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Development of CINPA1 analogs as novel and potent inverse agonists of constitutive androstane receptor

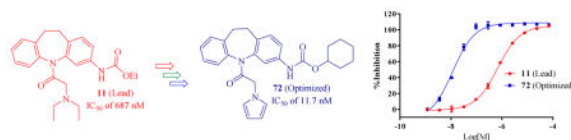
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Abstract

Constitutive androstane receptor (CAR, NR1I3) and pregnane X receptor (PXR, NR1I2) are master regulators of endobiotic and xenobiotic metabolism and disposition. Because CAR is constitutively active in certain cellular contexts, inhibiting CAR might reduce drug-induced hepatotoxicity and resensitize drug-resistant cancer cells to chemotherapeutic drugs. We recently reported a novel CAR inhibitor/inverse agonist CINPA1 (**11**). Here, we have obtained or designed 54 analogs of CINPA1 and used a time-resolved fluorescence resonance energy transfer (TR-FRET) assay to evaluate their CAR inhibition potency. Many of the 54 analogs showed CAR inverse agonistic activities higher than those of CINPA1, which has an IC_{50} value of 687 nM. Among them, **72** has an IC_{50} value of 11.7 nM, which is about 59-fold more potent than CINPA1 and over 10-fold more potent than clotrimazole (an IC_{50} value of 126.9 nM), the most potent CAR inverse agonist in a biochemical assay previously reported by others. Docking studies provide a molecular explanation of the structure-activity relationship (SAR) observed experimentally. To our knowledge, this effort is the first chemistry endeavor in designing and identifying potent CAR inverse agonists based on a novel chemical scaffold, leading to **72** as the most potent CAR inverse agonist so far. The 54 chemicals presented are novel and unique tools for characterizing CAR's function, and the SAR information gained from these 54 analogs could guide future efforts to develop improved CAR inverse agonists.

Graphical abstract



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Keywords

constitutive androstane receptor; CINPA1; inverse agonist; TR-FRET

1. Introduction

Constitutive androstane receptor (CAR, NR1I3) and pregnane X receptor (PXR, NR1I2) are members of the nuclear receptor (NR) superfamily and are master regulators of endobiotic and xenobiotic metabolism and disposition [1–3]. CAR and PXR are highly expressed in the liver, where most drug metabolism and clearance occurs [4]. CAR regulates the transactivation of many P450 enzymes and transporters, including CYP2Bs, CYP2Cs, CYP3As, multi-drug resistance protein 1 (MDR1), and multidrug resistance-associated proteins (MRPs) [5].

Depending on the cellular context, CAR may be constitutively active or activated by agonists [6]. CAR activation contributes to drug-induced hepatotoxicity [7], drug resistance [8], liver hypertrophy and liver tumor development in animals [9–11]. Therefore, small molecule inhibitors of CAR, either function as inverse agonists that decrease the constitutive activity, or as antagonists in the presence of agonists, or as deactivators that decrease the levels of CAR, will provide beneficial pharmacological effects by reducing the activity of CAR.

CAR has two main structural domains, the DNA-binding domain (DBD) and the ligand-binding domain (LBD) [12], which are common structural features of the NR family [13]. CAR recognizes a wide range of structurally diverse ligands because of its relatively large ligand binding pocket [14]. There are multiple splice variants of human CAR, with human CAR1 (wild-type CAR) being the most abundant and constitutively active isoform [15], and the target of our studies discussed here.

A panel of structurally diverse small molecules (Figure 1) has been reported as being CAR inverse agonists, inhibitors, deactivators, or antagonists. Androstanol (**1**), clotrimazole (**2**), and PK11195 (**3**) have been demonstrated to be CAR inverse agonists in a biochemical CAR-mediated PGC1 α coactivator recruitment/repression assay, with IC₅₀s of 1.2 μ M, 80 nM, and 390 nM, respectively [16]. In another report, clotrimazole displayed similar CAR inverse agonistic activity, with an IC₅₀ of 100 nM in a similar biochemical coactivator recruitment/repression assay (using SRC-1 instead of PGC1 α), and a K_i of ca. 100 nM in a Scintillation Proximity Binding Assay [17], however in a cell-based CAR inverse agonistic assay it exhibited a higher IC₅₀ value of 0.69 μ M. It was believed that cell permeability or metabolism of clotrimazole contributed to the activity discrepancy between the biochemical and cell-based assays. Among all reported CAR inverse agonists tested using various assay systems [18], clotrimazole was consistently the most potent CAR inverse agonist in the biochemical CAR-mediated coregulator recruitment/repression assays. However, the activity of clotrimazole against CAR in cell-based assays has varied from being inverse agonist [16, 17, 19] to agonist [16, 20, 21], most likely because of different cellular contexts, such as the levels of co-regulators in the different systems used [22]. Because of the complexity of cell-based assays, biochemical assays are more appropriate for structure-activity relationship

(SAR) analysis. Meclizine (**4**) was reported to be another CAR inverse agonist [23], but it failed to act as an effective hCAR inverse agonist or antagonist in cultured human hepatocytes [24]. As an estrogen receptor agonist, 17 α -ethynyl-3,17 β -estradiol (EE2, **5**) was shown to possess modest CAR inverse agonistic activity with an IC₅₀ of ca. 3 μ M in a cell-based assay [20]. S07662 (**6**) [25], TO901317 (TO901317, **7**) [26], Nigramide J (**8**) [27] and LY2090314 (**9**) [28] were also shown to be CAR inverse agonists with PXR agonistic activity. Although most CAR inverse agonists also act as PXR agonists, allyl isothiocyanate (AITC, **10**) was found to have inhibitory effects against both CAR and PXR [29], but only when very high concentrations (i.e., at 20 or 40 μ M) were used. Because of the relatively weak CAR inverse agonistic activities, no IC₅₀ value is available for other reported CAR inverse agonists. Recently, we discovered CINPA1 (**11**) [6] to be a potent CAR inverse agonist having a novel chemical scaffold. Uniquely, it does not activate PXR. Here, we present our efforts to develop CINPA1 analogs and evaluate their activities as inverse agonists of CAR.

Our efforts to identify more potent CAR inverse agonists are based on CINPA1 and were implemented in three stages. First, we obtained 10 close analogs of CINPA1 (**11**) that are commercially available and evaluated their CAR inverse agonistic activities and those of the lead compound **11** (CINPA1) in a biochemical CAR-mediated fluorescein-PGC1 α recruitment/repression assay [6, 16]. We chose to use the biochemical CAR-mediated fluorescent coactivator recruitment/repression assay because it provides consistent potency, regardless of whether SRC1 or PGC1 α peptide is used [16, 17]. Second, on the basis of the preliminary SAR information gained from these 11 compounds (i.e., CINPA1 and its 10 close analogs), we designed 23 novel analogs during our first-round chemistry effort to improve CAR inverse agonistic activities, which were evaluated by using the same biochemical CAR-mediated fluorescein-PGC1 α recruitment/repression assay. Third, the SAR information of the 23 novel analogs directed us to design 20 additional novel analogs and evaluate their activities. In summary, we have evaluated 54 chemicals (CINPA1, 10 commercially available analogs, and 43 novel analogs) and found that many of them have more potent CAR inverse agonistic activities than the lead chemical CINPA1 (**11**) does. Among them, the most potent analog **72** has an IC₅₀ value of 11.7 nM which is about 59-fold as potent as the lead compound CINPA1 (an IC₅₀ value of 687 nM) and it is over 10-fold as potent as the most potent and previously reported inverse agonist clotrimazole (with an IC₅₀ value of 126.9 nM from the same biochemical assay). To our knowledge, our effort is the first chemistry endeavor to develop and identify potent CAR inverse agonists based on a novel chemical scaffold identified from a compound screening campaign. The set of 54 compounds is a novel and useful tool for the study of CAR function.

2. Results and Discussion

2.1. CAR inverse agonistic activities and preliminary structure-activity relationship of CINPA1 and its 10 commercially available analogs

2.1.1. CAR inverse agonistic activities of CINPA1 and its 10 commercially available analogs—On the basis of the chemical scaffold of CINPA1, 3-amino-10,11-dihydro-5H-dibenz[*b,f*]azepine or saturated 3-amino-dibezapine (Figure 2), we searched for

commercially available close analogs of CINPA1 by using SciFinder[®] (provided by Chemical Abstracts Service) and identified and obtained 10 CINPA1 close analogs. We then evaluated their CAR inverse agonistic activities by using a biochemical CAR-mediated fluorescent PGC1 α coactivator recruitment/repression assay as previously reported [6, 16], with clotrimazole (positive control) and DMSO (negative control) used as controls. The chemical structures of CINPA1 and the 10 analogs, together with their CAR inverse agonistic activities, are summarized in Table 1. In our assay, clotrimazole had an IC₅₀ of 126.9 \pm 8.0 nM (Table 1), which is consistent with published results [16, 17]. CINPA1 (**11**) had an IC₅₀ of 687 \pm 71 nM.

CINPA1 has a chemical scaffold of 3-amino-10,11-dihydro-5*H*-dibenz[*b,f*]azepine or saturated 3-amino-dibezapine (Figure 2, with scaffold highlighted in bold and black) with a diethylamino substitute to the 5-nitrogen on the saturated dibezapine ring through a methylene carbonyl linker and an ethyl carbamate modification at the 3-amino group of the scaffold. These structure features provide CINPA1 with 3 major sites for chemical modifications (if the core scaffold remains unchanged): 1) substitutes (specified as site 1) attached to the 5-nitrogen on the saturated dibezapine ring by the methylene carbonyl linker; 2) the methylene carbonyl linker (specified as site 2); and 3) modifications on the 3-amino group site (specified as site 3) at the scaffold.

CINPA1 and the 10 commercially available analogs (Table 1) have varied modifications at all 3 major modifiable sites. CINPA1 (**11**) and chemicals **12** to **18** all have an ethyl carbamate group at the 3-amino position (site 3), but have different modifications at site 1 and site 2. However, chemical **19** has a methyl carbamate group at site 3, and chemicals **20** and **21** both have an isopropyl carbamate group at site 3. Although both CINPA1 (**11**) and chemical **12** have an ethyl carbamate modification at the 3-amino position, chemical **12** has a morpholino structure feature at site 1 instead of the diethylamino group presented in chemical **11** (CINPA1). This modification enhances the CAR inverse agonistic activity of chemical **12** (IC₅₀ of 235 nM) by about 3-fold over that of lead chemical **11** (IC₅₀ of 687 nM). The Morpholino group is similar in size to the diethylamino group but has reduced rotation flexibility because the diethylamino group in chemical **11** is locked in the form of a 6-member morpholino ring in chemical **12**. However, locking the diethylamino group conformation of chemical **11** in the form of an N-methyl-piperazino group resulted in a significantly weaker chemical **13** with a 56-fold higher IC₅₀ value (IC₅₀ of 38,755 nM) than that of chemical **11**. Compared to the Morpholino group, the N-methyl-piperazino group is bigger; an additional aliphatic tertiary amino group may allow chemical **13** to be protonated at a greater degree under assay conditions (pH 7.5), which may adversely affect ligand receptor interaction.

Chemicals **14** (IC₅₀ of 4,078 nM) and **15** (IC₅₀ > 70,000 nM) are much weaker CAR inverse agonists. Both have smaller substitutes at the position of the diethylamino group in chemical **11** (IC₅₀ of 687 nM), with a dimethyl amino group in chemical **14** and an amino group in chemical **15**. Therefore, substitutions with a smaller group at the position of the diethylamino group in chemical **12** contributed negatively to the CAR inverse agonistic activities.

Chemical **16** (IC₅₀ of 3,631 nM) has a secondary amino group (*tert*-butylamino) at the position of diethylamino group in chemical **11** (IC₅₀ of 687 nM), which reduced its CAR inverse agonistic activity (IC₅₀ increases by 5-fold). A secondary amino group such as *tert*-butylamino in chemical **16** at the position of diethylamino group in chemical **11** has a free proton on the nitrogen, which may contribute as a hydrogen bond donor. A hydrogen bond donor at this position may affect the CAR inverse agonistic activity negatively.

In comparison to chemical **12** (IC₅₀ of 235 nM) and chemical **14** (IC₅₀ of 4,078 nM), chemical **17** (IC₅₀ of 320 nM) and chemical **18** (IC₅₀ of 5,010 nM), which have an ethylene carbonyl linker, had slightly decreased CAR inverse agonistic activities, suggesting that a longer linker at site 2 may negatively affect the CAR inverse agonistic activity of the analogs. The only difference between chemical **17** (IC₅₀ of 320 nM) and chemical **19** (IC₅₀ of 288 nM) is at site 3, with an ethyl carbamate at 3-amino group in chemical **17** and a methyl carbamate in chemical **19**. Here a slightly increased CAR inverse agonistic activity was observed with a methyl carbamate rather than an ethyl carbamate at the 3-amino group. However, a methyl carbamate at the 3-amino group position was not taken into consideration in further development because the activity increase was marginal, and the configuration is less enzymatically and metabolically stable than an ethyl carbamate. More importantly, site 3 with a larger group such as an isopropyl carbamate in chemical **20**, endowed the analog with increased CAR inverse agonistic activity.

Chemical **18** (IC₅₀ of 5,010 nM) and chemical **20** (IC₅₀ of 1,792nM) have a difference only at site 3, with an ethyl carbamate in chemical **18** and an isopropyl carbamate in chemical **20**. The isopropyl carbamate at site 3 increased the potency of chemical **20** by about 3-fold compared to chemical **18**. Chemical **21** (IC₅₀ > 70,000 nM) has an even larger, protonable, and maybe more hydrophilic substitute (2-hydroxyethylpiperazino group) at site 1 and an ethylene carbonyl linker at site 2 which rendered it inactive as a CAR inverse agonist even though its isopropyl carbamate feature at site 3 could increase its CAR inverse agonistic activity.

2.1.2. Preliminary structure-activity relationship summary based on the CINPA1 and 10 commercially available analogs—Based on the CAR inverse agonistic activities gained from CINPA1 and 10 commercially available analogs, a preliminary SAR for the CINPA1 analogs was summarized (schematic CINPA1 structural features in Figure 2):

Scaffold: 3-amino-10,11-dihydro-5*H*-dibenz[*b,f*]azepine (saturated 3-amino-dibezapine) (bold and black in Figure 2);

Site 1: Medium constrained substitutes to the 5-nitrogen position through the methylene carbonyl linker are favorable; less charged or less protonated favorable; H-bond donor unfavorable;

Site 2: A methylene carbonyl linker between 5-nitrgen position of the saturated 3-amino-dibezapine and additional substitutes is preferred over an ethylene carbonyl linker (bold and red in Figure 2);

Site 3: Proper modification at the 3-amino group on the saturated dibezapine ring:
isopropyl carbamate > ethyl carbamate methyl carbamate.

2.2. Design, syntheses and activity evaluation of 23 CINPA1 analogs in the round 1 chemistry effort

2.2.1. Design of the 23 CINPA1 analogs in the round 1 chemistry effort—By using preliminary SAR gained from CINPA1 and 10 commercially available analogs as CAR inverse agonists, novel analogs were designed in a stepwise approach. Modifications were focused on site 1 (the substitutes to 5-nitrogen position through the methylene carbonyl linker), site 2 (the methylene carbonyl linker), and site 3 (the modifications at the 3-amino group at the saturated dibezapine ring), with the saturated 3-amino-dibezapine scaffold unchanged.

In the first-round chemistry effort, 23 analogs were designed, with CINPA1 as the lead compound; their structures are summarized in Table 2. Chemicals **22** to **30** have a variety of di-substituted amino group modifications at site 1: open rings, closed rings, aliphatic rings, or aromatic rings. Chemicals **31** and **32** have an ethylene carbonyl linker at site 2 to further explore and confirm the effect of the additional methylene insert within site 2. Chemicals **33** and **34** have a secondary amino group feature that was designed to further explore and confirm the hydrogen donor property at the site 1 position. Chemical **35** has a *tert*-butoxy group at the site 1 position instead of a regular tertiary amino group. A *tert*-butoxy group could eliminate the protonation possibility at site 1 under physiological conditions. Chemicals **36** to **44** have modifications on site 3 with various structure features of amide, carbamates, ureas, sulfamide, and carbamate heterocyclic isostere.

2.2.2. Syntheses of the 23 novel CINPA1 analogs in the first-round chemistry effort

2.2.2.1 Syntheses of starting materials of **11**, **46**, **47**, and **48** for the novel CINPA1

analogs: In preparing novel CINPA1 analogs for the 2 runs of chemistry efforts, compounds **46**, **47**, **48**, and **11** (CINPA1)—starting materials for various CINPA1 (**11**) analogs—were first synthesized according to published methods [30–32]. The procedures in preparing these 4 chemicals are summarized in Scheme 1. Briefly, compound **46** [30, 31] was subjected to subsequent reactions of ethoxycarbonylation to compound **47**, chloroacetylation to compound **48**, and amination to compound **11** [32].

In the first-run chemistry effort to prepare chemicals **22** to **44**, chemical **48** was used as the common starting material to prepare chemicals **22** to **32** (Scheme 2); chemical **47** was used as the common starting material to prepare chemicals **33** to **35** (Scheme 3); and chemical **11** was used as the common starting material to prepare chemicals **36** to **44** (Scheme 4). In the second-run chemistry effort to prepare chemicals **53** to **72**, chemical **46** was used as the common starting material to prepare chemicals **53** to **64** (Scheme 5) and **65** to **72** (Scheme 6).

2.2.2.2 Syntheses of the 23 CINPA1 analogs in the round 1 chemistry effort from

chemicals 11, 47, and 48: Chemicals **22** to **32** (Scheme 2) were prepared by using chemical

48 as the common starting material. In the presence of sodium hydride, alkylations of corresponding amines with the chloride chemical **48** at microwave-heated 80°C [33] or 0°C [34] yielded chemicals **22**, **23**, **27**, **31**, and **32** or **24**, **25**, **29**, and **30**, respectively. To prepare chemical **26**, the chloride chemical **48** was first converted to the more reactive species of iodide chemical **49** with sodium iodide [35] and then 2,2,6,6-tetramethylpiperidine was alkylated with the iodide chemical **49** to chemical **26** at 120°C in toluene [36]. To prepare chemical **28**, the chloride chemical **48** was first converted to the amino chemical **50** with ammonium hydroxide solution in methanol at 120°C under sealed conditions [37]; the amino chemical **50** was subsequently converted to chemical **28** by reacting with furan-2,5-dione in acetic acid at 100°C [38].

Chemicals **33** to **34** were prepared from chemical **47** (Scheme 3) with corresponding carboxylic acids in the presence of propylphosphonic anhydride at reflux or 55°C reaction conditions [39].

In the syntheses of chemicals **36** to **44** with site 3 modifications (Scheme 4), the chemical **11** was first converted to chemical **51** by acidic hydrolysis to remove the ethoxy carbonyl group [40]. Chemical **51** was further converted to chemicals **36** and **37** by reacting with acyl chlorides of butyryl chloride and isopropyl chloroformate, respectively, in methylene chloride in the presence of triethylamine at 10°C to 35°C [41] and then converted to chemical **38** by reacting with di-*tert*-butyl dicarbonate under similar conditions [42]. To prepare chemicals **39** to **42**, chemical **51** was first activated into the form of imidazole carboxamide **52** with carbonyldiimidazole [43] in methylene chloride in the presence of triethylamine, and the chemical **52** was then converted to chemicals **39** to **42** by reacting with corresponding amines in methylene chloride in the presence of triethylamine and 4-dimethylaminopyridine [44]. To prepare chemical **43**, the chemical **51** was reacted with freshly prepared *N*-ethyl-sulfamoyl chloride in methylene chloride in the presence of triethylamine at 15°C to 25°C [45]. To prepare chemical **44**, the chemical **51** was subsequently reacted with reagents of 1-(2-oxopyridine-1-carbothiopyridin-2-one, acetohydrazide, and EDC [46].

2.2.3. CAR inverse agonistic activities of the 23 CINPA1 analogs in the first-round chemistry effort—After we synthesized the 23 CINPA1 analogs, we determined their CAR inverse agonistic activities by using the biochemical CAR-mediated fluorescent PGC1 α coactivator recruitment/repression assay. The chemical structures and CAR inverse agonistic activities of the 23 analogs are summarized in Table 2 along with those of the lead chemical **11** (CINPA1).

Among the 23 analogs prepared in the first-round chemistry effort, chemicals **22** to **32** have modifications only at the site 1 position of the lead chemical CINPA1 (**11**)'s structure. Chemical **22** (IC₅₀ of 210 nM) has a dipropylamino group at the site 1 position of CINPA1. The introduction of this dipropylamino group increased the CAR inverse agonistic activity of chemical **22** to 3-fold more than that of lead chemical **11**, which has a diethylamino group at this position. However, a branching, disubstituted amino group is not favorable for CAR inverse agonistic activity, as chemical **23** (IC₅₀ of 3,050 nM), which has a diisopropyl amino

group at site 1, is much weaker, with an IC_{50} value more than 4-fold higher than that of lead chemical **11**.

