.5, 31.1, 30.1, 25.3, 24.2

I.R. (neat, cm^{-1}) 3222, 3091, 2928, 2854, 1663, 1584, 1487, 1448, 1405, 1377, 1225, 1031, 971

7-Bromo-4-(1-cyclopropylethyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (as a mixture of 2 diastereoisomers) (65)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (20 mg, 0.063 mmol) and cyclopropylmethylketone (8 µL, 0.076 mmol), 6.5 mg of the desired product was obtained (0.017 mmol, 27%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.92-9.82 (m, 1H), 7.52-6.82 (m, 8H), 5.45-5.40 (m, 1H), 3.62-3.17 (m, 2H), 2.17-1.99 (m, 1H), 1.17-1.08 (m, 3H), 0.92-0.75 (m, 1H), 0.55-0.36 (m, 2H), 0.29-0.22 (m, 1H), 0.17-0.82 (m, 1H), 0.52-0.02 (m, 2H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.2, 171.8, 143.2, 142.6, 137.2, 134.2, 133.6, 133.5, 130.9, 130.8, 128.2, 128.1, 127.5, 127.4, 126.9, 126.8, 122.8, 122.6, 115.5, 115.4, 65.6, 62.5, 62.3, 51.6, 51.5, 18.2, 16.3, 15.3, 14.8, 5.8, 4.8, 2.4, 2.2

I.R. (neat, cm^{-1}) 3312, 3197, 3075, 2974, 2880, 1741, 1655, 1581, 1489, 1400, 1374, 1351, 1332, 1228, 1136, 1099, 1076, 907, 878, 826

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{22}N_2OBr$ 385.0915 found 385.0919

7-Bromo-4-cyclopentyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (66)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (500 mg, 1.58 mmol) and cyclopentanone (167 µL, 1.89 mmol), 390 mg of the desired product was obtained (1.011 mmol, 64%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.98 (s, 1H), 7.50 (d, *J*=2.1 Hz, 1H), 7.48-7.43 (dd, *J*=8.5 Hz, *J*=2.2 Hz, 1H), 7.31-7.21 (m, 3H), 7.15-7.02 (t, *J*=8.4 Hz, 3H), 5.30 (s, 1H), 2.96-2.90 (m, 1H), 1.89-1.77 (m, 2H), 1.64-1.42 (m, 2H), 1.50-1.36 (m, 4H),

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.3, 142.3, 136.8, 133.8, 132.1, 131.0, 128.3, 127.7, 127.0, 122.4, 115.05, 66.5, 63.2, 53.02, 23.1, 23.9

I.R. (neat, cm^{-1}) 3194, 3070, 2993, 2961, 1648, 1579, 1491, 1449, 1421, 1401, 1377, 1357, 1324, 1294, 1253, 1225, 1179, 1131, 980, 870, 812

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{22}BrN_2O$ 385.0916 found 385.0915.

7-Bromo-4-(4-methylpentan-2-yl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (as a mixture of 2 diastereoisomers) (67)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (70 mg, 0.222 mmol) and isobutylmethylketone (28 µL, 1.89 mmol), 41 mg of the desired product was obtained (0.102 mmol, 46%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.83-9.72 (m, 1H), 7.72-6.83 (m, 8H), 5.24-5.22 (m, 1H), 3.40-3.07 (m, 2H), 2.89-2.85 (m, 1H), 1.58-1.05 (m, 3H), 1.01-0.93 (m, 3H), 0.89-0.79 (m, 3H), 0.73-0.69 (m, 3H)

^{13}C-NMR (100 MHz, ($CDCl_3$) δ (ppm) = 175.5, 174.5, 142.7, 141.7, 136.4, 135.0, 134.9, 133.7, 133.6, 131.2, 131.1, 128.5, 128.4, 128.1, 127.7, 127.5, 127.2, 123.0, 122.7, 117.5, 117.3, 68.4, 64.9, 56.4, 55.5, 51.7, 48.4, 44.9, 42.8, 25.3, 24.6, 23.6, 23.1, 22.6, 22.2, 17.9, 15.8

