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Development of tetrahydroindazole-based potent and selective sigma-2 receptor ligands

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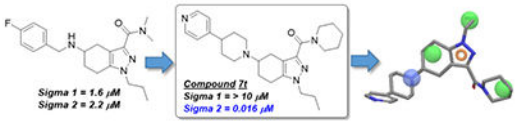
Abstract

The sigma-2 receptor has been shown to play important roles in a number of important diseases, including CNS disorders and cancer. However, mechanisms by which sigma-2 contributes to these diseases remain unclear. Development of new sigma-2 ligands that can be used to probe the function of this protein and potentially as drug discovery leads are therefore of great importance. Here we report the development of a series of tetrahydroindazole compounds that are highly potent and selective for sigma-2. The structure-activity relationship data was used to generate a pharmacophore model which summarizes the common features present in the potent ligands. Assays for solubility and microsomal stability showed that several members of this compound series possessed promising characteristics for further development of useful chemical probes or drug discovery leads.

Graphical Abstract



Medicinal chemistry optimization of a novel scaffold resulted in compound **7t** which has high potency and selectivity as a sigma-2 ligand. Molecular modeling produced a pharmacophore model that describes its key features and may be useful in further refining its bioactivity. Finally, microsomal stability and solubility data show this compound series has favorable drug-like characteristics as a chemical probes and drug discovery lead.

Keywords

sigma-1; sigma-2; tetrahydroindazole; pharmacophore; solubility

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Introduction

The sigma-2 receptor is a transmembrane protein previously thought to be the progesterone receptor membrane component 1 (PGRMC1) protein.[1] However, it has recently been established that sigma-2 is actually the endoplasmic reticulum (ER) transmembrane protein TMEM97.[2] While highly expressed in the brain[3] and several other tissues,[4] sigma-2 has been found to possess a wide range of functions. Supporting its myriad roles, sigma-2 has been shown to be localized to a number of organelles including the ER, lysosomes, and mitochondria.[5] This wide sub-cellular localization may contribute to the observed diverse ways in which the receptor has been shown to promote cell death, including by caspase-dependent[6] and caspase-independent[7] mechanisms.

The sigma-2 receptor has been studied for several decades due to its affinity to a variety of small molecule ligands and its diverse functions. Sigma-2 has been shown to be a biomarker for rapidly dividing cells[8] and has been used as a target for the development of new tumor imaging agents.[9] The protein has extensively been shown to be overexpressed in a variety of solid tumors, including lung,[10] brain,[11] and others.[8a, 8c, 12] Besides a potential role in inhibiting cancer cell proliferation through autophagy[13] and other mechanisms,[14] substantial evidence has shown that the sigma-2 receptor can regulate ion channels as well as the release of intracellular calcium,[15] both of which may contribute to the proliferation-modulating functions of sigma-2.

In addition to potential roles in cancer cell proliferation and apoptosis, sigma-2 has been shown to be involved in several CNS disorders as well. Sigma-2 antagonists prevent Aβ oligomer binding to neurons,[3a, 16] thereby reducing Aβ toxicity and improving cognition in mouse models of Alzheimer's disease.[3a] In other work, selective antagonism of sigma-2 was used to demonstrate a role of this protein in the action of cocaine.[15, 17] Finally, among its many reported roles, sigma-2 has also been found to bind to Niemann-Pick C1 (NPC1),[18] suggesting a role in the lysosomal storage disorder Niemann-Pick disease which is consistent with its long-studied role in cholesterol biosynthesis and homeostasis.[18–19]

Because of the important roles of sigma-2 receptors in CNS diseases and cancer, there have been a number of efforts aimed at developing potent and selective small molecule ligands. Tetrahydroisoquinoline derivatives,[20] triazoles,[21] and norbenzomorphans[22] have been used to develop potent sigma-2 ligands for use in animal models of Alzheimer's and other diseases. There have been other scaffolds developed as potent sigma-2 ligands for CNS and cancer indications as well (Figure 1).[22a, 23]