The preliminary SAR data from the 10 commercially available analogs suggested that reduced side chain flexibility at site 1 might improve the CAR inverse agonistic activity, as seen in chemical **12**. To further explore the effect of reducing side chain flexibility, chemicals **24** to **30** were designed to have ring structure features, which could reduce side chain rotation flexibility at site 1. Chemical **24** (IC_{50} of 1,580 nM) and chemical **25** (IC_{50} of 620 nM) were designed and prepared by directly constraining the diethylamino group in chemical **11** (IC_{50} of 687 nM) in the form of a pyrrolidinyl group or piperidinyl group, respectively. Compared to the CAR inverse agonistic potency of lead chemical **11**, that of chemical **24** decreased (IC_{50} increased by more than 2-fold) whereas that of chemical **25** was marginally increased. Therefore, constraint at site 1 may not always increase CAR inverse agonistic activities of the analogs. However, reasonable size at site 1 might be important as the piperidinyl group in chemical **25** is larger than the pyrrolidinyl group in chemical **24**, and chemical **25** exhibited higher CAR inverse agonistic potency than did chemical **24**.

An additional hydrogen-bond acceptor at the farthest end of the site 1 position may also give analogs improved CAR inverse agonistic activities. For example, chemical **12** (IC_{50} of 235 nM) has an additional oxygen atom as a hydrogen-bond acceptor at the very end of site 1 within its morpholino group, whereas chemical **25** (IC_{50} of 620 nM) has a similar 6-member ring structure to chemical **12** (IC_{50} of 235 nM) at site 1 but without a hydrogen-bond acceptor built in, and it is less active as a CAR inverse agonist. Chemical **26** (IC_{50} of 4,080 nM) has a 2,2,6,6-tetramethylpiperidinyl group at site 1: this group is branched at positions next to the nitrogen and is bulkier than the similar 6-member ring feature at this site in chemical **25** (IC_{50} of 620 nM). This difference in site 1 rendered chemical **26** significantly less active than chemical **25** (The IC_{50} of chemical **26** is over 6-fold more than that of chemical **25**).

Chemical **27** (IC_{50} of 1,410 nM) has a (1s,4s)-7-azabicyclo[2.2.1]heptanyl group at the site 1 position: this group branches at positions next to the nitrogen, with constraint in the form of an additional 5-member ring, and is slightly bulkier than the single 5-member ring in chemical **24** (IC_{50} of 1,580 nM). Chemical **27** is slightly more active than chemical **24**, suggesting that chemical groups at site 1 with appropriate bulkiness are desirable for improving the CAR inverse agonistic activities of analogs. Chemical **28** (IC_{50} of 39,810 nM) has a 2,5-dioxo-2,5-dihydro-1*H*-pyrrolyl substitute at site 1, making it branched at α -positions of the nitrogen atom but with a hydrogen-bond acceptor property. This structural feature rendered chemical **28** less active than chemical **24** as a CAR inverse agonist (the IC_{50} of chemical **28** is 25-fold lower than that of chemical **24**) even though a similar 5-member ring structure was maintained at site 1 in both chemical **24** and **28**, which may indicate hydrogen-bond acceptor property at α -positions of the nitrogen atom is not desirable for CAR inverse agonistic activities. Chemicals **29** (IC_{50} of 37 nM) and **30** (IC_{50} of 6,890 nM) have a similar 5-member aromatic ring structure feature at site 1 of the lead compound CINPA1, with a pyrrolyl group in chemical **29** and an imidazolyl group in

chemical **30**. Chemical **29** is the aromatic version of chemical **24** at the site 1 position but is 43- and 19-fold more potent than chemicals **24** and **11** (CINPA1), respectively. The aromatic feature at site 1 has the most significant positive impact for analogs as a CAR inverse agonist. However, when an additional heteroatom in the form of nitrogen was added to the 5-member aromatic ring in chemical **29** to make chemical **30** (which now has an imidazolyl group), a dramatic loss of CAR inverse agonistic activity was observed (the IC_{50} of chemical **30** is 186-fold less than that of chemical **29**). This observation further confirms that protonable groups at the site 1 position are indeed undesirable for CAR inverse agonistic activity because an imidazolyl group can be easily protonated under assay conditions (pH 7.5).

Chemical **31** (IC_{50} of 24,490 nM) has a mono-substituted amino group (ethylamino group) at site 1, with a proton attached to the nitrogen that can function as a hydrogen-bond donor. The extremely weak activity of chemical **31** further confirms that a hydrogen-bond donor feature at site 1 is undesirable (chemical **31** has an IC_{50} that is over 35-fold less than that of the lead chemical **11**) even though a smaller size at site 1 in chemical **31** may also partially contribute to the activity loss. However, chemical **32** (IC_{50} of 2,060 nM), which has a similar mono-substituted amino group (*tert*-pentylamino group) and reasonable bulkiness, only slightly lost its CAR inverse agonistic activity (IC_{50} is 3-fold less than that of the lead chemical **11**). In this example, having a hydrogen-bond donor feature at site 1 is still undesirable, but the suitable group size maintained at site 1 might have compensated for the negative effect of the hydrogen-bond donor and helped retain reasonable CAR inverse agonistic activity for chemical **32**.

Chemical **33** (IC_{50} of 2,430 nM) has an ethylene carbonyl linker at site 2 to replace the methylene carbonyl linker at the same site in lead chemical **11** (IC_{50} of 687 nM). This modification decreased the activity of chemical **33** (a 3.5-fold increase in the IC_{50} value over that of lead chemical **11**). Chemical **34** (IC_{50} of 3,180 nM) has a further modification at site 1, with a constrained pyrrolidinyl group to replace the diethylamino group in lead chemical **11** in addition to the ethylene carbonyl linker modification at site 2 as in chemical **33**. This constrained modification without enough bulkiness at site 1 caused a slight decrease in CAR inverse agonistic activity (IC_{50} of 2,430 nM for chemical **33** vs. IC_{50} of 3,180 nM for chemical **34**), which is consistent with the observed difference between chemical **11** (IC_{50} of 687 nM) and chemical **24** (IC_{50} of 1,580 nM). Chemical **35** (IC_{50} of 70.8 nM) has a *tert*-butoxy group at the site 1 position of lead chemical **11** (IC_{50} of 687 nM). This modification in chemical **35** increased its CAR inverse agonistic activity by 9.7-fold over that of lead chemical **11**, demonstrating that a non-protonable group with reasonable size at site 1 could improve CAR inverse agonistic activity. However the *tert*-butoxy group is not protonable under assay conditions (pH 7.5) but has reasonable size, and the diethylamino group in lead chemical **11** is protonable under similar conditions. The success of chemical **35** as a substantially more potent CAR inverse agonist than lead chemical **11** led us to focus on site 1 modifications with the *tert*-butoxy and diethylamino functional groups, as reflected in 9 of 20 novel chemicals in the second-round chemistry effort (discussed in section 2.3).

Chemicals **36** to **44** have modifications on site 3 of lead chemical **11**, with structural features of amide in chemical **36**, carbamates in chemicals **37** and **38**, ureas in chemicals **39** to **42**, sulfamide in chemical **43**, and carbamate heterocyclic isostere in chemical **44**. Chemical **36** (IC₅₀ of 2,320 nM) has a butyramide at site 3 instead of the ethyl carbamate of lead chemical **11** (IC₅₀ of 687 nM) at the same position. Butyramide and ethyl carbamate are similar in size and shape; the only difference between them is that the ethoxy oxygen (non-carbonyl oxygen) in lead chemical **11** was replaced by a methylene group in chemical **44**. The change of the ethoxy oxygen in chemical **11** to a methylene group in chemical **44** decreased the CAR inverse agonistic activity (IC₅₀ increased by about 3.4-fold). Therefore, it appears that the ethoxy oxygen in chemical **11** is beneficial for its CAR inverse agonistic activity, possibly because oxygen may serve as a hydrogen bond acceptor. Chemicals **37** and **38** have an isopropyl carbamate and a *tert*-butyl carbamate, respectively, at site 3 of lead chemical **11** (IC₅₀ of 687 nM), which has an ethyl carbamate at this site. The isopropyl carbamate modification maintains the CAR inverse agonistic activity for chemical **37** (IC₅₀ of 680 nM), which is consistent with the observed behavior of chemical **20**.

Therefore, an isopropyl carbamate at site 3 was further confirmed as being a more favorable structural feature than ethyl carbamate at the same site. An isopropyl group is slightly larger than an ethyl group, suggesting that a slightly larger chemical group at site 3 may be favored for CAR inverse agonistic activity. In addition, isopropyl carbamate should provide analogs with higher enzymatic and metabolic stability than ethyl carbamate does. Further increase in size at site 3 to a *tert*-butyl carbamate group in chemical **38** actually slightly decreased its activity (IC₅₀ of 1,030 nM) from that of lead chemical **11** (IC₅₀ of 687 nM). Compared to lead chemical **11** (IC₅₀ of 687 nM), urea, sulfamide, and carbamate heterocyclic isostere modifications at site 3 yielded chemicals **39** (IC₅₀ of 14,420 nM), **40** (IC₅₀ of 4,930 nM), **41** (IC₅₀ of 12,970 nM), **42** (IC₅₀ of 13,990 nM), **43** (IC₅₀ of 19,090 nM), and **44** (IC₅₀ of 18,790 nM), all with dramatically reduced CAR inverse agonistic activity. Therefore, these modifications at site 3 are unfavorable for CAR inverse agonistic activity, confirming that an alkoxy oxygen is optimal at site 3 to maintain the CAR inverse agonistic activity.

In addition, ureas in chemicals **39**, **40**, and **41** and sulfamide in chemical **43** both have a proton attached to the nitrogen atom, which could serve as a hydrogen-bond donor. The observation that a hydrogen-bond donor at site 3 may be unfavorable for CAR inverse agonistic activity is again seen in the chemical pair of **39** (IC₅₀ of 14,420 nM, with proton on nitrogen) and **42** (IC₅₀ of 13,990 nM, without a proton on nitrogen) in which chemical **42** is marginally more active than chemical **39**. However, a diethyl substitution in chemical **42** instead of a mono-ethyl substitution in chemical **39** might also contribute to the marginal difference. Chemical **44**, which has the non-carbonyl oxygen but the carbonyl oxygen is replaced with nitrogen, had dramatically lower activity (IC₅₀ of 18,790 nM) than did lead chemical **11** (IC₅₀ of 687 nM), indicating that a carbamate structure with both carbonyl oxygen and non-carbonyl oxygen (alkoxy oxygen) is required for optimal CAR inverse agonistic activity.

2.2.4. Structure-activity relationship summary from CINPA1, 10 commercial analogs and the 23 novel CINPA1 analogs prepared from the first-round

chemistry effort—Based on the overall CAR inverse agonistic activities from the 33 CINPA1 analogs, a brief SAR report of analogs with CAR inverse agonistic activities at least comparable to CINPA1 was summarized (structural features shown in Figures 2 and 3)

Scaffold of 3-amino-10,11-dihydro-5*H*-dibenz[*b,f*]azepine (saturated 3-amino-dibezapine).

Site 1: An unbranched disubstituted amino group is favorable; protonability is very undesirable; a suitably bulky group is desirable; an oxygen-contained group in the form of an alkoxy group may be better than a nitrogen-containing group in the form of an aliphatic tertiary amino group because an aliphatic amino group is susceptible to protonation under assay conditions (pH 7.5), which may be unfavorable for CAR inverse agonistic activity; a mono-nitrogen-containing aromatic ring is favorable because it is not as susceptible to protonation under assay conditions (pH 7.5) as an aliphatic tertiary amino group is.

Site 2: A methylene carbonyl linker is better than an ethylene carbonyl linker.

Site 3: A carbamate structure is favorable; a hydrogen-bond donor is unfavorable; medium-sized substitutes, such as isopropyl carbamate, are favorable.

2.3. Design, syntheses, and activity evaluation of 20 CINPA1 analogs in the second-round chemistry effort

2.3.1. Design of 20 novel CINPA1 analogs in the second-round chemistry effort—The SAR obtained from CINPA1, the 23 CINPA1 analogs of the first-round of chemistry, and the 10 analogs from commercial sources demonstrated that isopropyl carbamate at site 3 is desirable for CAR inverse agonistic activity. We, therefore, designed 12 novel analogs with isopropyl carbamate at site 3. In addition, 4 novel analogs with *tert*-butyl carbamate at site 3 were designed for comparison. Among these 16 novel analogs, 9 were given various alkoxy groups at site 1 because SAR from the 23 analogs in round 1 suggested that an alkoxy group at this site could improve CAR inverse agonistic activity. The other 7 analogs were given several site 1 and site 2 groups that are similar to those explored in the first-round chemistry effort in combination with either isopropyl carbamate or *tert*-butyl carbamate at site 3 to gain additional insight about the functional preference at site 3. To identify optimal groups at site 3, we designed 4 additional chemicals having novel carbamates at site 3 and the optimal pyrrolyl group at site 1. In total, 20 novel CINPA1 analogs were designed in the second-round chemistry effort.

2.3.2. Syntheses of the 20 novel CINPA1 analogs in the second-round chemistry effort—Chemical **46** was the common starting material to prepare all the analogs in the second-round chemistry effort. The syntheses of chemicals **53** to **64** are summarized in Scheme 5. To prepare chemical **53** [32], chemical **46** was first converted to chemical **73** by selectively acylating the primary 3-amino group at the saturated 3-amino-dibezapine ring in chemical **46** to form the isopropyl carbamate in chemical **73** with isopropyl chloroformate because this 3-primary amino group is more electronically and sterically reactive than is the secondary amino group (the 5-position nitrogen) on the saturated dibezapine ring. Chemical **73** was then subjected to further acylation with 3-

chloropropionyl chloride in toluene at 100°C to yield the chloride chemical **74**, which was then converted to chemical **53** by alkylating diethyl amine in toluene at 100°C. Chemical **73** was also converted to the chloride chemical **75** with 2-chloroacetyl chloride in toluene at 100°C [33]. Amines of dipropylamine, piperidine, pyrrole were alkylated with the chloride chemical **75** in dimethylformamide (DMF) in the presence of sodium hydride at 25°C to form the corresponding products of chemicals **54**, **55**, and **56** [34]. In addition, the chloride chemical **75** was converted to a more reactive alkylation chemical—the iodide chemical **76**—with sodium iodide in acetone at room temperature [35]. In the presence of sodium bis(trimethylsilyl)amide (NaHMDS) and under nitrogen atmosphere, respective alcohols were then alkylated with the iodide chemical **76** in tetrahydrofuran (THF) at 0°C to 25°C to yield corresponding chemicals **57** to **64** [47].

In the preparation of chemicals **65** to **72** (Scheme 6), chemical **46** was first converted to chemical **77** with benzyl chloroformate in ethanol at 0°C; chemical **77** was then further acylated into the chloride chemical **78** with 2-chloroacetyl chloride in toluene at 110°C. The chloride chemical **78** was further converted to chemicals **65** – **72** by using different synthetic approaches. To prepare chemicals **65** and **66**, corresponding dipropylamine and piperidine groups were first alkylated with the chloride chemical **78** to form the intermediate chemicals **79** and **80** [32], which were then subjected to de-carboxybenzyl (de-Cbz) [48] with 10% Pd-C catalyzed hydrogenolysis in methanol at room temperature and acylation with di-*tert*-butyl dicarbonate [42] in methanol at room temperature to give respective target chemicals. To prepare chemical **67**, 2-propanol was first deprotonated with sodium at temperatures from 90°C to 60°C to form sodium isopropoxide, which was alkylated with the chloride chemical **78** to form the intermediate chemical **81** [49]. The intermediate chemical **81** was then converted to chemical **67** [42] with di-*tert*-butyl dicarbonate in methanol at room temperature under 10% Pd-C-mediated catalytic hydrogenolysis conditions. During the preparation of chemicals **68** to **72**, pyrrole was de-protonated with sodium hydride in dimethylformamide at 0°C to 25°C under nitrogen atmosphere and then alkylated with the chloride chemical **78** to yield chemical **82** [50], which was then subjected to 10% Pd-C-mediated catalytic hydrogenolysis in ethyl acetate at room temperature to form chemical **83** [48], with the 3-amino group at the saturated dibenzapine being available for further carbamation. Chemicals **68** to **72** were then obtained by carbamation of the 3-amino group in chemical **83** with the corresponding alkyl chloroformates in tetrahydrofuran (THF) in the presence of triethylamine at temperatures of 0°C to 25°C [51].

2.3.3. CAR inverse agonistic activities of the 20 novel CINPA1 analogs in the second-round chemistry effort—We synthesized and evaluated the CAR inverse agonistic activities of these 20 novel CINPA1 analogs using the biochemical CAR-mediated fluorescent PGC1 α coactivator recruitment/repression assay. Their structures and CAR inverse agonistic activities are summarized in Table 3 along with those of lead chemical **11** (CINPA1).

Among the 20 novel CINPA1 analogs from the round 2 chemistry, chemical **53** is the only one with an ethylene carbonyl group modification and an isopropyl carbamate at site 3. Chemical **53** (IC₅₀ of 6,400 nM) is substantially less active than is lead chemical **11** (IC₅₀ of

687 nM) even though the isopropyl carbamate group at site 3 is expected to increase the CAR inverse agonistic activity as observed in other analogs with the isopropyl carbamate structural feature at site 3. The ethylene carbonyl linker at site 2 is again proven to be a less desirable group than the methylene carbonyl linker group.

Chemicals **22** (IC₅₀ of 210 nM), **54** (IC₅₀ of 116.4 nM), and **65** (IC₅₀ of 152.9 nM) are only different at site 3, with a corresponding ethyl, isopropyl, or *tert*-butyl carbamate group. Among this group of 3 chemicals, chemical **54**, which has an isopropyl carbamate at site 3, has the highest CAR inverse agonistic activity. This slight positive effect of a site 3 isopropyl carbamate on the CAR inverse agonistic activity was also observed among chemicals **29** (IC₅₀ of 37.0 nM), **56** (IC₅₀ of 20.9 nM), and **68** (IC₅₀ of 31.9 nM), with the only difference being their respective ethyl, isopropyl, or *tert*-butyl carbamate groups at site 3. In another group of chemicals —**25** (IC₅₀ of 620 nM), **55** (IC₅₀ of 249.0 nM), and **66** (IC₅₀ of 216.2 nM) — that had a corresponding ethyl, isopropyl, or *tert*-butyl carbamate group at site 3, **66** (with a *tert*-butyl carbamate) was only slightly more active than **55** (with an isopropyl carbamate), but both were substantially more active than **25**, which has an ethyl carbamate. Overall, an isopropyl carbamate is similar to a *tert*-butyl carbamate, but both are better than an ethyl carbamate at site 3 for the CAR inverse agonistic activity. Having an IC₅₀ of 20.9 nM, chemical **56** was the most active CAR inverse agonist and is 32.8-fold more potent than lead chemical **11** (IC₅₀ 687 nM).

Chemicals **57** to **64** and **67** have alkoxy groups at site 1 and an isopropyl or *tert*-butyl carbamate at site 3. These structural features improve the CAR inverse agonistic activities of the analogs compared to that of lead chemical **11**, with the exception of chemical **57** (IC₅₀ value 11,350 nM), which has an ethoxy group at site 1 that might be too small to maintain a high CAR inverse agonistic activity. Chemicals **58** (IC₅₀ 60.1 nM) and **67** (IC₅₀ 116.0 nM) both have an isopropoxy group at site 1 and an isopropyl or a *tert*-butyl carbamate, respectively, at site 3. Both **58** and **67** are substantially more active than lead chemical **11** (IC₅₀ of 687 nM). Here, the isopropyl carbamate at site 3 in chemical **58** again endowed the analog with higher CAR inverse agonistic activity than did the *tert*-butyl carbamate at the same site in chemical **67**. Increasing the size of the alkoxy group at site 1 from an ethoxy group in chemical **57** (IC₅₀ of 11,350 nM) and an isopropoxy group in chemical **58** (IC₅₀ of 60.1 nM) to a *sec*-butoxy group in chemical **59** (IC₅₀ of 35.3 nM) and a neopentyloxy group in chemical **60** (IC₅₀ of 21.2 nM) increases CAR inverse agonistic activity. Similar observations were made in the cyclic alkoxy group series at site 1: from chemical **61** (IC₅₀ of 81.5 nM) with a cyclopropoxy group to chemical **62** (IC₅₀ of 37.5 nM) with a cyclobutoxy group, and to chemical **63** (IC₅₀ of 19.5 nM) with a cyclopentyloxy group. However, a slight activity decrease was observed when the size of site 1 was further increased by incorporating a cyclohexyloxy group, as in chemical **64** (IC₅₀ of 24.1 nM). The highest CAR inverse agonistic activities were observed when a branched 5-carbon alkoxy group was at site 1, as in chemicals **60** and **63**.

Chemicals **69** to **72** were designed to further explore the effect of cyclic carbamates at site 3 on CAR inverse agonistic activities. Chemicals **69** (IC₅₀ of 22.1 nM), **70** (IC₅₀ of 20.1 nM), **71** (IC₅₀ of 13.9 nM), and **72** (IC₅₀ of 11.7 nM), which respectively have cyclopropyl,

cyclobutyl, cyclopentyl, and cyclohexyl carbamates at site 3 and the optimal pyrrolyl functional group at site 1, had substantially higher CAR inverse agonistic activities than did lead chemical **11** (IC₅₀ of 687 nM). These results demonstrate the significant contribution of cyclohexyl carbamates at site 3 to high CAR inverse agonistic activity. Chemical **72** is the most active CAR inverse agonist among chemicals **69** to **72**: having an IC₅₀ value of 11.7 nM, it is approximately 59-fold more potent than the lead compound **11** (CINPA1) and is the most active analog among all 54 analogs evaluated in this study.