I.R. (neat, cm^{-1}) 3203, 3061, 3027, 2954, 2926, 2867, 1668, 1579, 1595, 1484, 1468, 1450, 1393, 1384, 1366, 1248, 1225, 1159, 1127, 1028, 968, 823

HRMS *m/z* [$(M+H)^+$] calcd for $C_{21}H_{26}N_2OBr$ 401.1228 found 401.1238

7-Bromo-4-(3-methylcyclopentyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (as a mixture of diastereoisomers) (68)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (50 mg, 0.158 mmol) and 3-metylcyclopentanone (17 μL, 0.158 mmol), 36 mg of the desired product was obtained (0.090 mmol, 57%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.74-9.52 (m, 1H), 7.42-7.34 (m, 1H), 7.29-7.20 (m, 4H), 7.15-7.11 (m, 1H), 7.09-6.95 (m, 1H), 3.58-3.45 (m, 2H), 3.29-3.02 (m, 1H), 2.32-1.18 (m, 8H), 1.10 -0.92 (m, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 176.3, 176.2, 176.0, 141.7, 141.6, 141.5, 135.8, 134.3, 134.2, 131.5, 128.7, 128.6, 128.5, 128.4, 127.6, 127.5, 122.4, 122.3, 122.2, 116.5, 116.4, 67.9, 67.8, 67.7, 67.5, 62.4, 62.3, 61.8, 61.6, 52.7, 52.6, 52.4, 49.4, 48.9, 41.4, 41.0, 40.7, 39.3, 38.7, 32.7, 32.3, 32.2, 32.1, 31.9, 31.8, 31.0, 30.5, 27.0, 22.1, 22.0, 21.4, 21.1, 20.9, 20.1

I.R. (neat, cm^{-1}) 3204, 3083, 2950, 2866, 1657, 1579, 1488, 1449, 1401, 1365, 1224, 976, 907

HRMS *m/z* [$(M+H)^+$] calcd for $C_{21}H_{24}BrN_2O$ 399.1072 found 399.1071

7-Bromo-4-(2-methylcyclopentyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (as a mixture of diastereoisomers) (69)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** (50 mg, 0.158 mmol) and 2-methylcyclopentanone (25 µL, 0.237 mmol), 40 mg of the desired product was obtained (0.100 mmol, 63%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 10.03-9.83 (m, 1H), 7.62-7.02 (m, 8H), 5.30-5.26 (m, 1H), 3.38-3.14 (m, 2H), 2.85-2.75 (m, 1H), 1.90-1.10 (m, 6H), 0.97-0.85 (m, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 172.9, 172.3, 171.3, 143.2, 142.3, 141.8, 137.0, 136.7, 136.5, 134.1, 134.0, 133.8, 131.5, 131.1, 131.0, 128.4, 128.3, 128.0, 127.8, 127.7, 127.2, 127.1, 127.0, 126.6, 122.8, 122.3, 122.1, 115.6, 114.9, 114.8, 72.4, 66.4, 66.2, 64.8, 64.4, 64.1, 54.3, 53.7, 53.5, 36.1, 34.6, 33.9, 32.4, 30.7, 30.6, 28.3, 26.8, 26.7, 22.0, 19.9, 19.8, 19.7, 14.1, 13.3

I.R. (neat, cm^{-1}) 3193, 3063, 2957, 2869, 1654, 1597, 1579, 1489, 1448, 1419, 1400, 1376, 1320, 1288, 1224, 1179, 1075, 1026, 974, 872

HRMS *m/z* [$(M+H)^+$] calcd for $C_{21}H_{24}N_2OBr$ 399.1072 found 399.1075

7-Bromo-4-cyclopentyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (as a mixture of diastereoisomers) (70)