We recently reported the identification of a novel series of tetrahydroindazoles with unique bioactivity.[24] While evaluating the off-target activity of these compounds, we observed that one of them was a moderately potent ligand for the sigma-1 and sigma-2 receptors. As sigma-1 has been implicated in a wide varity of diseases,[23f, 25] subsequent medicinal chemistry was undertaken to further improve the sigma-1 activity of that series and this work was recenrly published.[26] Importantly, that work showed that potent and selective sigma-1 ligands could be developed by changing the *N-1* substituted tetrahydroindaolze to an *N-2* substituted ring. Because there are still many unanswered questions about the role of both

sigma-1 and sigma-2, we were interested in developing potent and selective ligands for both of them to use in future studies to differentiate the role of each of these proteins. Developing ligands selective for each of the sigma receptors based on the same scaffold could provide a powerful set of chemical tools for use in studying biology and developing new sigma-targeting therapeutics.

Here we report our medicinal chemsitry effort to develop more potent and selective sigma-2 inhibitors. The structure-activity relationships we observed were used to build a pharmacophore model that describes the observed bioactivity and can be used to support further optimization. The highly potent and selective sigma-2 ligands reported here will be useful as pharmacological tools and drug discovery leads to further evaluate sigma-2 as a valid drug target for CNS disorders and cancer.

Results and Discussion

Compounds were screened in a radioligand binding assay against sigma-1 and sigma-2 by the University of North Carolina Psychoactive Drug Screening Program (PDSP). Binding was assessed by displacement of [^{3}H]- Pentazocine for sigma-1 and [^{3}H]-DTG for sigma-2. New molecules were first screened at a single concentration of 10 μM ($pK_i = 5$) and those that showed > 50% inhibition were subsequently tested across a dose range to determine pK_i.

Substituents on the benzyl group were first studied by modifying the aromatic ring with electron-donating and - withdrawing groups. With the bulkier and less electron-withdrawing chloride (**7b**), a slight improvement in sigma-2 activity was observed along with a complete loss of activity against sigma-1. Electron-donating alkyl ether and hydroxyl substituents were also tested. The dimethoxy-containing derivative (**7c**) was 10-fold more potent towards sigma-2 than the original compound (**7a**) and showed no activity against sigma-1. In contrast, the 3-hydroxyl analog (**7d**), while more potent against sigma-2, was nearly equipotent towards sigma-1. A different hydrogen-bond donor, primary sulfonamide **7e**, produced an increase in sigma-2 potency while abolishing the sigma-1 activity seen in compound **7d**. When a 4-pyridine (**7f**) replaced the phenol moiety in compound **7d**, complete loss of both sigma-1 and sigma-2 activity was observed. However, a piperidine ring on the benzyl group (**7g**) afforded the most potent and selective sigma-2 compound in this series (Table 1). A lactam and a tetrahydropyran (**7j** and **7k**) also showed good inhibition of sigma-2, with both having $pK_i > 6.1$ M ($K_i < 1$ μM) for sigma-2 and no inhibition of sigma-1.

The effect of replacing the phenyl ring with acyclic groups and non-aromatic rings was next explored (Table 2). The conformationally-restricted tetrahydroisoquinoline **7l** and tertiary amine **7m** both possessed excellent sigma-2 inhibition while having no inhibitory activity against sigma-1. Notably, replacement with a smaller dibasic N-methyl piperazine group (**7n**) resulted in excellent potency against both sigma-1 and sigma-2, with pK_i of 6.7 M (K_i = 192 nM) and 7.5 M (K_i = 34 nM) respectively. Noting the potency of the N-methyl piperazine, other dibasic amines were also evaluated. Compared to the initial compound **7a**, substitution with a guanidine group (**7o**) led to loss of activity against sigma-1 while

retaining similar inhibition of sigma-2. Among the several dibasic groups tested, the more rigid 4-(4-pyridyl)-piperidine (**7t**) was the strongest inhibitor of sigma-2 (pK_i = 7.8) and was > 625-fold selective over sigma-1.