2.3.4. Structure-activity relationship summary of all 54 CINPA1 analogs

evaluated as CAR inverse agonists—Based on the overall CAR inverse agonistic activities of the 54 CINPA1 analogs, this brief summary of the SAR of modifications that affect CAR inverse agonistic activities (structural features in Figure 3) is provided:

Scaffold: 3-amino-10,11-dihydro-5*H*-dibenz[*b,f*]azepine (saturated 3-amino-dibezapine).

Site 1: medium size alkoxy groups or tertiary amino groups in aromatic rings with no additional substitute at α -position to the nitrogen; hydrogen-bond donor is highly unfavorable; protonability is very undesirable at this site.

Site 2: a methylene carbonyl linker is better than an ethylene carbonyl linker.

Site 3: a carbamate is better than an amide, urea, sulfamide, or carbamate heterocyclic isostere structure; a medium-sized aliphatic cyclic carbamate structure is favorable; a hydrogen-bond donor is unfavorable.

2.3.5. Representative molecular modeling study of compound **11** (CINPA1) and compound **72** (the most potent analog) to hCAR-LBD

—In order to predict the binding mode of CINPA1 (**11**) and the most potent analog (**72**) to the hCAR-LBD, we conducted docking studies using AutoDock Vina, which showed the ligands positioned in the binding pocket (Figure 4). The top ligand-poses were selected based on the superposition of the scaffold for both ligands and comparisons with the SAR data. In this model, the ethyl carbamate (position 3) of compound **11** (Figure 4A) and the cyclohexyl carbamate of compound **72** (Figure 4B) have the potential to form hydrogen bonding with Asn-165 or His-203. Because hydrogen bonds impart strong ligand-protein interactions, it is plausible that the postulated hydrogen bonding accounts for the necessity of the ethoxy and carbonyl oxygens.

In relation to site 1, the models agree with the SAR data with regard to the need of hydrophobic groups without moieties containing hydrogen bond donors or are incapable of protonation, because site 1 occupies a highly hydrophobic pocket in the protein ligand cavity. Aromatic groups such as the 5-member ring in compound **72** would potentially benefit of potential aromatic stacking with residues such as Phe-217 (Figures 4A and 4B).

Site 3 also occupies a very hydrophobic pocket with enough space to accommodate the cyclohexyl ring of compound **72** (Figure 4D), where groups containing hydrogen bond donors would negatively affect binding as observed in the SAR studies. In the same way as with site 1, larger groups at site 3 would provide stronger hydrophobic contacts with the

protein, presenting an explanation for the stronger potency of compound **72** compared to compound **11** (Figures 4C).

From the molecular modeling study, several important features were identified in terms of compounds **11** (CINPA1) and **72** interacting with the hCAR-LBD. The ligands reside at the binding pocket of hCAR-LBD, where site 1 and site 3 of the ligands occupy a highly hydrophobic environment, with potential for hydrogen bonding with Asn-165 or His-203. The larger moieties at sites 1 and 3 of compound **72** would correlate with its higher potency in comparison to compound **11** because of the stronger hydrophobic interactions with the protein. The models provide a molecular explanation of the SAR observed experimentally.

3. Conclusions

In this study, the following 54 CINPA1 analogs were obtained and evaluated for their CAR inverse agonistic activities in a SAR-guided stepwise approach: the lead compound and 10 commercial analogs; 23 novel analogs in the first-round chemistry effort; and 20 novel analogs in the second-round chemistry effort. Many analogs having improved CAR inverse agonistic activities were discovered. Among them, chemical **72** was the most active analog, with an IC₅₀ of 11.7 nM, which is approximately 59-fold more potent than the lead compound CINPA1 (an IC₅₀ value of 687 nM) and over 10-fold more potent than the most potent inverse agonist previously reported by others, clotrimazole (an IC₅₀ value of 126.9 nM in our assay). This set of 54 chemicals is a novel tool for investigating the function of CAR. The SAR data gained from these 54 analogs provides a basis to guide future efforts to develop additional CAR inverse agonists to study the function of CAR. To our knowledge, our effort is the first chemistry endeavor in developing highly active CAR inverse agonists based on a novel chemical scaffold discovered in a compound-screening campaign.

4. Experimental section

4.1. Chemistry

General procedures: Organic reagents were purchased from commercial suppliers unless otherwise noted and were used without further purification. All solvents were analytical- or reagent-grade. All reactions were carried out in flame-dried glassware under argon or nitrogen. Flash column chromatography was performed by using Sigma-Aldrich silica gel 60 (200–400 mesh) and carried out under moderate pressure, with columns of an appropriate size packed and eluted with appropriate eluents. All reactions were monitored by performing thin-layer chromatography (TLC) on precoated plates (silica gel HLF). TLC spots were visualized either by exposure to iodine vapor or by irradiation with UV light. Organic solvents were removed under vacuum by a rotary evaporator. The reactions, purities, or identities of final compounds were monitored or determined by performing TLC or using a Waters Acquity UPLC MS system with a C18 column in a 2-min gradient (H₂O + 0.1% formic acid → Acetonitrile + 0.1% formic acid) and detectors of PDA (215 – 400 nm), ELSD, and Acquity SQD ESI Positive MS. Preparative TLC separation was performed by using self-casted preparative TLC plates with Sigma-Aldrich silica gel 60 (200–400 mesh) on 20-cm × 20-cm glass plates. The purifications of reaction products were performed by using a Dionex APS 3000 dual purification/analytical LC/PDA/MS system with a C18

column in a 15-min gradient (H₂O with 0.05% NH₃•H₂O → Acetonitrile) and ESI Positive MS. High-resolution mass spectra were determined by using a Waters Acquity UPLC system with a C18 column (H₂O + 0.1% formic acid → acetonitrile + 0.1% formic acid gradient over 2.5 min) under Xevo G2Q-TOF ESI in positive, resolution mode. Compounds were internally normalized to leucine-enkephalin lock solution, with a calculated error of < 3 ppm. All ¹H NMR spectra were recorded on a Bruker ULTRASHIELD 400 plus NMR spectrometer and all ¹³C NMR spectra were recorded on a Bruker Ascend 126 MHz Fourier transform (FT) NMR spectrometer at room temperature. The chemical shift values are expressed in parts per million (ppm) relative to tetramethylsilane as the internal standard. Coupling constants (*J*) are reported in hertz (Hz).

4.1.1—Compounds **46** [30, 31], **47** [32], **48** [32] and **11** [32] were prepared by following published methods.

4.1.2. Ethyl (5-(dipropylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (22)—Dipropylamine (68 mg, 670 μmol) in DMF (2 mL) was added to compound **48** (200 mg, 550 μmol) in DMF (1 mL) in a microwavable reaction vessel. The vessel was sealed and heated to 80°C in a microwave for 0.5 hour, and LC-MS showed that starting material **48** was depleted. The mixture was extracted with ethyl acetate (20 mL × 2) and washed with brine (10 mL × 2). The organic layer was dried with anhydrous Na₂SO₄ and concentrated to give a residue. The residue was purified by preparative HPLC to give compound **22** (115.9 mg, 274 μmol, 49.8% yield, 99.4% purity). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.33 (br. s., 1H), 8.15 (s, 1H), 7.48 (s, 1H), 7.18–7.33 (m, 5H), 7.14 (d, *J* = 8.28 Hz, 1H), 4.14 (q, *J* = 7.15 Hz, 2H), 3.22 (br. s, 2H), 2.73–2.80 (m, 2H), 2.46 (t, *J* = 7.28 Hz, 4H), 1.21 – 1.36 (m, 7H), 0.73–0.85 (m, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 169.63, 163.37, 153.50, 141.25, 141.12, 140.82, 140.71, 137.98, 137.35, 137.11, 135.11, 130.46, 130.27, 130.06, 128.57, 128.34, 127.69, 127.27, 127.08, 126.25, 117.99, 117.42, 60.24, 60.13, 55.56, 54.96, 54.81, 30.06, 29.79, 29.43, 29.19, 20.31, 20.09, 20.01, 14.51, 11.68, 11.62. ESI-TOF HRMS: *m/z* 424.2604 (C₂₅H₃₃N₃O₃ + H⁺ requires 424.2602).

4.1.3. Ethyl (5-(diisopropylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (23)—Diisopropylamine (68 mg, 670 μmol) in DMF (2 mL) was added to compound **48** (200 mg, 550 μmol) in DMF (1 mL) in a microwavable reaction vessel. The reaction vessel was sealed and heated to 80°C in a microwave for 0.5 hour, and LC-MS showed that starting material **48** was depleted. The mixture was extracted with ethyl acetate (20 mL × 2) and washed with brine (10 mL × 2). The organic layer was dried with anhydrous Na₂SO₄ and concentrated to give a residue. The residue was purified by preparative HPLC to give compound **23** (45.2 mg, 106 μmol, 19.4% yield, 98.7% purity). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.31 (br. s., 1H), 8.14 (s, 1H), 7.46–7.53 (m, 1H), 7.17–7.35 (m, 5H), 7.13 (d, *J* = 8.41 Hz, 1H), 4.13 (q, *J* = 7.11 Hz, 2H), 3.21–3.28 (m, 4H), 2.99–3.04 (m, 2H), 2.72–2.80 (m, 2H), 1.25 (t, *J* = 7.03 Hz, 3H), 0.82–1.03 (m, 12H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 170.86, 163.44, 153.51, 141.16, 130.54, 130.29, 128.58, 128.35, 60.21, 48.99, 48.37, 47.60, 46.73, 29.96, 29.82, 29.31, 20.63, 20.48, 19.68, 17.56, 14.50. ESI-TOF HRMS: *m/z* 424.2602 (C₂₅H₃₃N₃O₃ + H⁺ requires 424.2602).

4.1.4. Ethyl (5-(2-(pyrrolidin-1-yl)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (24)—NaH (33.44 mg, 1.39 mmol) was added to a solution of pyrrolidine (50 mg, 703 μmol) in DMF (2 mL) at 0°C. The mixture was stirred at 0°C for 0.5 hour. Compound **48** (250 mg, 697 μmol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 0°C for 1 hour, and LC-MS showed that starting material **48** was depleted. The reaction was quenched with aqueous NH_4Cl solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to give a residue. The residue was dissolved in MeOH (3 mL) and purified by preparative HPLC to give compound **24** (42.7 mg, 108 μmol , 15.5% yield, 99.2% purity). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.29 (s, 1H), 8.18 (br. s., 1H), 7.49 (d, $J = 2.13$ Hz, 1H), 7.20–7.34 (m, 5H), 7.14 (d, $J = 8.28$ Hz, 1H), 4.14 (q, $J = 7.03$ Hz, 2H), 3.24–3.32 (m, 4H), 2.71–2.81 (m, 2H), 2.46–2.50 (m, 4H), 1.60–1.69 (m, 4H), 1.22–1.31 (m, 3H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 168.70, 163.67, 153.49, 141.20, 140.54, 140.38, 138.03, 137.35, 135.03, 130.79, 130.44, 130.11, 129.97, 128.63, 128.49, 127.60, 127.31, 127.12, 126.26, 118.36, 118.00, 117.48, 60.15, 56.61, 56.46, 53.37, 30.18, 29.67, 29.49, 29.00, 23.35, 14.51. ESI-TOF HRMS: m/z 394.2133 ($\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_3 + \text{H}^+$ requires 394.2132).

4.1.5. Ethyl (5-(2-(piperidin-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (25)

—NaH (33.44 mg, 1.39 mmol) was added to a solution of piperidine (60 mg, 704 μmol) in DMF (2 mL) at 0°C. The mixture was stirred at 0°C for 0.5 hour. Compound **48** (250 mg, 697 μmol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 0°C for 1 hour, and LC-MS showed that starting material **48** was depleted. The reaction was quenched with aqueous NH_4Cl solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to give a residue. The residue was dissolved in MeOH (3 mL) and purified by preparative HPLC to give compound **25** (85.4 mg, 209 μmol , 30.1% yield, 97.6% purity). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.22 (br. s., 1H), 8.16 (s, 1H), 7.48 (d, $J = 2.07$ Hz, 1H), 7.17–7.36 (m, 5H), 7.13 (d, $J = 8.48$ Hz, 1H), 4.14 (q, $J = 7.10$ Hz, 2H), 3.28 (br. s., 2H), 2.72–2.84 (m, 4H), 2.26–2.33 (m, 4H), 1.31–1.49 (m, 6H), 1.26 (t, $J = 7.06$ Hz, 3H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.10, 163.87, 154.00, 153.94, 141.87, 141.18, 141.05, 138.27, 137.85, 135.62, 131.30, 130.83, 130.44, 130.31, 129.12, 129.04, 128.71, 127.81, 127.36, 126.71, 118.64, 118.44, 117.96, 117.65, 60.70, 60.62, 54.26, 30.51, 30.40, 29.86, 29.75, 25.78, 23.88, 14.98. ESI-TOF HRMS: m/z 408.2287 ($\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_3 + \text{H}^+$ requires 408.2289).

4.1.6. Ethyl (5-(2-(2,2,6,6-tetramethylpiperidin-1-yl)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (26)—NaI (376 mg, 2.51 mmol) was added to compound **48** (300 mg, 836 μmol) in acetone (5 mL) in one portion at 25°C under N_2 . The mixture was stirred at 25°C for 10 hours, and TLC showed that the reaction was completed. The mixture was filtered, and the filtrate was concentrated under vacuum to afford compound **49** (325 mg, 721.8 μmol) as a yellow solid. A mixture of compound **49** (150 mg, 333.2 μmol) and 2,2,6,6-tetramethylpiperidine (2.00 g, 14.16 mmol) in toluene (10 mL) was heated to 120°C and stirred for 16 hours. LC-MS showed that starting material **49** was depleted. The mixture was cooled to 25°C and concentrated under reduced pressure at 50°C.

The residue was purified by preparative HPLC to afford compound **26** (10.00 mg, 12.94 μmol , 6.48% yield, 95.6% purity) as a yellow solid. ^1H NMR (400 MHz, chloroform-*d*) δ (ppm) 1.12–1.51 (m, 15 H), 1.68 (d, $J = 5.77$ Hz, 2 H), 2.78–2.93 (m, 3 H), 3.07–3.33 (m, 4 H), 3.56 (d, $J = 17.82$ Hz, 1 H), 4.19 (ddt, $J = 10.54, 7.03, 3.64, 3.64$ Hz, 3 H), 5.05 (d, $J = 17.82$ Hz, 1 H), 7.16–7.32 (m, 5 H), 7.36–7.49 (m, 1 H), 8.00 (d, $J = 7.78$ Hz, 1 H), 8.17 (br. s., 1 H), 9.27–9.52 (m, 1 H). ESI-TOF HRMS: m/z 464.2918 ($\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_3 + \text{H}^+$ requires 464.2915).

4.1.7. Ethyl (5-(2-((1*s*,4*s*)-7-azabicyclo[2.2.1]heptan-7-yl)acetyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-3-yl)carbamate (27)—7-Azabicyclo[2.2.1]heptane (65 mg, 669 μmol) in DMF (2 mL) was added to compound **48** (200 mg, 550 μmol) in DMF (1 mL) in a microwavable reaction vessel. The vessel was sealed and heated to 80°C in a microwave for 0.5 h, and LC-MS showed that starting material **48** was depleted. The mixture was extracted with ethyl acetate (20 mL \times 2) and washed with brine (10 mL \times 2). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to give a residue. The residue was purified by preparative HPLC to give compound **27** (75.7 mg, 180 μmol , 32.8% yield, 98.9% purity). ^1H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.32 (br. s., 1H), 8.18 (br. s., 1H), 7.49 (s, 1H), 7.17–7.37 (m, 5H), 7.12 (d, $J = 8.28$ Hz, 1H), 4.06–4.18 (m, 2H), 3.20–3.29 (m, 2H), 3.02–3.15 (m, 4H), 2.65–2.83 (m, 2H), 1.44–1.62 (m, 4H), 1.13–1.30 (m, 7H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 169.65, 164.07, 153.98, 141.81, 141.18, 141.03, 138.30, 138.00, 137.82, 135.72, 131.48, 130.81, 130.49, 130.36, 129.24, 129.03, 128.82, 127.98, 127.80, 127.43, 126.70, 118.42, 117.97, 60.61, 60.45, 49.58, 30.49, 30.39, 29.83, 28.25, 27.98, 14.98. ESI-TOF HRMS: m/z 420.2291 ($\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_3 + \text{H}^+$ requires 420.2289).

4.1.8. Ethyl (5-(2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-3-yl)carbamate (28)— $\text{NH}_3 \cdot \text{H}_2\text{O}$ (10 mL) was added to compound **48** (200 mg, 557 μmol) in MeOH (3 mL) in one portion at 25°C in a sealable reaction tube. The reaction mixture was sealed and stirred at 120°C for 12 hours. TLC showed that the reaction was completed. The mixture was concentrated under vacuum to afford compound **50** (185.00 mg, 545.10 μmol , 97.80% yield) as a yellow solid. Furan-2,5-dione (260 mg, 2.65 mmol) was added to compound **50** (180 mg, 530 μmol) in AcOH (5 mL) in one portion at 25°C. The mixture was stirred at 100°C for 12 hours, and TLC showed that the reaction was completed. The mixture was cooled to 25°C and concentrated under reduced pressure. The residue was poured into saturated NaHCO_3 solution (5 mL) and stirred for 3 min. The aqueous phase was extracted with ethyl acetate (3 mL \times 3). The combined organic phase was washed with saturated brine (3 mL), dried with anhydrous Na_2SO_4 , filtered, and concentrated under vacuum. The residue was purified by performing silica gel chromatography (Petroleum ether/ethyl acetate = 3/1 to 1/1) to afford the compound **28** (14.00 mg, 33.38 μmol , 6.29% yield, 96.7% purity) as an off-white solid. ^1H NMR (400 MHz, chloroform-*d*) δ (ppm) 1.23–1.42 (m, 3 H), 2.79–2.93 (m, 2 H), 3.29–3.43 (m, 1 H), 3.48–3.61 (m, 1 H), 3.82–3.99 (m, 1 H), 4.17–4.31 (m, 2 H), 4.35–4.57 (m, 1 H), 6.50–6.69 (m, 1 H), 6.76 (s, 2 H), 7.07–7.23 (m, 2 H), 7.25–7.39 (m, 7 H), 7.43 (d, $J = 7.53$ Hz, 1 H), 7.58 (s, 1 H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 171.00, 170.97, 166.09, 165.98, 154.02, 153.89, 140.67, 140.32, 140.28, 140.25, 138.90, 137.93, 137.88, 135.57,

135.43, 131.23, 131.05, 130.88, 130.85, 129.62, 128.85, 128.71, 128.20, 128.14, 128.09, 126.91, 60.82, 60.62, 30.44, 29.91, 29.87, 29.42, 14.98, 14.96. ESI-TOF HRMS: m/z 420.1566 ($C_{23}H_{21}N_3O_5 + H^+$ requires 420.1561).

4.1.9. Ethyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (29)—NaH (33.5 mg, 1.39 mmol) was added to a solution of pyrrole (47 mg, 700 μ mol) in DMF (2 mL) at 0°C. The mixture was stirred at 0°C for 0.5 hour. Compound **48** (250 mg, 696 μ mol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 0°C for 1 hour, and LC-MS showed that starting material **48** was depleted. The reaction was quenched with aqueous NH_4Cl solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to give a residue. The residue was dissolved in MeOH (3 mL) and purified by preparative HPLC to give compound **29** (81.4 mg, 209 μ mol, 30.0% yield, 99.5% purity). 1H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.53 (br. s., 1H), 7.27–7.43 (m, 3H), 6.99–7.22 (m, 3H), 6.62 (d, $J = 14.43$ Hz, 1H), 6.47 (d, $J = 9.79$ Hz, 2H), 6.11 (t, $J = 1.94$ Hz, 2H), 4.47–4.72 (m, 2H), 4.16–4.29 (m, 2H), 3.16 (br. s., 2H), 2.73 (d, $J = 9.41$ Hz, 2H), 1.27–1.37 (m, 3H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 167.65, 154.08, 153.91, 140.79, 140.66, 138.66, 138.02, 137.89, 135.62, 131.52, 130.95, 130.84, 129.35, 129.09, 128.83, 128.21, 128.01, 127.84, 126.80, 122.37, 119.47, 118.15, 108.06, 108.00, 60.78, 60.61, 51.03, 50.83, 30.48, 30.22, 29.84, 29.59, 14.98. ESI-TOF HRMS: m/z 390.1823 ($C_{23}H_{23}N_3O_3 + H^+$ requires 390.1819).