According to procedure (c) at 60 °C: From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** 100 mg, 0.318 mmol) and 3,3-dimethylcyclopentanone (35 mg, 0.381 mmol), 94 mg of the desired product was obtained (0.228 mmol, 72%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.56-9.51 (s, 1H), 7.44-7.36 (m, 1H), 7.33-7.08 (m, 6H), 7.01 (d, *J* = 7.7 Hz, 1H), 5.27-5.22 (s, 1H), 3.54-3.40 (m, 2H), 3.27-3.15 (m, 1H), 2.05-1.92 (m, 1H), 1.82-1.72 (m, 2H), 1.64-1.47 (m, 2H), 1.43-1.39 (m, 1H), 1.13-1.11 (s, 3H), 0.95-0.92 (s, 3H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 176.0, 175.9, 141.6, 135.8, 134.3, 134.2, 131.5, 128.6, 128.5, 127.6, 122.3, 116.5, 116.4, 67.7, 67.5, 61.9, 61.8, 52.7, 52.5, 46.9, 46.5, 39.4, 39.1, 37.8, 37.5, 31.5, 31.2, 31.0, 30.7, 30.4

HRMS *m/z* [$(M+H)^+$] calcd for $C_{22}H_{26}N_2OBr$ 413.1228 found 413.1224

Procedure (h) 4-Allyl-7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (71)

To a solution of 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (200 mg, 0.632 mmol) in THF (6 mL) was added allyl iodide (319 µL, 1.896 mmol). The mixture was stirred at room temperature overnight after which the solvent was evaporated. The crude extract was purified by flash chromatography (cyclohexane-ethyl acetate: 9-1 to 5-1), affording 107 mg of the desired compound (0.300 mmol, 47%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.10 (s, 1H), 7.46 (dd, *J* = 2.3 Hz, *J* = 8.5 Hz, 1H), 7.39-7.24 (m, 5H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 2.1 Hz, 1H), 5.89-5.73 (m, 1H), 5.17 (d, *J* = 18.2 Hz, 1H), 5.16 (d, *J* = 9.7 Hz, 1H), 4.95 (s, 1H), 3.38-3.22 (m, 2H), 3.38-3.12 (m, 2H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.7, 140.1, 136.3, 134.5, 133.6, 133.0, 131.3, 128.6, 128.4, 127.8, 122.2, 118.6, 117.3, 67.5, 56.7, 52.9

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{18}N_2OBr$ 357.0602 found 357.0615

7-Bromo-4-(2-methoxyethyl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (72)

According to procedure (c): From 7-bromo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **4** 50 mg, 0.158 mmol) and 3-methoxy-propionaldehyde (24 µL, 0.237 mmol), 60 mg of the desired product was obtained (0.158 mmol, 100%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.08 (s, 1H), 7.46 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.39-7.20 (m, 5H), 7.09 (d, *J* = 2.0 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 4.99 (s, 1H), 3.41-3.12 (m, 5H), 2.63-2.56 (m, 2H), 1.74-1.65 (m, 2H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 174.3, 140.5, 137.1, 136.3, 133.9, 132.9, 131.5, 128.8, 128.7, 127.9, 122.3, 117.2, 70.3, 68.7, 58.7, 53.0, 50.5

I.R. (neat, cm^{-1}) 3203, 3062, 2923, 2871, 1660, 1596, 1579, 1480, 1449, 1383, 1258, 1225, 1113, 1028, 908

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{22}N_2O_2Br$ 389.0865 found 389.0852

7-Bromo-5-phenyl-4-(prop-2-yn-1-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (73)

Procedure (h): To a solution of 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (100 mg, 0.316 mmol) in DMF (3 mL, 0.316 mmol) was added propargyl bromide (85 µL, 0.948 mmol). The mixture was stirred at 60 °C for 48 hours and then purified by flash chromatography (cyclohexane-ethyl acetate, 5-1 to 1-1), furnishing the desired compound (62 mg, 55%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.08 (s, 1H), 7.47 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.42-7.22 (m, 5H), 7.04 (d, *J* = 2.1 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 5.05 (s, 1H), 3.52-3.12 (m, 5H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 172.4, 139.4, 136.5, 133.9, 133.2, 131.7, 129.0, 128.5, 128.3, 118.0, 78.9, 73.8, 67.1, 54.0, 44.1

I.R. (neat, cm^{-1}) 3296, 3219, 3062, 2923, 1671, 1596, 1579, 1481, 1449, 1380, 1284, 1258, 1173, 1085, 1028, 908

HRMS *m/z* [$(M+H)^+$] calcd for $C_{18}H_{16}BrN_2O$ 355.0446 found 355.0442

7-Bromo-4-(but-3-yn-1-yl)-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (74)

According to procedure (c): From 7-bromo-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one **5** (100 mg, 0.316 mmol) and 1-bromo-4-butyne (89 µL, 0.948 mmol), 45 mg of the desired product was obtained (0.120 mmol, 38%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 10.09 (s, 1H), 7.47 (dd, *J* = 2.2 Hz, *J* = 8.4 Hz, 1H), 7.41-7.21 (m, 5H), 7.09 (d, *J* = 2.1 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 5.05 (s, 1H), 3.52-3.12 (m, 3H), 2.83-2.53 (m, 4H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.8, 140.0, 136.2, 133.9, 132.8, 131.7, 128.8, 128.7, 128.1, 122.4, 117.4, 82.1, 69.7, 68.4, 53.1, 52.2, 18.2

I.R. (neat, cm^{-1}) 3300, 3204, 3062, 2921, 2849, 1660, 1596, 1578, 1484, 1449, 1379, 1327, 1285, 1256, 1176, 1028, 975

HRMS *m/z* [$(M+H)^+$] calcd for $C_{19}H_{18}BrN_2O$ 369.0603 found 369.0613

4-Cyclopentyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (75)

Procedure (a): From 2-aminobenzophenone (2 g, 10.14 mmol) and bromoacetyl bromide (883 µL, 10.14 mmol), 1.9 g of the desired product was obtained (8.11 mmol, 80%).

Then procedure (c) at 60 °C: From 5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (100 mg, 0.423 mmol) and cyclopentanone (45 µL, 0.507 mmol), 72 mg of the desired product was obtained (0.232 mmol, 55%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.86 (s, 1H), 7.32-7.02 (m, 9H), 5.24 (s, 1H), 3.23 (s, 2H), 3.01-2.92 (m, 1H), 1.88-1.77 (m, 2H), 1.68-1.56 (m, 2H), 1.47-1.39 (m, 4H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.1, 142.9, 137.3, 131.8, 129.4, 128.3, 128.1, 127.6, 126.8, 123.3, 120.4, 67.1, 63.3, 53.1, 30.6, 30.3, 23.3, 22.9

I.R. (neat, cm^{-1}) 3197, 3064, 2992, 2956, 1649, 1585, 1489, 1432, 1392, 1360, 1060

HRMS *m/z* [$(M+H)^+$] calcd for $C_{20}H_{23}N_2O$ 307.1810 found 307.1797

4-Cyclopentyl-7-fluoro-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (76)

To a solution of 2-aminobenzophenone (250 mg, 2.53 mmol) in acetonitrile (13 mL) was added N-fluorosuccinimide (400 mg, 2.53 mmol) at room temperature. The mixture was stirred overnight. Flash chromatography (cyclohexane-ethyl acetate, 1-1) gave an inseparable mixture of the desired compound and the starting material (1.27 mmol).

Procedure (a): From (2-amino-5-fluorophenyl)(phenyl)methanone (1.27 mmol) and bromoacetyl bromide (132 µL, 1.52 mmol).

Then procedure (c) at 60 °C: From 7-fluoro-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (1.27 mmol) and cyclopentanone (337 µL, 3.81 mmol), 10 mg of the desired product was obtained (0.030 mmol, 3% over 3 steps) after purification by HPLC.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.84 (s, 1H), 7.32-7.10 (m, 8H), 5.25 (s, 1H), 3.42-3.19 (m, 2H), 2.98 (m, 1H), 1.98-1.72 (m, 2H), 1.69-1.55 (m, 2H), 1.52-1.33 (m, 4H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 171.6, 159.2, 156.8, 142.5, 133.9, 132.0, 131.9, 128.2, 127.5, 126.9, 122.2, 122.1, 117.8, 117.6, 115.2, 114.9, 66.5, 63.6, 53.1, 30.5, 30.2, 23.2, 22.9

I.R. (neat, cm^{-1}) 3309, 3197, 3087, 2996, 2957, 2908, 2859, 1650, 1620, 1597, 1514, 1501, 1495, 1450, 1425, 1409, 1389, 1369, 1231, 1129, 1152, 1090, 976

7-Chloro-4-cyclopentyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (77)

Procedure (a): From 2-amino-5-chlorobenzophenone (300 mg, 1.298 mmol) and bromoacetyl bromide (135 µL, 1.557 mmol).