4.1.10. Ethyl (5-(2-(1H-imidazol-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (30)—NaH (33.5 mg, 1.39 mmol) was added to a solution of imidazole (48 mg, 705 μ mol) in DMF (2 mL) at 0°C. The mixture was stirred at 0°C for 0.5 hour. Compound **48** (250 mg, 697 μ mol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 0°C for 1 hour, and LC-MS showed that starting material **48** was depleted. The reaction was quenched with aqueous NH_4Cl solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to give a residue. The residue was dissolved in MeOH (3 mL) and purified by preparative HPLC to give compound **30** (91.5 mg, 234 μ mol, 33.7% yield, 99.1% purity). 1H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.27 (br. s., 1H), 7.59 (s, 1H), 7.45 (s, 2H), 7.25–7.33 (m, 4H), 7.18 (d, $J = 8.29$ Hz, 1H), 7.03 (s, 1H), 6.85 (s, 1H), 4.79 (d, $J = 9.80$ Hz, 2H), 4.15 (q, $J = 6.97$ Hz, 2H), 3.20–3.31 (m, 2H), 2.72–2.81 (m, 2H), 1.26 (t, $J = 7.06$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 166.99, 154.08, 153.91, 140.58, 140.52, 140.49, 140.40, 138.84, 138.71, 138.12, 137.90, 135.50, 131.70, 130.99, 130.88, 129.48, 129.04, 128.82, 128.18, 128.12, 128.05, 127.89, 126.81, 121.29, 121.26, 119.67, 118.12, 60.78, 60.61, 60.23, 48.44, 48.29, 30.60, 30.21, 29.90, 29.54, 21.24, 14.98, 14.56. ESI-TOF HRMS: m/z 391.1775 ($C_{22}H_{22}N_4O_3 + H^+$ requires 391.1772).

4.1.11. Ethyl (5-(ethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (31)—Ethylamine (31 mg, 687 μ mol) in DMF (2 mL) was added to compound **48** (200 mg, 550 μ mol) in DMF (1 mL) in a microwavable vessel. The vessel was sealed and heated to 80°C in a microwave for 0.5 hour, and LC-MS showed that starting

material **48** was depleted. The mixture was extracted with ethyl acetate (20 mL × 2) and washed with brine (10 mL × 2). The organic layer was dried with anhydrous Na₂SO₄ and concentrated to yield a residue. The residue was purified by preparative HPLC to yield compound **31** (104.5 mg, 284 μmol, 51.7% yield, 99.1% purity). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.29 (br. s., 1H), 8.17 (br. s., 1H), 7.48 (s, 1H), 7.18–7.35 (m, 5H), 7.15 (d, *J* = 8.48 Hz, 1H), 4.14 (q, *J* = 6.97 Hz, 2H), 3.20–3.21 (m, 2H), 2.78 (d, *J* = 9.42 Hz, 2H), 2.51–2.59 (m, 4H), 2.09 (s, 1H), 1.25 (t, *J* = 7.06 Hz, 3H), 0.96 (t, *J* = 7.06 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 169.70, 169.49, 164.83, 154.00, 153.93, 140.77, 140.56, 140.50, 138.65, 137.93, 137.85, 135.49, 131.24, 131.03, 130.96, 130.88, 130.70, 129.34, 128.91, 128.01, 127.83, 126.84, 119.19, 118.19, 60.76, 60.62, 49.96, 49.89, 43.16, 30.62, 30.01, 29.95, 29.39, 14.97, 14.43, 14.27. ESI-TOF HRMS: *m/z* 368.1980 (C₂₁H₂₅N₃O₃ + H⁺ requires 368.1976).

4.1.12. Ethyl (5-(tert-pentylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (32)—*Tert*-Amylamine (59 mg, 677 μmol) in DMF (2 mL) was added to compound **48** (200 mg, 550 μmol) in DMF (1 mL) in a microwavable vessel. The vessel was sealed and heated to 80°C in a microwave for 0.5 hour, and LC-MS showed that starting material **48** was depleted. The mixture was extracted with ethyl acetate (20 mL × 2) and washed with brine (10 mL × 2). The organic layer was dried with anhydrous Na₂SO₄ and concentrated to yield a residue. The residue was purified by preparative HPLC to yield compound **32** (96.3 mg, 235 μmol, 42.8% yield, 95.0% purity). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.35 (br. s., 1H), 8.18 (s, 1H), 7.50 (s, 1H), 7.22–7.34 (m, 5H), 7.15 (d, *J* = 8.28 Hz, 1H), 4.10–4.16 (m, 2H), 3.18–3.27 (m, 4H), 2.71–2.81 (m, 2H), 1.20–1.27 (m, 5H), 0.95 (s, 1H), 0.86 (s, 6H), 0.73–0.80 (m, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 171.08, 170.95, 164.40, 154.00, 153.93, 141.09, 140.94, 140.68, 138.55, 137.87, 135.54, 131.17, 131.00, 130.87, 130.60, 129.24, 128.93, 127.97, 127.89, 127.75, 126.80, 119.04, 118.31, 118.06, 117.66, 60.75, 60.67, 60.61, 52.81, 52.44, 44.13, 44.04, 33.07, 33.02, 30.59, 29.99, 29.44, 26.37, 26.20, 26.14, 26.08, 22.62, 14.97, 8.64, 8.58, 8.33. ESI-TOF HRMS: *m/z* 410.2455 (C₂₄H₃₁N₃O₃ + H⁺ requires 410.2445).

4.1.13. Ethyl (5-(3-(diethylamino)propanoyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (33)—A mixture of 3-(diethylamino)propionic acid (124 mg, 854 μmol) and compound **47** (200 mg, 708 μmol) in T₃P (5 mL) was stirred at reflux for 12 hours, and LC-MS showed that starting material **47** was depleted. The mixture was concentrated to yield a residue that was then purified by preparative HPLC to yield compound **33** (147.3 mg, 360 μmol, 50.8% yield, 97.7% purity) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.32 (br. s., 1H), 8.16 (s, 1H), 7.50 (br. s., 1H), 7.19–7.35 (m, 5H), 7.15 (d, *J* = 8.28 Hz, 1H), 4.14 (q, *J* = 7.15 Hz, 2H), 3.19–3.28 (m, 2H), 2.69–2.81 (m, 4H), 2.32–2.43 (m, 6H), 1.26 (t, *J* = 7.09 Hz, 3H), 0.85–0.94 (m, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 171.09, 171.06, 164.37, 153.99, 153.92, 141.90, 141.88, 141.21, 141.07, 138.58, 137.84, 137.65, 135.60, 131.08, 130.76, 130.73, 130.63, 129.12, 128.99, 128.95, 128.33, 127.78, 127.67, 126.71, 118.84, 118.41, 117.98, 117.82, 60.73, 60.58, 48.53, 48.48, 46.66, 46.63, 31.13, 31.10, 30.53, 30.23, 29.86, 29.59, 14.99, 14.96, 11.36, 11.33. ESI-TOF HRMS: *m/z* 410.2446 (C₂₄H₃₁N₃O₃ + H⁺ requires 410.2445).

4.1.14. Ethyl (5-(3-(pyrrolidin-1-yl)propanoyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (34)—A mixture of 1-pyrrolidinepropanoic acid (122 mg, 852 μmol) and compound **47** (200 mg, 708 μmol) in T_3P (5 mL) was stirred at reflux for 12 hours, and LC-MS showed that starting material **47** was depleted. The mixture was concentrated to yield a residue. The residue was purified by preparative HPLC to yield compound **34** (197.4 mg, 485 μmol , 68.5% yield, 99.0% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.25 (br. s., 1H), 8.14 (s, 1H), 7.47 (s, 1H), 7.10–7.30 (m, 5H), 4.13 (q, $J = 6.97$ Hz, 2H), 3.03–3.15 (m, 2H), 2.70 (t, $J = 6.97$ Hz, 4H), 2.20–2.44 (m, 6H), 1.65 (td, $J = 3.30, 6.59$ Hz, 4H), 1.25 (t, $J = 7.06$ Hz, 3H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 170.81, 170.78, 164.27, 154.00, 153.92, 141.78, 141.74, 141.21, 141.09, 138.54, 137.83, 137.69, 135.74, 131.11, 130.81, 130.73, 130.68, 130.66, 129.23, 128.95, 128.33, 127.81, 127.68, 126.71, 118.86, 118.38, 117.98, 117.86, 60.74, 60.59, 53.83, 51.62, 51.54, 32.70, 32.63, 30.44, 30.27, 29.80, 29.65, 23.41, 14.99, 14.97. ESI-TOF HRMS: m/z 408.2290 ($\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_3 + \text{H}^+$ requires 408.2289).

4.1.15. Ethyl (5-(2-(tert-butoxy)acetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (35)—A mixture of compound **47** (150 mg, 530 μmol) and 2-*tert*-butoxyacetic acid (105 mg, 796 μmol) in propylphosphonic acid (T_3P) (5 mL) was stirred at 55°C for 12 hours and, LC-MS showed that starting material **47** was depleted. The mixture was washed with NaHCO_3 aqueous solution (20 mL \times 3). The organic layer was dried with anhydrous Na_2SO_4 and concentrated under vacuum to yield a residue. The residue was purified by preparative HPLC to give compound **35** (50.00 mg, 126.11 μmol , 23.74% yield, 99.5% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.63 (br. s., 1 H), 7.03–7.61 (m, 7 H), 4.04–4.22 (m, 2 H), 3.87 (d, $J = 8.91$ Hz, 2 H), 3.10–3.32 (m, 2 H), 2.63–2.87 (m, 2 H), 1.23 (t, $J = 7.09$ Hz, 3 H), 0.92 (br. s., 9 H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.08, 153.96, 141.68, 140.95, 138.35, 138.17, 137.81, 130.90, 130.48, 130.26, 128.95, 127.82, 127.40, 126.73, 118.37, 118.01, 73.79, 62.07, 60.65, 30.44, 30.17, 29.82, 29.60, 27.16, 14.97. ESI-TOF HRMS: m/z 419.1952 ($\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_4 + \text{Na}^+$ requires 419.1949).

4.1.16. 1-(3-amino-10,11-dihydro-5H-dibenzo[*b,f*]azepin-5-yl)-2-(diethylamino)ethan-1-one (51)—Compound **11** (2.5 g, 6.32 mmol) in $\text{H}_2\text{SO}_4/\text{AcOH}$ (40 mL) was stirred at 120°C for 4 hours, and LC-MS showed that starting material **11** was depleted. The mixture was basified to pH 7 with Na_2CO_3 aqueous solution and extracted with ethyl acetate (50 mL \times 3). The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give compound **51** (2.0 g, 97.8% yield) as a light yellow oil. LCMS: MS +1=324.2. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 7.17–7.36 (m, 4H), 6.76–6.99 (m, 1H), 6.36–6.59 (m, 2H), 4.92–5.15 (m, 2H), 3.02–3.22 (m, 4H), 2.51–2.81 (m, 4H), 2.44 (br. s., 2H), 0.75–0.87 (m, 6H).

4.1.17. *N*-(5-(diethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)butyramide (36)—A mixture of **51** (180 mg, 556 μmol), butyryl chloride (118.6 mg, 1.11 mmol) and Et_3N (141 mg, 1.39 mmol) in dichloromethane (3 mL) was stirred at 10°C to 35°C for 16 hours, and LC-MS showed that starting material **51** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC

to give compound **36** (95.2 mg, 242 μmol , 43.4% yield, 98.8% purity). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.55 (br. s., 1H), 8.15 (s, 1H), 7.61 (d, $J = 1.88$ Hz, 1H), 7.40 (dd, $J = 2.07, 8.29$ Hz, 1H), 7.18–7.36 (m, 4H), 7.14 (d, $J = 8.29$ Hz, 1H), 3.21 (d, $J = 1.13$ Hz, 4H), 2.72–2.84 (m, 2H), 2.52–2.59 (m, 4H), 2.28 (t, $J = 7.25$ Hz, 2H), 1.58–1.70 (m, 2H), 0.94 (t, $J = 7.44$ Hz, 3H), 0.81–0.89 (m, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 206.58, 171.62, 171.47, 170.99, 169.88, 163.86, 141.83, 141.57, 141.13, 141.03, 138.43, 137.90, 137.78, 135.55, 131.80, 130.79, 130.52, 130.40, 129.65, 129.03, 128.82, 128.08, 127.75, 127.50, 126.70, 119.40, 118.72, 61.49, 54.63, 47.19, 42.78, 38.69, 38.65, 30.52, 30.19, 30.01, 29.70, 21.44, 18.99, 16.97, 14.07, 13.93, 12.25, 12.22. ESI-TOF HRMS: m/z 394.2501 ($\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_2 + \text{H}^+$ requires 394.2496).

4.1.18. Isopropyl (5-(diethylglycyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (37)—A mixture of **51** (180 mg, 556 μmol), isopropyl chloroformate (118.6 mg, 1.11 mmol), and Et_3N (1401 mg, 1.4 mmol) in dichloromethane (3 mL) was stirred at 10°C to 35°C for 16 hours, and LC-MS showed that starting material **51** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC to give **37** (61.1 mg, 149 μmol , 26.8% yield, 97.9% purity). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.16 (br. s., 1H), 8.15 (br. s., 1H), 7.49 (s, 1H), 7.17–7.35 (m, 5H), 7.12 (d, $J = 8.48$ Hz, 1H), 4.91 (td, $J = 6.22, 12.43$ Hz, 1H), 3.18–3.27 (m, 4H), 2.71–2.80 (m, 2H), 2.51–2.58 (m, 4H), 1.27 (dd, $J = 1.41, 6.31$ Hz, 6H), 0.85 (t, $J = 7.16$ Hz, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.90, 164.06, 153.56, 141.80, 141.66, 141.28, 141.17, 138.48, 137.91, 137.71, 135.60, 131.00, 130.77, 130.61, 130.45, 129.00, 128.78, 128.11, 127.74, 127.48, 126.71, 118.65, 118.39, 117.89, 68.04, 67.92, 54.72, 54.50, 47.18, 30.51, 30.26, 29.87, 29.65, 22.42, 12.30, 12.21. ESI-TOF HRMS: m/z 410.2444 ($\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_3 + \text{H}^+$ requires 410.2445).

4.1.19. tert-Butyl (5-(diethylglycyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (38)—A mixture of compound **51** (100 mg, 310 μmol), Boc_2O (135 mg, 619 μmol), and Et_3N (78 mg, 772 μmol) in dichloromethane (2 mL) was stirred at 20°C to 25°C for 24 hours, and LC-MS showed that starting material **51** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC to give compound **38** (50 mg, 118.05 μmol , 38.18% yield, 99.6% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 8.95 (br. s., 1H), 8.16 (br. s., 1H), 7.49 (d, $J = 1.70$ Hz, 1H), 7.16–7.36 (m, 5H), 7.11 (d, $J = 8.48$ Hz, 1H), 3.20 (s, 4H), 2.71–2.84 (m, 2H), 2.51–2.58 (m, 4H), 1.49 (s, 9H), 0.86 (t, $J = 7.06$ Hz, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.85, 163.91, 153.16, 141.80, 141.60, 141.27, 141.15, 138.70, 138.13, 137.67, 135.58, 130.76, 130.65, 130.49, 129.04, 128.75, 128.12, 127.72, 127.47, 126.69, 118.64, 118.27, 117.91, 117.70, 79.68, 79.49, 54.70, 54.45, 47.19, 30.56, 30.29, 29.84, 29.62, 28.58, 12.29, 12.20. ESI-TOF HRMS: m/z 424.2603 ($\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_3 + \text{H}^+$ requires 424.2602).

4.1.20. N-(5-(diethylglycyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)-1H-imidazole-1-carboxamide (52)—A mixture of compound **51** (500 mg, 1.55 mmol), CDI (250.6 mg, 1.55 mmol), and Et_3N (312.9 mg, 3.09 mmol) in dichloromethane (5 mL) was stirred at 20°C to 25°C for 2 hours, and LC-MS showed that starting material **51** was depleted. The mixture was used directly for the next step without further purification.

4.1.21. 1-(5-(Diethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)-3-ethylurea (39)—A mixture of ethylamine (26 mg, 577 μmol), compound **52** (120 mg, 287 μmol), DMAP (17.6 mg, 143 μmol), and Et_3N (29.1 mg, 287.42 μmol) in dichloromethane (2 mL) was stirred at 20°C to 25°C for 12 hours, and LC-MS showed that starting material **52** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC to give compound **39** (82.3 mg, 208 μmol , 72.6% yield, 97.6% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 8.22 (br. s., 1H), 8.14 (s, 1H), 7.44 (br. s., 1H), 7.13–7.36 (m, 5H), 7.06 (d, $J = 8.41$ Hz, 1H), 5.95 (br. s., 1H), 3.07–3.18 (m, 6H), 2.69–2.80 (m, 2H), 2.50–2.58 (m, 4H), 0.99–1.14 (m, 3H), 0.77–0.88 (m, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.76, 169.62, 163.96, 155.51, 141.86, 141.53, 141.20, 141.08, 139.91, 139.25, 137.83, 135.63, 130.77, 130.68, 130.43, 129.46, 129.00, 128.77, 128.06, 127.69, 127.45, 126.65, 118.10, 117.81, 117.38, 117.22, 54.62, 54.35, 47.29, 47.24, 34.40, 34.28, 30.65, 30.31, 29.85, 29.56, 15.93, 12.25, 12.14. ESI-TOF HRMS: m/z 395.2450 ($\text{C}_{23}\text{H}_{30}\text{N}_4\text{O}_2 + \text{H}^+$ requires 395.2449).

4.1.22. 1-(5-(Diethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)-3-isopropylurea (40)—A mixture of *iso*-propylamine (34 mg, 575 μmol), compound **52** (120.00 mg, 287 μmol), DMAP (17.6 mg, 143 μmol), and Et_3N (29.08 mg, 287.42 μmol) in dichloromethane (2 mL) was stirred at 20°C to 25°C for 12 hours, and LC-MS showed that starting material **52** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC to give compound **40** (52.7 mg, 128 μmol , 44.9% yield, 99.7% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 8.07 (s, 1H), 7.44 (d, $J = 1.88$ Hz, 1H), 7.12–7.35 (m, 5H), 7.03–7.09 (m, 1H), 5.77 (d, $J = 7.35$ Hz, 1H), 3.78 (qd, $J = 6.59, 13.59$ Hz, 1H), 3.16–3.34 (m, 4H), 2.69–2.82 (m, 2H), 2.51–2.56 (m, 4H), 1.04–1.19 (m, 6H), 0.86 (t, $J = 7.06$ Hz, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 170.10, 169.99, 154.87, 142.04, 141.73, 141.30, 141.20, 139.78, 139.12, 137.82, 135.64, 130.71, 130.42, 129.50, 129.05, 128.69, 128.09, 127.65, 127.43, 126.63, 117.95, 117.82, 117.27, 117.16, 54.75, 54.45, 47.14, 47.09, 41.39, 41.29, 30.65, 30.31, 29.87, 29.57, 23.44, 23.41, 12.45, 12.34. ESI-TOF HRMS: m/z 409.2607 ($\text{C}_{24}\text{H}_{32}\text{N}_4\text{O}_2 + \text{H}^+$ requires 409.2605).

4.1.23. 1-(*tert*-Butyl)-3-(5-(diethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)urea (41)—A mixture of *tert*-butylamine (42 mg, 574 μmol), compound **52** (120.00 mg, 287 μmol), DMAP (17.6 mg, 143 μmol), and Et_3N (29 mg, 287 μmol) in dichloromethane (2 mL) was stirred at 20°C to 25°C for 12 hours, and LC-MS showed that starting material **52** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC to give **41** (71.7 mg, 170 μmol , 59.2% yield, 99.8% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 8.17 (s, 1H), 7.98 (br. s., 1H), 7.49 (s, 1H), 7.17–7.39 (m, 5H), 7.09 (d, $J = 8.16$ Hz, 1H), 3.25–3.42 (m, 8H), 2.68–2.82 (m, 2H), 2.52–2.57 (m, 4H), 0.99–1.31 (m, 6H), 0.75–0.93 (m, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.77, 169.63, 163.95, 154.76, 141.88, 141.59, 141.18, 139.92, 139.25, 137.78, 135.60, 130.77, 130.65, 130.44, 129.37, 129.06, 128.75, 128.10, 127.68, 127.45, 127.27, 126.64, 117.88, 117.56, 117.10, 116.93, 54.63, 54.31, 49.86, 47.28, 47.23, 30.68, 30.33, 29.84, 29.48, 12.25, 12.16. ESI-TOF HRMS: m/z 423.2762 ($\text{C}_{25}\text{H}_{34}\text{N}_4\text{O}_2 + \text{H}^+$ requires 423.2762).