Then procedure (c) at 60 °C: From 7-chloro-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (1.298 mmol) and cyclopentanone (138 µL, 1.557 mmol), 202 mg of the desired product was obtained (0.739 mmol, 57% over 2 steps).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.98 (s, 1H), 7.42-7.06 (m, 8H), 5.29 (s, 1H), 3.24 (s, 2H), 3.01-2.92 (m, 1H), 1.88-1.77 (m, 2H), 1.68-1.56 (m, 2H), 1.47-1.38 (m, 4H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.1, 142.2, 136.3, 131.6, 130.9, 128.2, 128.0, 127.6, 127.0, 126.9, 121.9, 66.4, 63.2, 52.9, 30.6, 30.2, 23.1, 22.8

I.R. (neat, cm^{-1}) 3309, 3195, 3078, 2993, 2962, 2873, 2857, 2830, 1650, 1599, 1583, 1492, 1450, 1423, 1405, 1379, 1367, 1358, 1255, 1226, 1162, 970, 873

HRMS *m/z* [(M+H)$^+$] calcd for $C_{20}H_{22}ClN_2O$ 341.1421 found 341.1416

7-Iodo-4-cyclopentyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one (78)

To a solution of 2-aminobenzophenone (500 mg, 2.53 mmol) in EtOH (25 mL) were added I_2 (966 mg, 7.59 mmol) and $AgSO_4$ (3.16 g, 9.36 mmol). The mixture was stirred overnight at room temperature. Flash chromatography (cyclohexane-ethyl acetate, 9-1 to 7-1) afforded 289 mg of the monoiodinated compound (0.885 mmol, 35%) along with some diiodinated compound (ortho and para positions).

Then procedure (a): From (2-amino-5-iodophenyl)(phenyl)methanone (289 mg, 0.894 mmol) and bromoacetyl bromide (93 µL, 1.072 mmol), 215 mg of the desired compound was obtained (0.596 mmol, 67%).

Then procedure (c) at 60 °C: From 7-iodo-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (0.298 mmol) and cyclopentanone (30 µL, 0.327 mmol), 23 mg of the desired product was obtained (0.053 mmol, 18%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ (ppm) = 9.94 (s, 1H), 7.61-7.51 (m, 2H), 7.28 (t, , *J* =, 1H), 7.21-7.20 (d, J = Hz, 1H), 7.06 (d, *J*=8.4 Hz, 2H), 6.91 (d, J = Hz, 1H), 5.25 (s, 1H), 3.21 (s, 2H), 2.93-2.82 (m, 1H), 1.85-1.72 (m, 2H), 1.69-1.55 (m, 2H), 1.49-1.32 (m, 4H)

^{13}C-NMR (100 MHz, $(CD_3)_2SO$) δ (ppm) = 172.4, 142.4, 139.6, 137.3, 132.1, 128.2, 127.7, 127.0, 122.5, 87.1, 66.4, 63.1, 52.9, 30.7, 30.4, 23.2, 22.8

I.R. (neat, cm^{-1}) 3192, 3067, 2992, 2959, 2856, 1652, 1577, 1488, 1398, 1449, 1420, 1398, 1373, 1362, 1226, 1132, 1047, 947

HRMS *m/z* $[(M+H)^+]$ calcd for $C_{20}H_{22}N_2OI$ 433.0777 found 433.0786

Chemicals for *in vitro* experiments

The following products were from the indicated commercial sources: [^{14}C]-leucine (PerkinElmer), Shiga-like toxin 2 (Stx, List Biological Laboratories, Inc.), DMSO (Sigma), fetal bovine serum (Sigma), glutamine, pyruvate, non-essential amino acids and antibiotic solutions (Gibco). Alexa-488-StxB was prepared as previously described.[23]