4.1.24. 3-(5-(Diethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)-1,1-diethylurea (42)—A mixture of diethylamine (42 mg, 575 μmol), compound **52** (120 mg, 287.42 μmol), DMAP (17.6 mg, 143.8 μmol), and Et_3N (29 mg, 287.4 μmol) in dichloromethane (2 mL) was stirred at 20°C to 25°C for 12 hours, and LC-MS showed that starting material **52** was depleted. The mixture was concentrated to give a residue. The residue was purified by preparative HPLC to give **42** (102.7 mg, 243 μmol , 84.8% yield, 95.2% purity) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 8.05-8.23 (m, 2H), 7.44 (br. s., 1H), 7.32 (br. s., 1H), 7.16-7.27 (m, 3H), 7.00-7.12 (m, 2H), 5.81 (br. s., 1H), 3.26-3.37 (m, 4H), 2.67-2.80 (m, 2H), 2.51-2.56 (m, 4H), 1.29 (s, 9H), 0.77-0.90 (m, 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 169.89, 169.74, 164.11, 154.75, 141.86, 141.20, 140.84, 139.98, 139.40, 137.74, 135.62, 130.76, 130.48, 130.12, 129.95, 129.02, 128.74, 128.09, 127.66, 127.44, 126.62, 120.16, 120.04, 119.75, 119.47, 54.54, 54.45, 47.25, 47.18, 30.66, 30.36, 29.83, 29.59, 14.35, 12.30, 12.22, 11.53. ESI-TOF HRMS: m/z 423.2769 ($\text{C}_{25}\text{H}_{34}\text{N}_4\text{O}_2 + \text{H}^+$ requires 423.2762).

4.1.25. 1-(5-(Diethylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)-3-ethylsulfamide (43)—Ethanamine (10 g, 222 mmol) in dichloromethane (30 mL) was added dropwise to a suspension of sulfuryl chloride (100 g, 741 mmol) at 0°C to 10°C. After addition, the mixture was heated to 75°C for 15 hours and then cooled to 20°C. The mixture was concentrated to dryness to yield compound *N*-ethyl-sulfamoyl chloride (50 g, crude), which was freshly used directly in the next step. *N*-Ethyl-sulfamoyl chloride (532.7 mg, 3.71 mmol) was added to a mixture of compound **51** (200 mg, 618.4 μmol) in dichloromethane (6 mL), and Et_3N (18.8 mg, 185.52 μmol) was added dropwise. The mixture was stirred at 15°C to 25°C for 1 hour, and LC-MS showed that starting material **51** was depleted. The mixture was concentrated, dried, and purified by preparative HPLC to give compound **43** (76.6 mg, 178 μmol , 28.8% yield, 94.4% purity) as a light yellow solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 9.62 (br. s., 1H), 6.94-7.57 (m, 8H), 3.03-3.28 (m, 4H), 2.64-2.93 (m, 4H), 2.44 (br. s., 4H), 0.96 (t, $J = 7.22$ Hz, 3H), 0.79 (br. s., 6H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ (ppm) 170.23, 170.07, 141.93, 141.38, 141.29, 138.55, 137.82, 137.62, 135.67, 131.06, 130.83, 130.72, 130.48, 130.38, 129.18, 128.98, 128.77, 128.13, 127.71, 127.48, 126.70, 119.00, 118.14, 116.78, 54.82, 47.06, 37.51, 30.47, 30.20, 29.90, 29.63, 15.01, 12.30. ESI-TOF HRMS: m/z 431.2120 ($\text{C}_{22}\text{H}_{30}\text{N}_4\text{O}_3\text{S} + \text{H}^+$ requires 431.2119).

4.1.26. 2-(Diethylamino)-1-(3-((5-methyl-1,3,4-oxadiazol-2-yl)amino)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-5-yl)ethan-1-one (44)—1-(2-Oxopyridine-1-carbothiopyridin-2-one (344.7 mg, 1.48 mmol) in dichloromethane (0.5 mL) was added dropwise to a solution of compound **51** (320 mg, 989.39 μmol) in dichloromethane (1 mL) at 0°C. The mixture was stirred at 20°C for 16 hours and then concentrated and purified by performing silica gel chromatography (dichloromethane: MeOH = 30:1). A mixture of acetohydrazide (146.6 mg, 1.98 mmol) and purified product in THF (1 mL) was stirred at 20°C for 16 hours and then concentrated; EDC (307.19 mg, 1.98 mmol), TEA (200.23 mg, 1.98 mmol), and DMF (1 mL) were then added, and the reaction was stirred at 20°C for 16 hours. The residue was further purified by preparative HPLC to give compound **44** (39.8 mg, 98 μmol , 9.9% yield, 94.1% purity) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) 10.28 (br. s., 1H), 9.68 (br. s., 1H), 7.80 - 7.20 (m, 7H), 4.32 (br. s., 1H), 3.94 - 3.47

(m, 2H), 3.26 (br. s., 5H), 2.92 - 2.75 (m, 2H), 2.40 (s, 3H), 1.24 (br. s., 6H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 160.21, 159.94, 157.68, 157.51, 138.59, 137.82, 137.70, 135.61, 131.55, 131.42, 131.11, 131.01, 130.34, 129.82, 128.53, 128.10, 128.04, 127.87, 127.02, 117.37, 116.35, 116.28, 49.03, 45.73, 30.45, 30.23, 29.79, 29.58, 10.86, 9.78, 8.86. ESI-TOF HRMS: m/z 406.2247 ($\text{C}_{23}\text{H}_{27}\text{N}_5\text{O}_2 + \text{H}^+$ requires 406.2245).

4.1.27. Isopropyl (10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (73)—

Isopropyl carbonochloridate (4.20 g, 34.24 mmol) was added dropwise to a solution of compound **46** (3.60 g, 17.12 mmol) in ethanol (36 mL) at 0°C for approximately 20 min. A solution of sodium carbonate (1.81 g, 17.12 mmol) in water (36 mL) was then added dropwise while the temperature was maintained below 15°C. The reaction was stirred at 25°C for 1 hour. TLC showed that starting material **46** was depleted. The reaction solution was poured into water and filtered yielding pure compound **73** (5.20 g, crude) as a green solid. LC-MS: MS + 1 = 297.15. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.35 (s, 1H), 8.28 (s, 1H), 7.24 (s, 1H), 7.01-6.96 (m, 3H), 6.87- 6.85(d, J = 8.0 Hz, 1H), 6.66-6.64 (m, 2H), 4.91-4.85 (m, 1H), 2.93-2.84 (m, 4H), 1.26-1.25 (d, J = 4.0 Hz, 6H).

4.1.28. Isopropyl (5-(3-(diethylamino)propanoyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (53)—

A mixture of compound **73** (200 mg, 674.85 μmol) and 3-chloropropionyl chloride (128.5 mg, 1.01 mmol) in toluene (2 mL) was stirred at 100°C for 3 hours. TLC showed that the reaction was complete. The mixture was concentrated to afford compound **74** as gray gum, which was directly used in the next step. A mixture of compound **74** (261 mg, 675 μmol) and diethyl amine (247 mg, 3.38 mmol) in toluene (2 mL) was stirred at 100°C for 10 hours. TLC showed that the reaction was complete. The solvent was removed, and the residue was first purified by flash column chromatography (petroleum ether/EtOAc = 10/1 to 100% EtOAc) and then further purified by preparative HPLC to afford compound **53** (60 mg, 382.48 μmol , 56.66% yield, 96.4% purity) as a colorless oil. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.50-9.79 (d, J = 34 Hz, 1 H) 8.93 (br, 1 H) 7.73-7.07 (m, 7 H) 4.81-4.95 (m, 1 H) 3.15-3.36 (m, 5 H) 3.05-3.15 (m, 4 H) 2.86-2.99 (m, 1 H) 2.71-2.85 (m, 2 H) 2.33-2.45 (m, 1 H) 1.24 (d., J = 5.8 Hz, 6 H) 1.09-1.20 (m, 6 H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 169.36, 169.35, 158.73, 158.47, 158.22, 157.96, 153.66, 153.52, 141.39, 141.37, 140.55, 140.25, 138.81, 137.95, 137.76, 135.34, 131.35, 131.04, 130.95, 130.79, 130.76, 129.28, 128.94, 128.87, 128.16, 127.98, 127.91, 126.73, 119.36, 118.57, 118.21, 117.81, 116.19, 68.17, 67.97, 48.03, 47.42, 47.39, 47.26, 47.14, 47.11, 30.72, 30.04, 29.94, 29.32, 22.43, 22.41, 9.04, 8.98, 8.94, 8.91, 8.87. ESI-TOF HRMS: m/z 424.2604 ($\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_3 + \text{H}^+$ requires 424.2602).

4.1.29. Isopropyl (5-(2-chloroacetyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (75)—

A mixture of compound **73** (5.2 g, 17.55 mmol) and 2-chloroacetyl chloride (2.97 g, 26.32 mmol) in toluene (52 mL) was stirred at 100°C for 3 hours. TLC showed that the reaction was complete. The mixture was concentrated to afford compound **75** (5.20 g, 13.95 mmol, 79.47% yield) as a green solid. LC-MS: MS + 1 = 373.12. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.62-9.59 (d, J = 12.0 Hz, 1H), 7.63-7.11 (m, 7H), 4.91-4.85 (m, 1H), 4.34-4.09 (m, 2H), 3.21-3.02 (m, 2H), 2.77-2.68 (m, 2H), 1.25-1.24 (m, 6H).

4.1.30. Isopropyl (5-(dipropylglycyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (54)—NaH (12 mg, 500 μmol) was added to a mixture of dipropylamine (60 mg, 592 μmol) in DMF (2 mL) at 25°C. The mixture was stirred at 25°C for 0.5 hour. Compound **75** (100 mg, 268 μmol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 25°C for 2.5 hours, and LC-MS showed that starting material **75** was mostly depleted. The reaction was quenched with aqueous NH_4Cl solution. The product was extracted with EtOAc (50 mL \times 3), and the combined organic layer was washed with brine (20 mL \times 2), dried with anhydrous Na_2SO_4 , filtered, and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL), purified by preparative HPLC to give the compound **54** (52 mg, 119 μmol , 44.4% yield, 98.6% purity). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm) 9.68 (s, 1H), 7.61-7.32 (m, 7H), 4.97 (s, 1H), 3.54-3.30 (m, 8H), 2.83 (s, 2H), 1.33 (s, 10H), 0.85 (s, 6H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ (ppm) 170.16, 153.56, 141.76, 141.58, 141.31, 141.20, 138.53, 137.91, 137.55, 135.58, 130.97, 130.73, 130.52, 129.04, 128.78, 128.17, 127.72, 127.53, 126.71, 118.67, 118.43, 117.87, 68.06, 67.91, 56.03, 55.78, 55.46, 55.28, 30.53, 30.28, 29.89, 29.66, 22.41, 20.78, 20.61, 20.52, 12.16, 12.09. ESI-TOF HRMS: m/z 438.2759 ($\text{C}_{26}\text{H}_{35}\text{N}_3\text{O}_3 + \text{H}^+$ requires 438.2758).

4.1.31. Isopropyl (5-(2-(piperidin-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (55)—NaH (12 mg, 500 μmol) was added to a mixture of piperidine (50 mg, 587 μmol) in DMF (2 mL) at 25°C. The reaction was stirred at 25°C for 0.5 hour. Compound **75** (100 mg, 268 μmol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 25°C for 2.5 hours, and LC-MS showed that starting material **75** was mostly depleted. The reaction was quenched with aqueous NH_4Cl solution. The product was extracted with EtOAc (50 mL \times 3), and the combined organic layer was washed with brine (20 mL \times 2), dried with anhydrous Na_2SO_4 , filtered, and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give the compound **55** (83.6 mg, 198 μmol , 73.8% yield, 99.1% purity). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm) 9.62-9.59 (m, 1H), 7.60-7.10 (m, 7H), 4.89-4.86 (m, 1H), 3.34-3.16 (m, 6H), 2.77-2.74 (m, 4H), 1.50 (s, 4H), 1.35 (s, 2H), 1.26-1.24 (dd, $J_1 = 4.0$ Hz, $J_2 = 4.0$ Hz, 6H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ (ppm) 162.76, 153.59, 153.53, 138.46, 137.97, 137.86, 135.55, 131.26, 130.88, 130.50, 128.96, 127.81, 127.49, 126.75, 118.14, 117.62, 68.06, 67.94, 54.12, 36.25, 31.24, 30.54, 30.34, 29.84, 29.65, 25.36, 22.42, 1.63. ESI-TOF HRMS: m/z 422.2452 ($\text{C}_{25}\text{H}_{31}\text{N}_3\text{O}_3 + \text{H}^+$ requires 422.2445).

4.1.32. Isopropyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (56)—NaH (12 mg, 500 μmol) was added to a mixture of pyrrole (40 mg, 596 μmol) in DMF (2 mL) at 25°C. The mixture was stirred at 25°C for 0.5 hour. Compound **75** (100 mg, 268 μmol) in DMF (1 mL) was then added dropwise. The mixture was stirred at 25°C for 2.5 hour, and LC-MS showed that starting material **75** was mostly depleted. The reaction was quenched with aqueous NH_4Cl solution. The product was extracted with EtOAc (50 mL \times 3), and the combined organic layer was washed with brine (20 mL \times 2), dried by anhydrous Na_2SO_4 , filtered, and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL), purified by preparative HPLC to give the compound **56** (50 mg, 124 μmol , 46.3% yield, 99.4% purity). ^1H NMR (400 MHz, $\text{Methanol}-d_4$) δ (ppm) 7.67 (s, 1H), 7.50 (s, 1H), 7.36-7.31 (m, 2H), 7.24 (s, 2H),

7.13-7.11 (d, $J = 8.0$ Hz, 1H), 6.52-6.49 (d, $J = 12.0$ Hz, 2H), 6.03 (s, 2H), 4.76-4.59 (m, 3H), 3.24-3.20 (m, 2H), 2.79-2.77 (d, $J = 8.0$ Hz, 2H), 1.33-1.29 (m, 6H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 167.65, 153.67, 153.52, 140.79, 140.67, 138.74, 138.00, 135.61, 131.43, 130.97, 130.85, 129.34, 129.01, 128.83, 128.22, 128.00, 127.83, 126.80, 122.38, 119.43, 118.12, 108.05, 108.00, 68.14, 67.94, 55.39, 51.03, 50.83, 30.49, 30.23, 29.83, 29.58, 22.42. ESI-TOF HRMS: m/z 404.1978 ($\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_3 + \text{H}^+$ requires 404.1976).

4.1.33. Isopropyl (5-(2-iodoacetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (76)—Sodium iodide (1.21 g, 8.05 mmol) was added to a mixture of compound **75** (3.00 g, 8.05 mmol) in acetone (30 mL) in one portion at 25°C. The mixture was stirred at 25°C for 12 hours, and LC-MS showed that starting material **75** was depleted. The mixture was concentrated, and water (80 mL) was added to the residue. The aqueous phase was extracted with ethyl acetate (200 mL \times 3). The combined organic phase was washed with saturated brine (200 mL \times 2), dried with anhydrous sodium sulfate, filtered, and vacuum concentrated to afford compound **76** (3.00 g, 6.46 mmol, 80.25% yield) as a gray solid. LC-MS: MS + 1 = 465.1.

4.1.34. Isopropyl (5-(2-ethoxyacetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (57)—Ethanol (90 mg, 1.95 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **57** (51 mg, 133 μmol , 20.6% yield, 99.5% purity). ^1H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.08-7.54 (m, 7H), 6.71 (s, 1H), 5.01 (br, s, 1H), 4.16-4.30 (m, 1H), 3.76-3.08 (m, 1H), 3.54 (q, $J = 8.0$ Hz, 2H), 3.35 (br, s, 2H), 2.77-2.82 (m, 2H), 1.30 (d, $J = 8.0$ Hz, 6H), 1.20 (t, $J = 4.0$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 168.73, 153.57, 140.70, 137.94, 130.89, 130.56, 129.15, 128.99, 127.90, 127.65, 126.78, 118.34, 118.06, 68.72, 67.99, 66.40, 30.61, 30.10, 29.95, 29.48, 22.42, 15.40. ESI-TOF HRMS: m/z 383.1970 ($\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4 + \text{H}^+$ requires 383.1973).

4.1.35. Isopropyl (5-(2-isopropoxyacetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (58)—Isopropanol (117 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL \times 3) and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **58** (52 mg, 131 μmol , 20.3% yield, 99.1% purity). ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.58 (s, 1H), 7.52-7.11 (m, 7H), 4.91-4.86 (m, 1H), 4.07-3.78 (m, 3H),

3.24 (m, 2H), 2.75 (m, 2H), 1.26-1.24 (d, $J = 8.0$ Hz, 6H), 0.99-0.98(d, $J = 4.0$ Hz, 6H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 168.92, 153.56, 140.82, 137.90, 130.86, 130.66, 130.46, 129.01, 127.84, 127.57, 126.74, 118.32, 118.02, 71.76, 67.97, 66.61, 33.40, 30.56, 30.13, 29.92, 29.52, 22.42, 22.07. ESI-TOF HRMS: m/z 397.2129 ($\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_4 + \text{H}^+$ requires 397.2129).

4.1.36. Isopropyl (5-(2-(sec-butoxy)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (59)—2-Butanol (144 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 h. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **59** (17 mg, 41.4 μmol , 6.4% yield, 95.0% purity). ^1H NMR (400 MHz, chloroform- d) δ (ppm) 7.62-6.96 (m, 6H), 6.66 (br. s., 1H), 5.10-4.88 (m, 1H), 4.39-3.65 (m, 2H), 3.54-3.18 (m, 3H), 2.93-2.66 (m, 2H), 1.61-1.48 (m, 1H), 1.46-1.34 (m, 1H), 1.30 (d, $J = 6.3$ Hz, 6H), 1.07 (br. s., 3H), 0.87 (t, $J = 7.5$ Hz, 3H). ESI-TOF HRMS: m/z 411.2287 ($\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_4 + \text{H}^+$ requires 411.2286).

4.1.37. isopropyl (5-(2-(neopentyloxy)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (60)—2,2-Dimethyl-1-Propanol (171 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL), purified by preparative HPLC to give compound **60** (102.8 mg, 242 μmol , 37.5% yield, 99.4% purity). ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.59 (s, 1H), 7.53 (s, 1H), 7.45-7.10 (m, 6H), 4.91-4.85 (m, 1H), 4.19-4.16 (d, $J = 12.0$ Hz, 1H), 3.83-3.72 (m, 1H), 3.23 (s, 2H), 2.80-2.75(m, 2 H), 1.26-1.25 (d, $J = 4.0$ Hz, 6H), 0.82 (s, 9H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 168.70, 153.57, 140.85, 140.66, 137.88, 131.18, 130.88, 130.55, 129.08, 128.87, 128.78, 127.88, 127.64, 127.03, 126.73, 118.38, 118.02, 81.56, 69.73, 67.96, 32.22, 30.63, 30.54, 30.17, 29.99, 29.56, 27.41, 26.96, 22.42. ESI-TOF HRMS: m/z 425.2451 ($\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_4 + \text{H}^+$ requires 425.2442).

4.1.38. Isopropyl (5-(2-cyclopropoxyacetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (61)—Cyclopropanol (113 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium

chloride solution, extracted with ethyl acetate (20 mL × 3), and washed with brine (20 mL × 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **61** (82 mg, 208 μmol, 32.2% yield, 98.6% purity). ¹H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.38–7.86 (m, 7H), 5.32 (br, s, 1H), 4.51–4.65 (m, 1H), 4.13–4.24 (m, 1H), 3.68–3.84 (m, 4H), 3.08–3.15 (m, 2H), 1.61 (d, *J* = 4.0 Hz, 6H), 0.88 (br, s, 2H), 0.74–0.75 (m, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 168.34, 153.56, 140.90, 140.77, 140.59, 137.94, 135.48, 131.21, 130.97, 130.75, 130.55, 129.25, 128.99, 128.83, 127.92, 127.71, 126.78, 119.09, 118.30, 118.08, 117.55, 68.82, 68.11, 67.95, 53.75, 30.61, 30.02, 29.95, 29.39, 22.41, 5.79, 5.76. ESI-TOF HRMS: *m/z* 395.1974 (C₂₃H₂₆N₂O₄ + H⁺ requires 395.1973).

4.1.39. Isopropyl (5-(2-cyclobutoxyacetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (62)—Cyclobutanol (140 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL × 3) and washed with brine (20 mL × 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **62** (52 mg, 127 μmol, 19.7% yield, 98.5% purity). ¹H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.08–7.52 (m, 7H), 6.69 (s, 1H), 5.01 (br, s, 1H), 3.69–4.18 (m, 3H), 2.07 (br, s, 2H), 2.77–2.82 (m, 2H), 2.14–2.23 (m, 2H), 1.90 (t, *J* = 8.0 Hz, 2H), 1.61–1.69 (m, 1H), 1.37–1.49 (m, 1H), 1.30 (d, *J* = 8.0 Hz, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 168.49, 153.56, 140.96, 140.74, 137.92, 131.18, 130.92, 130.70, 130.52, 129.16, 128.96, 127.90, 127.64, 126.76, 118.29, 118.05, 73.12, 67.96, 65.97, 65.93, 30.60, 30.13, 30.00, 29.43, 22.41, 22.32, 12.36. ESI-TOF HRMS: *m/z* 409.2138 (C₂₄H₂₈N₂O₄ + H⁺ requires 409.2129).