Intoxication assays

HeLa cells were maintained at 37 °C under 5% CO_2 in DMEM (Dulbecco's modified Eagle's medium, Invitrogen), supplemented with 10% fetal bovine serum, 4.5 g/L glucose, 100 U/mL penicillin, 100 µg/mL streptomycin, 4 mM glutamine, 5 mM pyruvate. The cells were plated at a density of 50,000 cells per well in 96-well Cytostar-T™ scintillating microplates (PerkinElmer) with scintillator incorporated into the polystyrene plastic. After incubation with either 30 µM or various concentrations of compounds (or 0.1% DMSO) for 4 hours at 37 °C, cells were challenged with increasing doses of Stx in the continued presence of the compounds. After incubation for 20 hours, the medium was removed and replaced with DMEM without leucine (Eurobio) containing 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1% penicillin/streptomycin supplemented by 0.5 µCi/mL [^{14}C]-leucine. The cells were grown for an additional 6 hours at 37 °C in an atmosphere of 5% CO_2 and 95% air. Protein biosynthesis was

then determined by measuring the incorporation of radiolabeled leucine into cells using a Wallac 1450 MicroBeta liquid scintillation counter (PerkinElmer).

The mean percentage of protein biosynthesis was determined and normalized from duplicate wells. Data were fitted with Prism v5 software (Graphpad Inc., San Diego, CA) to obtain the 50% inhibitory toxin concentration (IC_{50}), i.e. the concentration of toxin that is required to kill 50% of cells. IC_{50} values and protection factor R (R = IC_{50} drug/IC_{50} DMSO) were determined by the software's nonlinear regression "dose-response EC_{50} shift equation". The goodness of fit for Stx alone (carrier) or with drug was assessed by r^2 and confidence intervals. The percentage of cell protection was calculated for each compound after determination of the R value (Rdrug) and compared to the R value of Retro-1 (Rref):

$$\%\, protection = \frac{Rdrug - 1}{Rref - 1} \times 100$$

All compounds were tested at 30 µM and Retro-1 compound equals 100% protection at 30 µM.

Determination of EC_{50} values

For compounds that displayed a percentage of protection equal to or greater than 100%, EC_{50} represents the concentration of a compound that is required for 50% of its full inhibitory effect against Stx. EC_{50} was used to compare the efficacy of compounds because it is more precise than R values and the associated percentage protection. This is due to the fact that R values may fluctuate between cell experiments using different 96-well plates corresponding to compounds tested on different days. In contrast, the EC_{50} value for a single compound is calculated from experimental data obtained using a single 96-well plate. Cell assays were performed with various concentrations of the inhibitor. For each concentration, a percentage protection was determined

from R values calculated with Prism software with Rmax corresponding to the higher value of R of the series:

$$\%\, protection = \frac{R - 1}{Rmax - 1} \times 100$$

Drug concentration was plotted against the corresponding percentage protection of cells and the half-maximal effective concentration (EC_{50}) was calculated by non-linear regression using the Prism software package.

Fluorescent staining

For fluorescence experiments, compound-treated HeLa cells were pre-incubated for 1 hour in the continued presence of the compounds (1 μM). Compound-treated cells were then incubated with Alexa 488-StxB (0.1 μg/mL) for 30 min on ice, followed by 45 min at 37°C in the continued presence of the compounds (1 μM). After washing, cells were fixed with a solution of paraformaldehyde (4%, 5 min), labeled with phalloidin-Atto-550 (1/1000, Sigma) for actin staining or immunolabeled for giantin (1/1000, ab53542, Abcam) and with DAPI (1 μg/mL, Sigma) dissolved in the mounting medium for nuclei staining. Samples were imaged on an inverted SP8x confocal microscope (Leica) using a 63x oil immersion objective, NA 1.4. Maximum projections of optical Z slices are shown.

ASSOCIATED CONTENT

Supporting Information.

NMR of the compounds in experimental section, UPLC/UV analysis, HPLC separation of enantiomers, colocalization of Stx B and Golgi apparatus (±)-Retro-1.1 (PDF)

Molecular formula strings (CSV)

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ABBREVIATIONS

Stx, Shiga toxin; HUS, hemolytic uremic syndrome; Gb3, globotriaosylceramide; ER, endoplasmic reticulum; TGN, trans-Golgi network; NBS, N-bromosuccinimide; HRMS, high-resolution mass spectrometry.

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