4.1.40. Isopropyl (5-(2-(cyclopentylloxy)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (63)—Cyclopentanol (167 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μmol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL × 3), and washed with brine (20 mL × 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **63** (80.5 mg, 190.6 μmol, 29.5% yield, 99.3% purity). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.59 (s, 1H), 7.52–7.10 (m, 7H), 4.91–4.85 (m, 1H), 3.99 (s, 1H), 3.78 (s, 2H), 3.24–3.18 (m, 2H), 2.75 (m, 2H), 1.51–1.41 (d, *J* = 40.0 Hz, 8H), 1.26–1.24 (d, *J* = 8.0 Hz, 6H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 168.70, 153.56, 141.20, 141.03, 140.76, 138.53, 137.92, 131.19, 130.89, 130.62, 130.43, 129.01, 127.83, 127.55, 126.74,

118.93, 118.33, 118.03, 117.55, 81.62, 67.97, 67.38, 31.93, 30.57, 30.12, 29.92, 29.50, 23.48, 22.41. ESI-TOF HRMS: m/z 423.2288 ($C_{25}H_{30}N_2O_4 + H^+$ requires 423.2286).

4.1.41. Isopropyl (5-(2-(cyclohexyloxy)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (64)—Cyclohexanol (194 mg, 1.94 mmol) was added to NaHMDS (1 M, 2.07 mL, 2.07 mmol) at 0°C under nitrogen. The mixture was stirred at 0°C for 0.5 hour. Compound **76** (300 mg, 646 μ mol) in THF (3 mL) was then added dropwise. The mixture was stirred at 25°C for 1 hour. TLC showed that starting material **76** was depleted. The reaction was quenched with aqueous saturated ammonium chloride solution, extracted with ethyl acetate (20 mL \times 3), and washed with brine (20 mL \times 2). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a residue. The residue was dissolved in acetonitrile (3 mL) and purified by preparative HPLC to give compound **64** (28 mg, 64.2 μ mol, 9.9% yield, 99.3% purity). 1H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.09–7.52 (m, 7H), 6.60 (s, 1H), 5.01 (d, J = 4.0 Hz, 1H), 3.84–4.25 (m, 2H), 3.28–3.30 (m, 3H), 2.79–2.82 (m, 2H), 1.86–1.88 (m, 3H), 1.68 (br, s, 2H), 1.51 (br, s, 1H), 1.17–1.31 (m, 10H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 168.99, 153.56, 141.14, 140.94, 140.77, 137.90, 131.17, 130.88, 130.65, 130.45, 129.02, 127.84, 127.58, 126.74, 118.34, 118.01, 77.34, 67.95, 66.41, 31.78, 30.58, 30.14, 29.93, 29.52, 25.73, 23.75, 22.41. ESI-TOF HRMS: m/z 437.2445 ($C_{26}H_{32}N_2O_4 + H^+$ requires 437.2442).

4.1.42. Benzyl (10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (77)

—Benzyl chloroformate (16.23 g, 95.12 mmol) was added dropwise to a solution of compound **46** (10 g, 47.56 mmol) in EtOH (100 mL) at 0°C over a period of 1 hour. A solution of Na_2CO_3 (5.04 g, 47.56 mmol) in H_2O (50 mL) was then added dropwise while the reaction temperature was maintained below 15°C. The reaction was stirred for another hour until TLC demonstrated that starting material **46** was depleted. The reaction solution was poured into water (500 mL) and filtered to give the crude product. The crude product was triturated with petroleum ether/ethyl acetate = 100/1, filtered, and concentrated to give compound **77** (15.00 g, crude) as a white solid. 1H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 2.91 (d, J = 5.77 Hz, 4 H), 5.15 (s, 2 H), 6.61 - 6.71 (m, 2 H), 6.87 (d, J = 8.03 Hz, 1 H), 6.95 - 7.03 (m, 2 H), 7.25 (br. s., 1 H), 7.31 - 7.49 (m, 6 H), 8.30 (s, 1 H), 9.57 (s, 1 H).

4.1.43. Benzyl (5-(2-chloroacetyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (78)

—A mixture of compound **77** (15 g, 43.55 mmol) and 2-chloroacetyl chloride (7.38 g, 65.33 mmol) in toluene (150 mL) was stirred at 110°C for 3 hours, and LCMS showed that compound **77** was depleted. The mixture was concentrated, and the residue was purified by re-crystallization with petroleum ether and EtOAc (500 mL, 10/1), filtered, and concentrated to give compound **78** (14.00 g, crude) as a white solid. LCMS: MS + 1 = 420.12.

4.1.44. *tert*-Butyl (5-(dipropylglycyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepin-3-yl)carbamate (65)

—A mixture of compound **78** (500 mg, 1.19 mmol) and dipropylamine (120 mg, 1.19 mmol) in toluene (1.8 mL) was stirred at 110°C for 6 hours, and LCMS showed that starting material **78** was depleted. The mixture was concentrated and purified by pre-HPLC (FA) to give compound **79**. Then, 10% Pd/C (65.00 mg) was added to the

solution of compound **79** that was just prepared in MeOH (5 mL), and the mixture was stirred at room temperature under H₂ (15psi) for 12 hours. TLC showed that compound **79** was depleted. (Boc)₂O (260 mg, 1.19 mmol) was then added at 25°C, and the mixture was stirred at 25°C for 3 hours; LCMS showed that the reaction was complete. The mixture was filtered and concentrated to give crude product that was further purified by preparative HPLC to give compound **65** (116 mg, 257 μmol, 21.6% yield, 99.5% purity). ¹H NMR (400 MHz, chloroform-*d*) δ (ppm) 0.826 (t, *J* = 7.2 Hz, 6 H), 1.36 (s, 4 H), 1.51 (s, 9 H), 2.53–2.55 (m, 3 H), 2.74–2.80 (m, 2 H), 3.11–3.45 (m, 4 H), 6.47 (s, 1 H), 7.06–7.49 (m, 7 H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 170.16, 153.18, 153.15, 141.77, 141.55, 141.32, 141.19, 138.75, 138.13, 137.51, 135.57, 130.73, 130.62, 130.60, 129.08, 128.74, 128.20, 127.70, 127.51, 126.69, 118.66, 118.31, 117.88, 117.73, 79.68, 79.48, 56.04, 55.45, 55.23, 30.58, 30.31, 29.87, 29.64, 28.57, 20.63, 20.53, 12.16. ESI-TOF HRMS: *m/z* 452.2914 (C₂₇H₃₇N₃O₃ + H⁺ requires 452.2915).

4.1.45. *tert*-Butyl (5-(2-(piperidin-1-yl)acetyl)-10,11-dihydro-5*H*-

dibenzo[*b,f*]azepin-3-yl)carbamate (66)—A mixture of compound **78** (500 mg, 1.19 mmol) and piperidine (101 mg, 1.19 mmol) in toluene (1.8 mL) was stirred at 110°C for 6 hours, and LCMS showed that starting material compound **78** was depleted. The mixture was concentrated and purified by pre-HPLC (FA) to give compound **80**. Then, 10% Pd/C (65.00 mg) was added to the solution of compound **80** that was just prepared in MeOH (5 mL), and the mixture was stirred at room temperature under H₂ (15psi) for 12 hours. TLC showed that compound **80** was depleted. (Boc)₂O (260 mg, 1.19 mmol) was then added at 25°C, and the mixture was stirred at 25°C for 3 hours; LCMS showed that the reaction was complete. The mixture was filtered and concentrated to give crude product that was further purified by preparative HPLC to give compound **66** (100 mg, 229.7 μmol, 19.3% yield, 99.6% purity). ¹H NMR (400 MHz, chloroform-*d*) δ (ppm) 1.30 - 1.91 (m, 15 H), 2.11 - 2.90 (m, 5 H), 2.96 - 3.73 (m, 4 H), 6.28 - 6.58 (m, 1 H), 6.95 - 7.69 (m, 7 H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 169.23, 153.16, 141.20, 141.04, 138.58, 138.13, 137.83, 135.58, 131.01, 130.82, 130.67, 130.28, 129.08, 128.80, 128.64, 127.83, 127.72, 127.33, 126.68, 118.57, 118.31, 117.92, 117.56, 79.65, 79.49, 60.80, 54.28, 30.59, 30.44, 29.84, 29.68, 28.58, 25.86, 23.94. ESI-TOF HRMS: *m/z* 436.2604 (C₂₆H₃₃N₃O₃ + H⁺ requires 436.2602).

4.1.46. *tert*-Butyl (5-(2-isopropoxyacetyl)-10,11-dihydro-5*H*-

dibenzo[*b,f*]azepin-3-yl)carbamate (67)—Na (0.5 g, 21.7 mmol) was added to 2-propanol (20 mL), and the reaction mixture was heated at 90°C until Na was completely consumed. The reaction mixture (6 mL) was then added to a solution of compound **78** (2.0 g, 4.75 mmol) in 2-propanol (20 mL) at 60°C. After 30 min, TLC showed that compound **78** was depleted, and the desired compound **81** was detected by LCMS. Water (20 mL) was added, and the reaction mixture was extracted with EtOAc (20 mL × 3). The combined organic phase was washed with brine, dried with anhydrous Na₂SO₄, and concentrated to give a brown oil that was purified by prep-HPLC (TFA) to give compound **81** (400 mg, 18.9 % yield) as a white solid. A mixture of compound **81** (400 mg, 0.90 mmol), (Boc)₂O (237 mg, 1.09 mmol), and 10% Pd/C (40 mg) in MeOH (10 mL) was stirred under H₂ atmosphere (15 psi) at 25°C for 2 hours. TLC showed that compound **81** was depleted, and the desired

compound **67** was detected by LCMS. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated to give a crude product that was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 10/1 to 3/1) to afford compound **67** (103 mg, 27.9 % yield, 95.6% purity) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) 9.39 (br. s., 1 H), 7.02–7.67 (m, 6 H), 3.97–4.21 (m, 1 H), 3.70–3.89 (m, 1 H), 3.38–3.52 (m, 1 H), 3.21 (d, *J* = 11.80 Hz, 2 H), 2.75 (br. s., 2 H), 1.47 (s, 9 H), 0.99 (d, *J* = 5.52 Hz, 6 H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 170.80, 168.90, 153.16, 141.14, 140.78, 138.14, 137.87, 130.80, 130.54, 129.03, 128.63, 128.14, 127.99, 127.84, 127.56, 126.72, 118.92, 118.21, 118.00, 117.53, 79.54, 71.74, 71.65, 66.64, 60.22, 30.61, 30.15, 29.92, 29.50, 28.58, 22.42, 22.07, 21.24, 14.56. ESI-TOF HRMS: *m/z* 411.2289 (C₂₄H₃₀N₂O₄ + H⁺ requires 411.2286).

4.1.47. Benzyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (82)—NaH (836.4 mg, 20.91 mmol) was added to a mixture of pyrrole (1.4 g, 20.91 mmol) in DMF (30 mL) in portions at 0°C under N₂. The mixture was stirred at 0°C for 1 hour, then a solution of compound **78** (8.0 g, 19.01 mmol) in DMF (70 mL) was added dropwise at 0°C. The mixture was allowed to warm to room temperature (25°C) and stirred for 12 hours. LCMS showed that the reaction was complete. The mixture was diluted with water and extracted with EtOAc (50 mL \times 3). The combined organic layers were washed twice with water, dried with anhydrous Na₂SO₄, and concentrated to afford crude product that was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 4/1 to 3/2) to afford compound **82** (2.30 g, 5.1 mmol, 26.8% yield) as a yellow solid. LCMS: MS + 1 = 452.1. ¹H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.63-7.01 (m, 11H), 6.81 (d, *J* = 17.7 Hz, 1H), 6.47 (d, *J* = 8.9 Hz, 2H), 6.12 (t, *J* = 2.0 Hz, 2H), 5.21 (d, *J* = 16.9 Hz, 2H), 4.74-4.41 (m, 2H), 3.26-3.07 (m, 2H), 2.86-2.65 (m, 2H).

4.1.48. 1-(3-Amino-10,11-dihydro-5H-dibenzo[*b,f*]azepin-5-yl)-2-(1H-pyrrol-1-yl)ethan-1-one (83)—First, 10% Pd/C (200.00 mg) was added to a solution of compound **82** (2.30 g, 5.1 mmol) in EtOAc (50 mL) at 25°C. The mixture was degassed under vacuum and purged with H₂. The mixture was then stirred under H₂ (15 psi) for 4 hours. TLC (Petroleum ether/Ethyl acetate = 2/1) showed that the reaction was complete. After the solution was filtered over celite pad, the filtrate was concentrated to afford compound **83** (1.20 g, crude) as a yellow solid. LCMS: MS+1 = 318.1.

4.1.49. *tert*-Butyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-

dibenzo[*b,f*]azepin-3-yl)carbamate (68)—A solution of *tert*-butoxycarbonyl chloride (65 mg, 475 μ mol) in THF (0.5 mL) was added dropwise to a solution of compound **83** (150 mg, 475 μ mol) in THF (0.5 mL) at 0°C. A solution of Et₃N (48 mg, 475 μ mol) in THF (1 mL) was then added dropwise at 0°C. The mixture was allowed to warm to room temperature (25°C) and stirred for 2 hours. The mixture was filtered, and the filtrate was concentrated. The residue was purified by preparative TLC (petroleum ether/ethyl acetate = 2/1) to give compound **68** (103 mg, 247 μ mol, 52.0% yield, 99.4% purity) as a white solid. ¹H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.64-6.98 (m, 6H), 6.62-6.37 (m, 3H), 6.12 (s, 2H), 4.75-4.51 (m, 2H), 3.24-3.08 (m, 2H), 2.82-2.67 (m, 2H), 1.53 (d, *J* = 12.4 Hz,

9H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 167.63, 153.24, 153.12, 140.83, 140.77, 140.63, 138.97, 138.20, 138.00, 135.57, 131.24, 130.84, 130.76, 129.31, 128.92, 128.87, 128.74, 128.23, 127.98, 127.82, 126.78, 122.38, 119.41, 118.11, 117.98, 108.04, 107.98, 79.77, 79.52, 51.04, 50.82, 30.55, 30.24, 29.83, 29.53, 28.59. ESI-TOF HRMS: m/z 440.1960 ($\text{C}_{25}\text{H}_{27}\text{N}_3\text{O}_3 + \text{Na}^+$ requires 440.1952).

4.1.50. Cyclopropyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (69)—A solution of cyclopropyl chloroformate (58 mg, 475 μmol) in THF (0.5 mL) was added dropwise to a solution of compound **83** (150 mg, 475 μmol) in THF (0.5 mL) at 0°C. A solution of Et_3N (48 mg, 475 μmol) in THF (1 mL) was then added dropwise at 0°C. The mixture was allowed to warm to room temperature (25°C) and stirred for 2 hours. The mixture was filtered, and the filtrate was concentrated. The residue was purified by preparative TLC (petroleum ether/ethyl acetate = 2/1) to give compound **69** (58 mg, 144.5 μmol , 30.4% yield, 95.1% purity) as a white solid. ^1H NMR (400 MHz, chloroform- d) δ (ppm) 7.69 - 6.98 (m, 6H), 6.62 (br. s., 1H), 6.48 (d, $J=7.5$ Hz, 2H), 6.12 (s, 2H), 4.71-4.51 (m, 2H), 4.21-4.06 (m, 1H), 3.23-3.09 (m, 2H), 2.81-2.66 (m, 2H), 0.76 (d, $J=12.4$ Hz, 4H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 167.65, 154.43, 154.25, 140.77, 138.41, 138.03, 137.64, 135.62, 131.68, 130.99, 130.83, 129.36, 129.26, 128.82, 128.20, 128.02, 127.85, 126.81, 122.38, 119.50, 118.20, 108.05, 108.00, 55.39, 51.02, 50.85, 49.36, 49.23, 30.45, 30.19, 29.85, 29.60, 5.28. ESI-TOF HRMS: m/z 402.1821 ($\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_3 + \text{H}^+$ requires 402.1819).

4.1.51. Cyclobutyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (70)—A solution of cyclobutyl chloroformate (64 mg, 475 μmol) in THF (0.5 mL) was added dropwise to a solution of compound **83** (150 mg, 475 μmol) in THF (0.5 mL) at 0°C. A solution of Et_3N (48 mg, 475 μmol) in THF (1 mL) was then added dropwise at 0°C. The mixture was allowed to warm to room temperature (25°C) and stirred for 2 hours. The mixture was filtered, and the filtrate was concentrated. The residue was purified by preparative TLC (petroleum ether/ethyl acetate = 2/1) to give compound **70** (65 mg, 156.5 μmol , 32.9% yield, 96.1% purity) as a white solid. ^1H NMR (400 MHz, chloroform- d) δ (ppm) 7.62-6.95 (m, 6H), 6.68-6.40 (m, 3H), 6.19-6.04 (m, 2H), 5.16-4.94 (m, 1H), 4.78-4.47 (m, 2H), 3.24-3.07 (m, 2H), 2.84-2.65 (m, 2H), 2.40 (br. s., 2H), 2.22-2.05 (m, 2H), 1.92-1.76 (m, 1H), 1.73-1.63 (m, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) 167.64, 153.29, 153.11, 140.77, 138.56, 138.02, 137.79, 135.60, 131.57, 130.94, 130.83, 129.35, 129.10, 128.92, 128.83, 128.21, 128.00, 127.84, 126.80, 122.38, 118.13, 108.04, 107.99, 68.60, 51.02, 50.84, 30.51, 30.20, 29.85, 29.58, 13.40. ESI-TOF HRMS: m/z 416.1978 ($\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}_3 + \text{H}^+$ requires 416.1976).

4.1.52. Cyclopentyl (5-(2-(1H-pyrrol-1-yl)acetyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate (71)—A solution of cyclopentyl chloroformate (71 mg, 475 μmol) in THF (0.5 mL) was added dropwise to a solution of compound **83** (150 mg, 475 μmol) in THF (0.5 mL) at 0°C. A solution of Et_3N (48 mg, 475 μmol) in THF (1 mL) was then added dropwise at 0°C. The mixture was allowed to warm to room temperature (25°C) and stirred for 2 hours. The mixture was filtered, and the filtrate was concentrated. The residue was purified by preparative TLC (petroleum ether/ethyl acetate = 2/1) to give

compound **71** (87 mg, 202.7 μmol , 42.7% yield, 97.8% purity) as a white solid. ^1H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.72-6.97 (m, 6H), 6.69-6.40 (m, 3H), 6.12 (s, 2H), 5.21 (d, $J = 15.8$ Hz, 1H), 4.79-4.47 (m, 2H), 3.27-3.07 (m, 2H), 2.82-2.64 (m, 2H), 2.04-1.64 (m, 8H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 167.64, 153.88, 153.74, 140.79, 138.73, 138.01, 135.61, 131.41, 130.95, 130.84, 129.33, 128.98, 128.92, 128.82, 128.20, 128.00, 127.83, 126.80, 122.37, 119.42, 118.10, 108.05, 107.99, 77.37, 77.21, 55.39, 51.03, 50.83, 32.77, 30.49, 30.22, 29.83, 29.58, 23.73. ESI-TOF HRMS: m/z 430.2137 ($\text{C}_{26}\text{H}_{27}\text{N}_3\text{O}_3 + \text{H}^+$ requires 430.2132).

4.1.53. Cyclohexyl (5-(2-(1*H*-pyrrol-1-yl)acetyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-3-yl)carbamate (72**)**—

A solution of cyclohexyl chloroformate (77 mg, 475 μmol) in THF (0.5 mL) was added dropwise to a solution of compound **83** (150 mg, 475 μmol) in THF (0.5 mL) at 0°C. A solution of Et₃N (48 mg, 475 μmol) in THF (1 mL) was then added dropwise at 0°C. The mixture was allowed to warm to room temperature (25°C) and stirred for 2 hours. The mixture was filtered, and the filtrate was concentrated. The residue was purified by preparative TLC (petroleum ether/ethyl acetate = 2/1) to give compound **72** (134 mg, 302.3 μmol , 63.6% yield, 98.0% purity) as a white solid. ^1H NMR (400 MHz, chloroform-*d*) δ (ppm) 7.67-6.95 (m, 6H), 6.67-6.39 (m, 3H), 6.12 (s, 2H), 4.82-4.49 (m, 3H), 3.27-3.03 (m, 2H), 2.85-2.63 (m, 2H), 2.01-1.86 (m, 2H), 1.83-1.71 (m, 2H), 1.55-1.28 (m, 6H). ^{13}C NMR (126 MHz, DMSO-*d*₆) δ (ppm) 165.25, 151.21, 151.06, 138.39, 138.32, 136.34, 135.64, 135.58, 133.24, 129.02, 128.59, 128.51, 128.45, 126.95, 126.61, 126.41, 125.81, 125.62, 125.44, 124.41, 119.98, 117.06, 115.76, 105.66, 105.61, 70.59, 70.43, 48.63, 48.45, 29.67, 28.09, 27.83, 27.45, 27.20, 22.98, 21.49. ESI-TOF HRMS: m/z 444.2290 ($\text{C}_{27}\text{H}_{29}\text{N}_3\text{O}_3 + \text{H}^+$ requires 444.2289).

4.2. Biology

Materials: Tb-anti-GST antibody, GST-hCAR-LBD, Fluorescein-PGC1 α coactivator peptide, TR-FRET coregulator buffer G, and 1 M DTT were purchased from Invitrogen (Carlsbad, CA, USA). Clotrimazole was purchased from Sigma (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Black 384-well low-volume assay plates were purchased from Corning (Tewksbury, MA, USA). Chemicals **10**, **13**, **14**, **15**, **19**, and **20** were purchased from ChemDiv (San Diego, CA, USA). Chemicals **11**, **12**, **16**, **17**, and **18** were purchased from ChemBridge (San Diego, CA, USA).

4.2.1. CAR-mediated fluorescent PGC1 α coactivator recruitment/repression assay

—In black 384-well low-volume assay plates, titrations of chemicals (final concentrations in assay: clotrimazole, 1-to-3 dilutions from 42 μM to 0.71 nM for 11 concentration levels; other compounds, 1-to-3 dilutions from 70 μM to 1.18 nM for 11 concentration levels; DMSO, 0.7%) were mixed with fluorescein-PGC1 α coactivator peptide (125 nM), GST-hCAR-LBD (5 nM), and Tb-anti-GST antibody (5 nM) in TR-FRET coregulator buffer G supplemented with 5 mM DTT at 20 μl per well. In addition, DMSO (final concentration: 0.7%) and clotrimazole (final concentration: 42 μM with 0.7% DMSO) were included in each plate and served as negative control (0% Inhibition) and positive control (100% Inhibition), respectively. The assay plates were then briefly spun down and

incubated for 1 hour at room temperature (25°C). The TR-FRET signals for individual wells were collected by using a PHERAstar *FS* plate reader (BMG Labtech; Durham, NC, USA) with a 340-nm excitation filter, 100- μ s delay time, and 200- μ s integration time to measure the fluorescence emission ratio (10,000 \times 520 nm/490 nm). The data were then normalized to positive control (42 μ M clotrimazole, 100% inhibition) and negative control (DMSO, 0% inhibition) values by using Equation 1 to derive the %Inhibition for individual chemicals at respective concentrations.

$$\% \text{Inhibition} = 100\% - 100\% \times \frac{(\text{signal}_{\text{Chemical}} - \text{Signal}_{42 \mu\text{M clotrimazole}})}{(\text{Signal}_{\text{DMSO}} - \text{Signal}_{42 \mu\text{M Clotrimazole}})} \quad \text{Equation 1}$$

Where applicable, the data were fit into a sigmoidal dose-response equation to derive IC₅₀ values by using the graphic software GraphPad Prism 5.04 (GraphPad Software, La Jolla, CA, USA).

4.3. Molecular Modeling

The hCAR-LBD protein crystal structure was obtained from the RCSB Protein Data Bank (<http://www.rcsb.org>) in PDB format (PDB code 1XVP) [52]. All water molecules and ligand from the protein structure were removed using the molecular graphics systems Pymol (<http://www.pymol.org>). The 3D-ligand structures with energy-minimization were generated in ChemBio3D (CambridgeSoft Corporation). The PDBQT files for docking (protein and ligands) were created in AutoDockTools (ADT) version 1.5.6 (<http://mgltools.scripps.edu>). The protein search space with XYZ dimensions 22 Å \times 22 Å \times 22 Å was centered at coordinates 34.231 (x), 59.568 (y), 78.066 (z). Docking of the ligands to hCAR-LBD was carried out with the program AutoDock Vina version 1.1.1 [53], with the exhaustiveness value set to 300. The docking results were analyzed and the figures created in Pymol with assistance of Ligplot+ [54].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CAR	constitutive androstane receptor
PXR	pregnane X receptor
NR	nuclear receptor
CYP2Bs	cytochrome P450, family 2, subfamily B proteins

CYP2Cs	cytochrome P450, family 2, subfamily C proteins
CYP3As	cytochrome P450, family 3, subfamily A proteins
MDR1	multi-drug resistance protein 1
MRPs	multi-drug resistance-associated proteins
TCPOBOP	1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene
DBD	DNA-binding domain
LBD	ligand-binding domain
PK11195	1-(2-Chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide
IC₅₀	half maximal inhibitory concentration
K_i	binding affinity of an inhibitor
EE2	17 α -ethynyl-3,17 β -estradiol
S07662	N-[(2-Methyl-3-benzofuranyl)methyl]-N'-(2-thienylmethyl)urea
TO901317 (T0901317)	N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-benzenesulfonamide
LY2090314	3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
AITC	allyl isothiocyanate
CINPA1	ethyl (5-(diethylglycyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl)carbamate
T₃P	propylphosphonic anhydride
DMF	dimethylformamide
NaHMDS	sodium bis(trimethylsilyl)amide
THF	tetrahydrofuran
SRC1	nuclear receptor coactivator 1
PGC1α	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
SAR	structure-activity relationship
DMSO	dimethyl sulfoxide
GST	glutathione S-transferase
TR-FRET	time-resolved fluorescence resonance energy transfer

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Highlights

- We study a novel scaffold in CINPA1 to discover better CAR inverse agonists
- We have obtained, or designed and synthesized 54 analogs of CINPA1
- We have evaluated the CAR inverse agonistic activity of all analogs
- Chemical **72** is the most potent CAR inverse agonist so far ($IC_{50} = 11.7$ nM)
- This is the first chemical endeavor in systematically exploring CAR inverse agonism

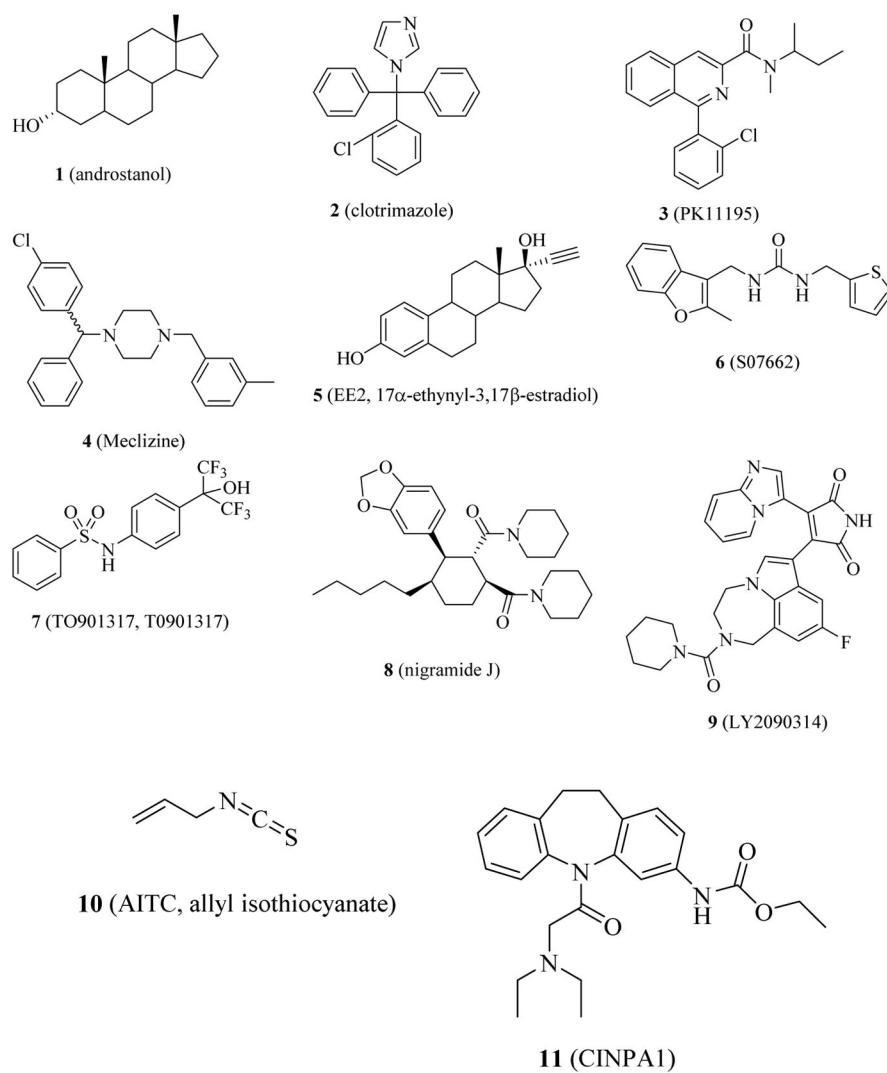


Figure 1.
Chemical structures of a panel of representative CAR inverse agonists

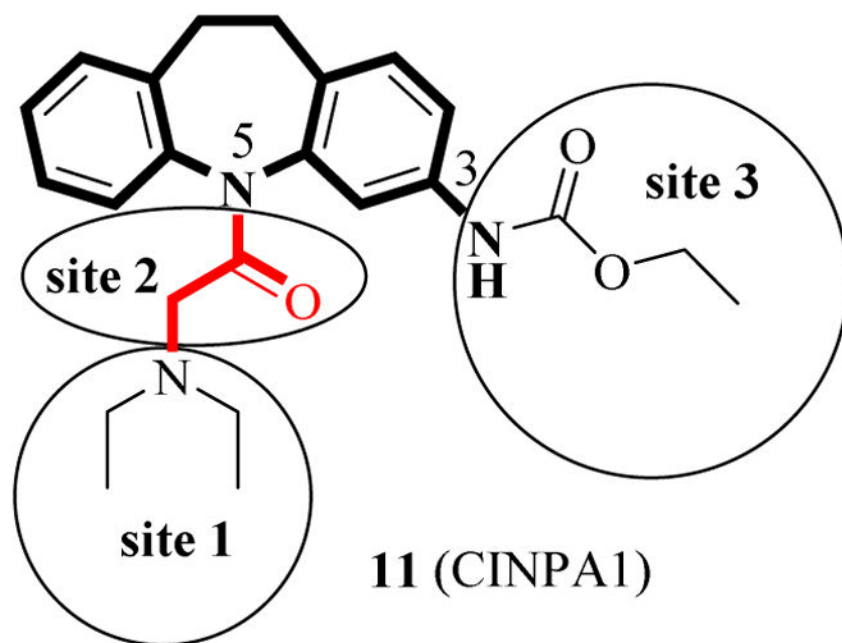


Figure 2.
Structural features of **11** (CINPA1)

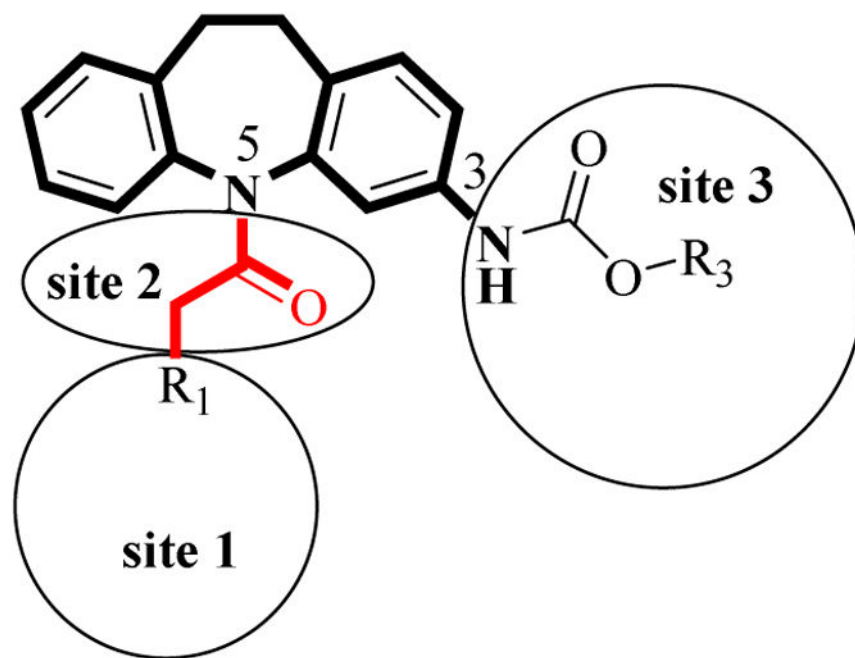


Figure 3.
Structural features of CINPA1 analogs as potent CAR inverse agonists

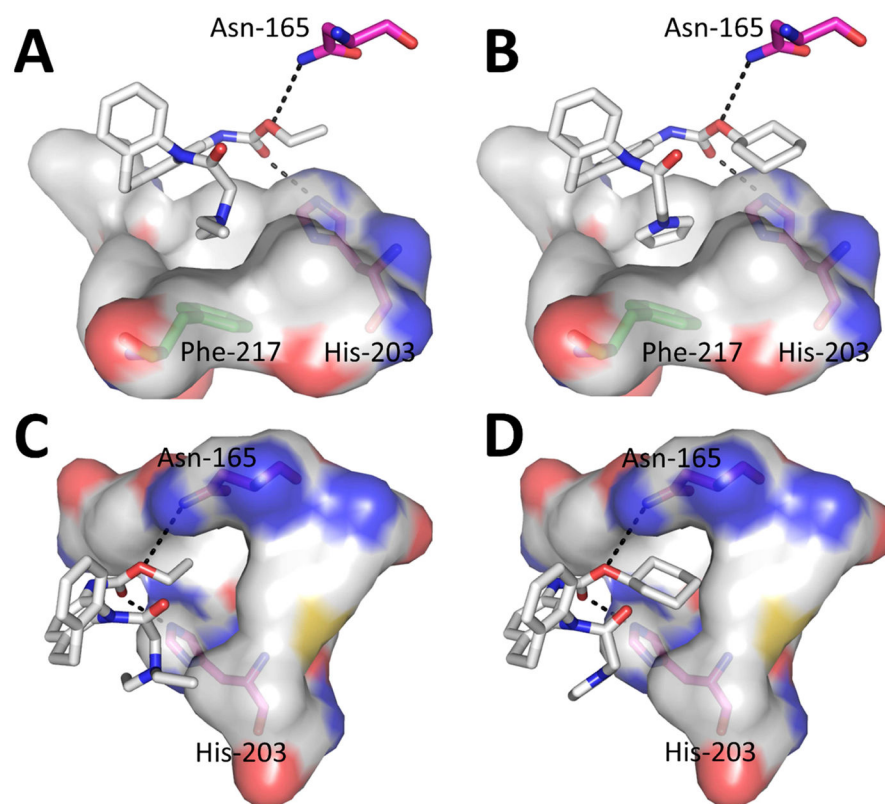
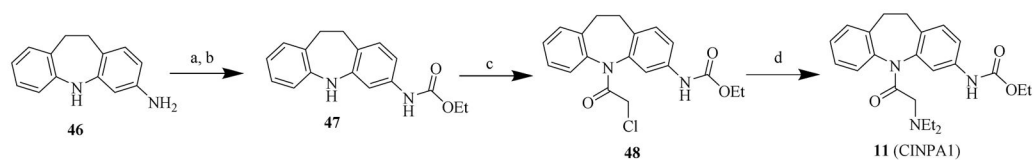
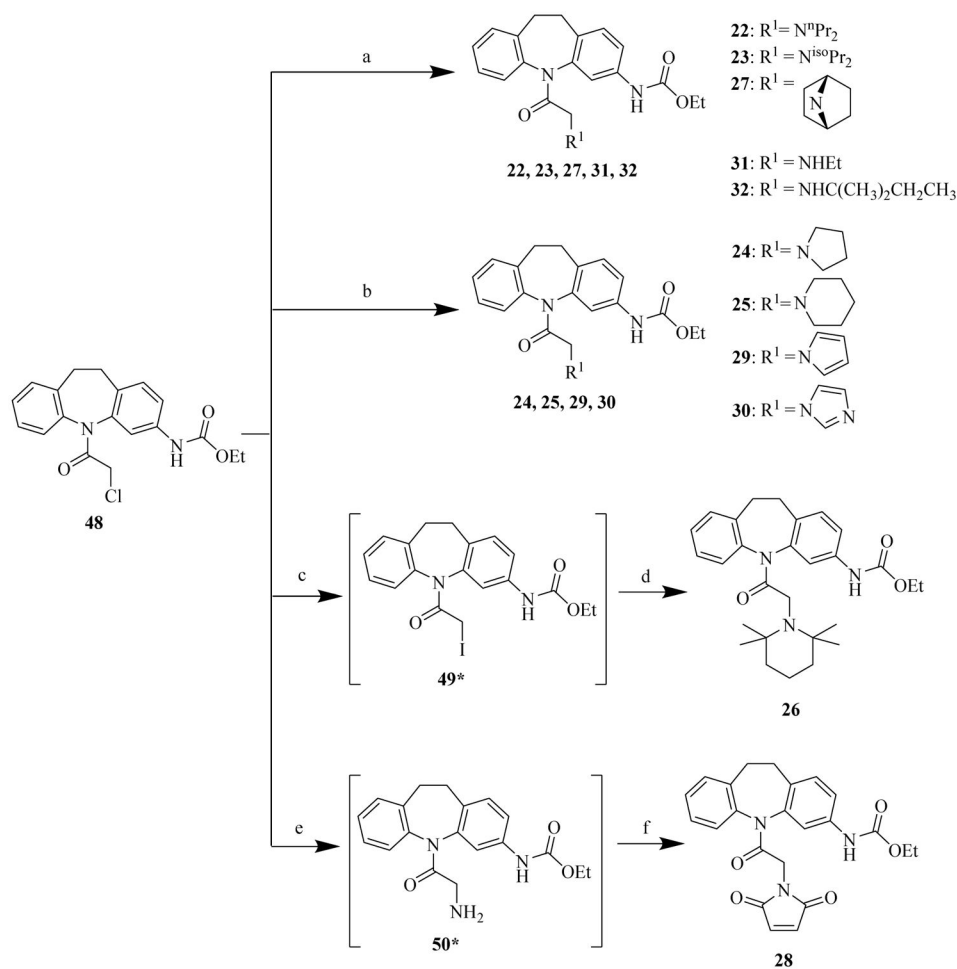


Figure 4. Docking of CINPA1 (**11**) and compound **72** to hCAR-LBD depicting interactions of site 1 of compound **11** (A), site 1 of compound **72** (B), site 3 of compound **11** (C) and site 3 of compound **72** (D). The ligands are illustrated as sticks surrounded by protein residues (surface representation). Carbon, oxygen, nitrogen and sulfur atoms are shown in grey, red, blue and yellow, respectively. The carbon atoms of Asn-165 and His-203 are represented in pink, while carbon atoms of Phe-217 are shown in green. Potential hydrogen bonding is indicated with dash lines.

**Scheme 1. Syntheses of intermediate chemicals 46, 47, 48, and 11**

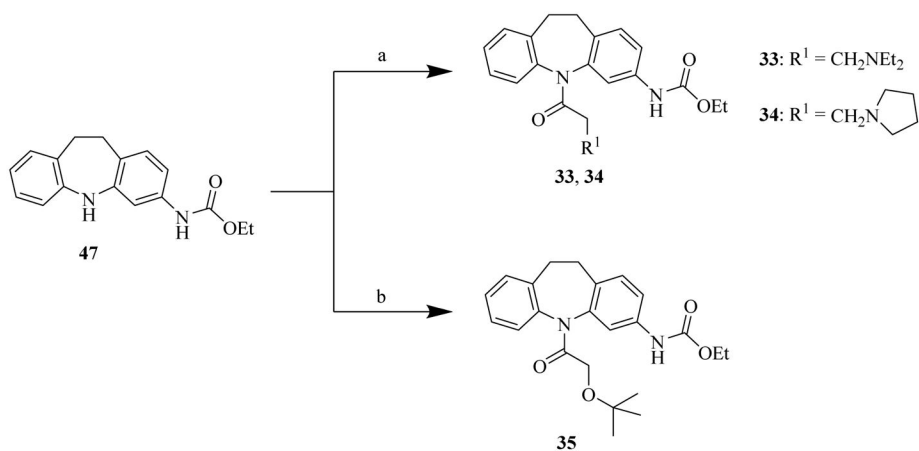
Reagents and conditions: (a) ethyl chloroformate, EtOH, 5°C to 7°C; (b): Na₂CO₃/H₂O, 2 h; (c): 2-chloroacetyl chloride, anhydrous toluene, reflux, 1 h; (d): Et₂NH, anhydrous toluene, reflux, 4 h.



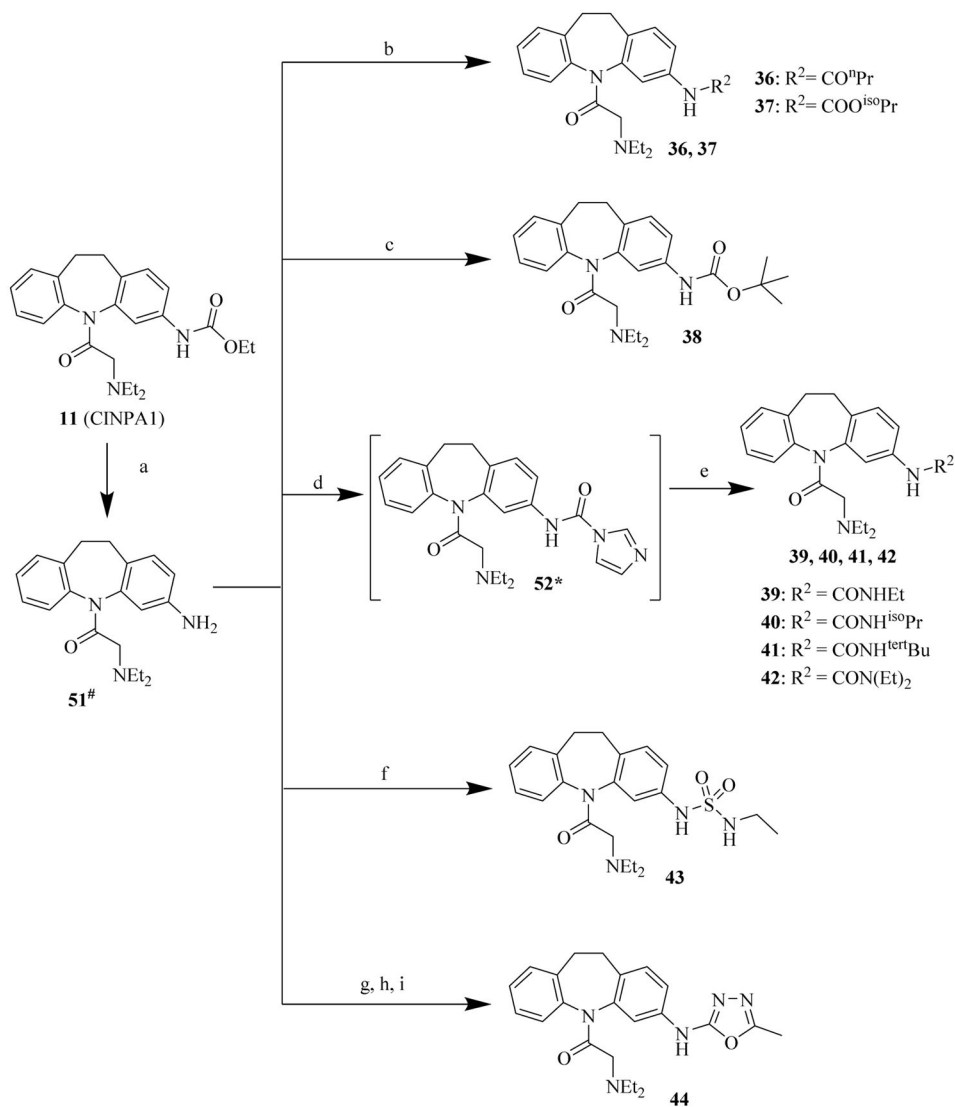
Scheme 2. Syntheses of CINAP1 analog compounds 22 to 32

Reagents and conditions: (a) R¹H, NaH, DMF, microwave, 80°C, 0.5 h; (b): R¹H, NaH, DMF, 0°C, 1.5 h; (c): NaI, acetone, N₂, 25°C, 12 h; (d): 2,2,6,6-tetramethylpiperidine, toluene, 120°C, 16 h; (e): NH₃·H₂O, MeOH, 120°C, 12 h; (f): furan-2,5-dione, AcOH, 100°C, 12 h.

*This intermediate chemical was prepared (purified or unpurified) and used as raw starting material for the next step without structural characterization.

**Scheme 3. Syntheses of CINPA1 analog compounds 33 to 35**

Reagents and conditions: (a) $R^1\text{CH}_2\text{COOH}$, T_3P , reflux, 12 h; (b) 2-*tert*-butoxyacetic acid, T_3P , 55°C, 12 h.

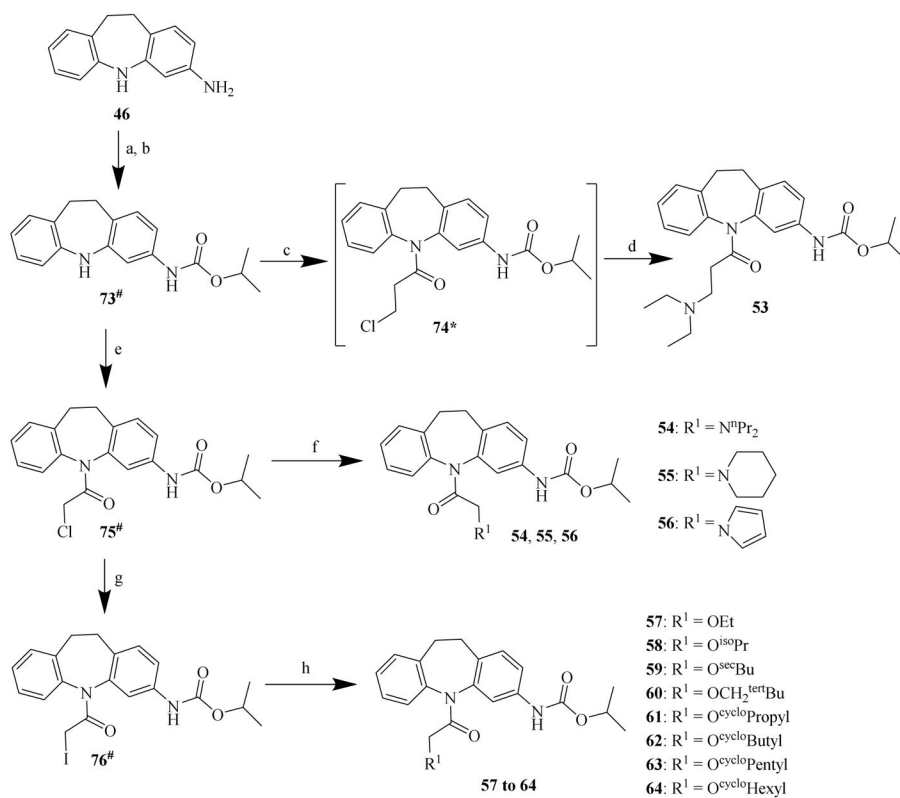


Scheme 4. Syntheses of CINPA1 analog compounds 36 to 44

Reagents and conditions: (a) H₂SO₄, AcOH, 120°C, 4 h; (b) R²Cl, Et₃N, dichloromethane, 10°C to 35°C, 16 h; (c) Boc₂O, Et₃N, dichloromethane, RT, 24 h; (d) CDI, Et₃N, dichloromethane, RT, 2 h; (e): corresponding primary or secondary amine, DMAP, Et₃N, dichloromethane, RT, 12 h; (f): EtNHSO₂Cl, dichloromethane, Et₃N, 15°C to 25°C, 1 h; (g): 1-(2-oxopyridine-1-carbothioyl)pyridin-2-one, dichloromethane, 20°C, 16 h; (h): acetohydrazide, THF, 20°C, 16 h; (i): EDC, Et₃N, DMF, 20°C, 16 h.

[#]Purified or unpurified intermediate with partial structural characterization (¹H NMR, MS, or both).

*This intermediate chemical was prepared (purified or unpurified) and used as raw starting material for the next step without structural characterization.

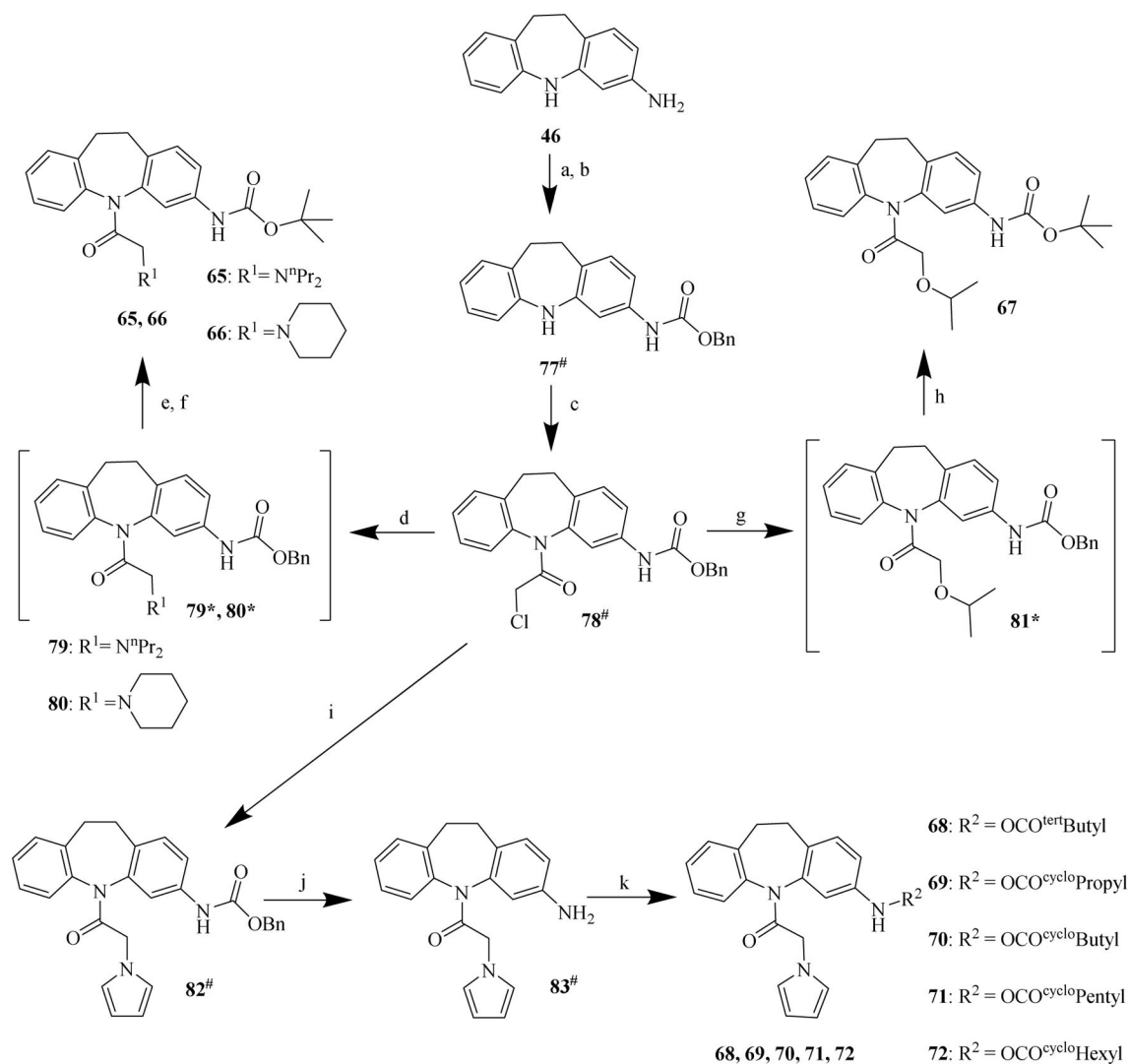


Scheme 5. Syntheses of CINPA1 analogs: chemicals 53 to 64

Reagents and conditions: (a) isopropyl chloroformate, EtOH, 0°C, 20 min; (b) Na₂CO₃, H₂O, 15°C to 25°C, 1 h; (c) 3-chloropropionyl chloride, toluene, 100°C, 3 h; (d) diethyl amine, toluene, 100°C, 10 h; (e) 2-chloroacetyl chloride, toluene, 100°C, 3 h; (f) R¹H, DMF, NaH, 25°C, 3 h; (g) NaI, acetone, 25°C, 12 h; (h) R¹H, NaHMDS, N₂, THF, 0°C to 25°C, 1.5 h.

#Purified or unpurified intermediate, with partial structural characterization (¹H NMR, MS, or both).

*An intermediate chemical prepared, purified or unpurified, and used as raw starting material for the next step without structural characterization.



Scheme 6. Syntheses of CINPA1 analogs: chemicals 65 to 72

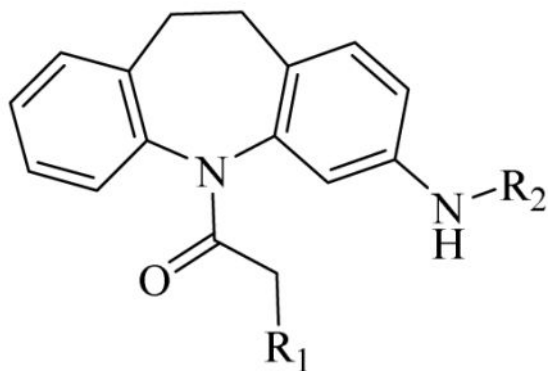
Reagents and conditions: (a) benzyl chloroformate, EtOH, 0°C, 1 h; (b) Na₂CO₃, H₂O, 15°C, 1 h; (c) 2-chloroacetyl chloride, toluene, 110°C, 3 h; (d) R¹H, toluene, 110°C, 6 h; (e) 10% Pd/C, MeOH, H₂ (15 psi), 25°C, 12 h; (f) (Boc)₂O, MeOH, 25°C, 3 h; (g) 2-propanol, Na, 90°C → 60°C, 30 min; (h) Boc₂O, Pd/C, MeOH, H₂ (15 psi), 25°C, 2 h; (i) pyrrole, DMF, NaH, N₂, 0°C to 25°C, 12 h; (j) Pd/C, H₂ (15 psi), EtOAc, 25°C, 4 h; (k) alkyl chloroformate, THF, Et₃N, 0°C to 25°C, 2 h.

#Purified or unpurified intermediate, with partial structural characterization (¹H NMR, MS, or both).

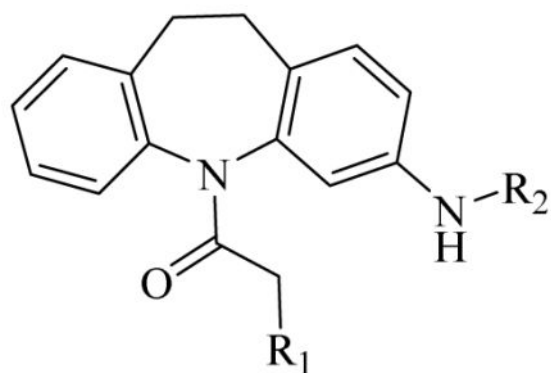
*An intermediate chemical prepared, purified or unpurified, and used as raw starting material for the next step without structural characterization.

Table 1

CAR inverse agonistic activities of CINPA1 and 10 commercially available analogs



Compound	R ₁	R ₂	IC ₅₀ (nM)
2 (clotrimazole)	NA	NA	126.9 ± 8.0
11 (CINPA1)			687 ± 71
12			235 ± 20
13			38,755 ± 5,451
14			4,078 ± 239
15	NH ₂	(12 to 18)	> 70,000
16			3,631 ± 187
17			320 ± 37

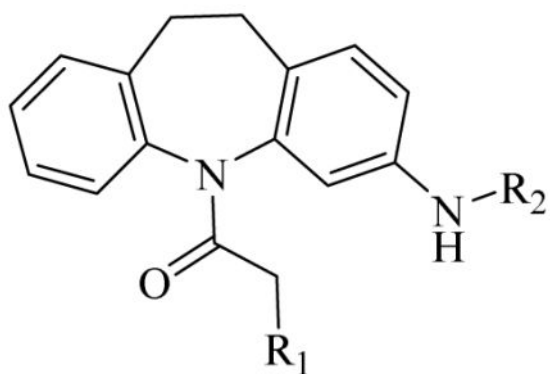


Compound	R ₁	R ₂	IC ₅₀ (nM)
18			5,010 ± 84
19			288 ± 13
20			1,792 ± 87
		(20 and 21)	
21			> 70,000

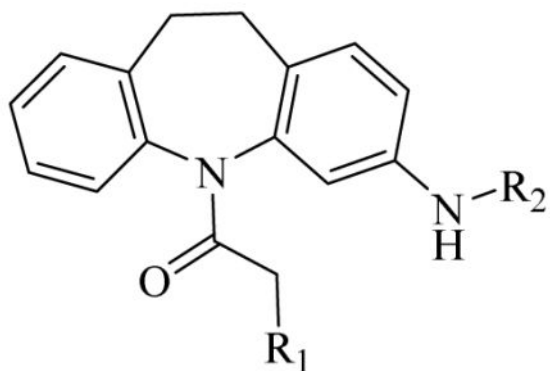
NA: not applicable

Table 2

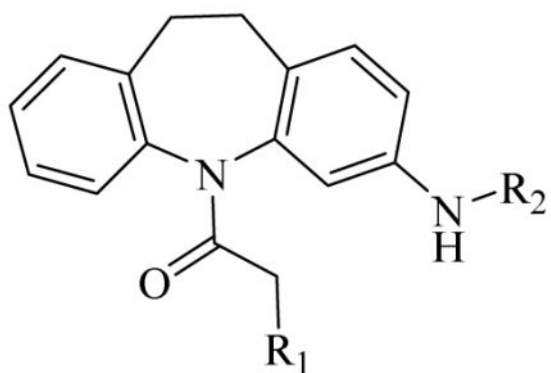
CAR inverse agonistic activities of CINPA1 and its 23 analogs from the first-round chemistry effort



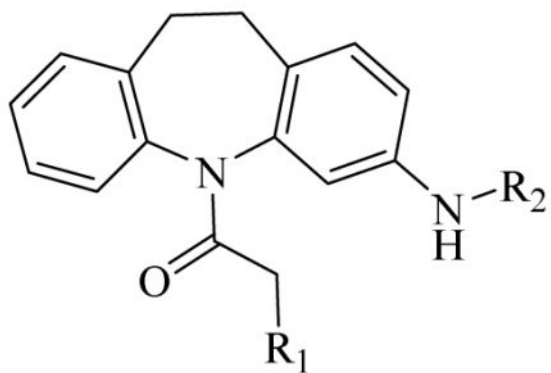
Compound	R ₁	R ₂	IC ₅₀ (nM)
11 (CINPA1)			687 ± 71
22			210 ± 10
23			3,050 ± 125
		22 to 35	
24			1,580 ± 88
25			620 ± 29
26			4,080 ± 162
27			1,410 ± 41



Compound	R ₁	R ₂	IC ₅₀ (nM)
28			39,810 ± 4,384
29			37.0 ± 1.5
30			6,890 ± 350
31			24,490 ± 845
32			2,060 ± 163
33			2,430 ± 114
34			3,180 ± 131



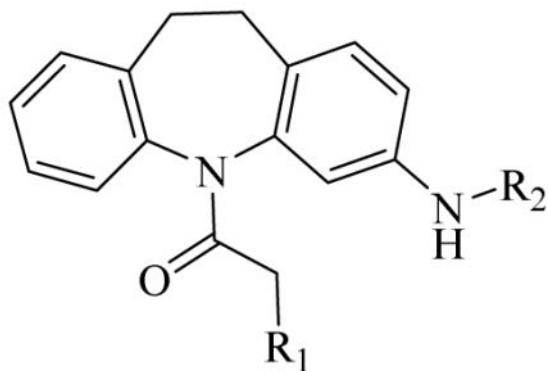
Compound	R ₁	R ₂	IC ₅₀ (nM)
35			70.8 ± 1.0
36	 36 to 44		2,320 ± 95
37			680 ± 31
38			1,030 ± 38
39			14,420 ± 742
40			4,930 ± 146
41			12,970 ± 183



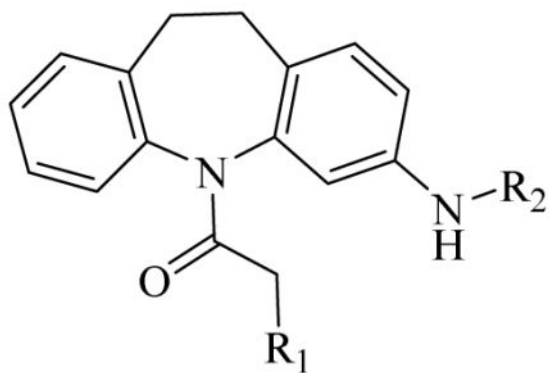
Compound	R ₁	R ₂	IC ₅₀ (nM)
42			13,990 ± 318
43			19,090 ± 1,397
44			18,790 ± 1,585

Table 3

CAR inverse agonistic activities of CINPA1 and its novel 20 analogs from the second-round chemistry effort

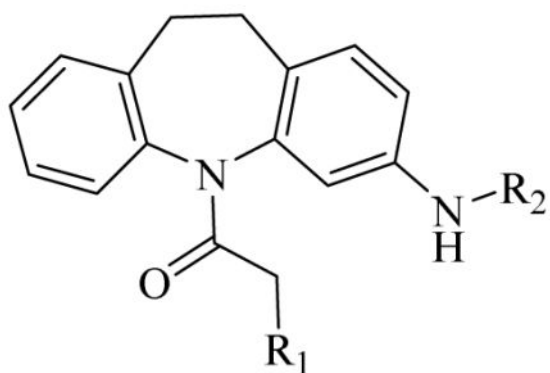


Compound	R ₁	R ₂	IC ₅₀ (nM)
11 (CINPA1)			687 ± 71
53			6,400 ± 124
54			116.4 ± 8.6
		53 to 64	
55			249.0 ± 16.6
56			20.9 ± 2.6
57			11,350 ± 670
58			60.1 ± 3.4



Compound	R ₁	R ₂	IC ₅₀ (nM)
59			35.3 ± 4.8
60			21.2 ± 1.9
61			81.5 ± 7.4
62			37.6 ± 2.8
63			19.5 ± 1.5
64			24.1 ± 1.4
65			152.9 ± 11.1

65 to 68



Compound	R ₁	R ₂	IC ₅₀ (nM)
66			216.2 ± 15.6
67			116.0 ± 7.3
68			31.9 ± 2.2
69			22.1 ± 1.3
70			20.1 ± 2.8
71	 69 to 72		13.9 ± 0.9
72			11.7 ± 0.8