The sigma-2 crystal structure has not yet been solved. Hence, a common-features based pharmacophore was built using the sigma-2 binding data of our compounds to further define the essential structural features. The Pharmacophore Alignment and Scoring Engine (PHASE)[27] tool implemented in the Schrödinger software suite was used to build the model. A total of 10 hypotheses were generated and the best hypothesis was found to be a 5-point pharmacophore model with 3 hydrophobic features (HHH), one positive ionization (P), and one ring aromatic (R) feature (Figure 2). This model is shown overlaid with potent sigma-2 ligand **7t** in Figure 2.

To evaluate the pharmaceutical properties and potential for future therapeutic development, a selection of five structurally diverse and potent and/or selective ligands were chosen for microsomal stability and solubility analyses. The thermodynamic solubility of compounds in pH=7.4 PBS buffer was evaluated in a high-throughput manner on a Synergy HTX plate reader. All compounds had solubilities > 400 μM, and three of the compounds had solubilities > 1 mM in PBS buffer (Table 3). The same set of five compounds tested in the solubility assay were screened for their *in vitro* stability towards mouse liver microsomes to evaluate their potential *in vivo* metabolic stability. Each compound was measured after a 60-minute incubation period at 37 °C. The tertiary amine compound **7m** was completely consumed under these conditions. Compound **7a** and **7l** were also rapidly degraded after 60 minutes, with less than 25% of the parent compound remaining. Compounds **7g** and **7s** had 47% and 54% remaining after a 60-minute incubation, respectively (Table 3).

Synthesis of compounds **7a** – **7t** is depicted in Scheme 1. Starting with commercial 1,4-dioxaspiro[4.5]decan-8-one (**1**), acylation with diethyloxalate was achieved using LDA at −78 °C to provide **2** in 68% yield. Formation of the tetrahydroindazole to give compound **3** in 82% yield was achieved by treatment with propylhydrazine. The ester was then hydrolyzed to give acid **4** in 84% yield. The acid was coupled to either dimethylamine or piperidine to give amides **5a** and **5b**, respectively, and the ketal protecting group was removed using 3N HCl. Finally, reductive amination of the resulting ketone with diverse amines afforded the desired final compounds in variable yields (Scheme 1).

Conclusions

Based on our initial identification of tetrahydroindazole **7a** with moderate activity against sigma-1 and sigma-2 receptors, an SAR study was carried out to improve potency and selectivity for sigma-2 from this chemotype. Compounds could be readily diversified using an efficient synthetic route that used late-stage amide formation and reductive amination as key steps. We significantly improved potency and selectivity towards sigma-2 by adding a dibasic amine on the *C5* position of the tetrahydroindazole, with compound **7t** found to be the most potent and selective (pK_i = 7.8 M for sigma-2 and < 5 M for sigma-1) ligand for this chemotype. Furthermore, these compounds were shown to have good to excellent thermodynamic solubility, with **7m** having solubility of 2.4 mM in PBS buffer. Some of

these tetrahydroindazoles, particularly **7g** and **7s**, also showed good mouse liver microsomes stability after 60 minutes. Finally, we also developed a common-features pharmacophore of these compounds for sigma-2 receptor to help rationalize the observed SAR.

Sigma-1 and sigma-2 receptors have been implicated in a wide variety of diseases, including cancer and CNS disorders. Because of the potential for developing new therapeutics targeting sigma-2, there has been substantial effort to develop potent ligands for this protein. However, many of the ligands described to-date lack high selectivity for sigma-2 over sigma-1, complicating efforts to use them as pharmacological tools to dissect the roles of these two proteins. This lack of selectivity also presents significant challenges in trying to further develop these compounds as drug discovery leads. In this report, we describe a novel scaffold that was developed into highly potent and selective sigma-2 ligands. Our most promising compound (**7t**) is a 16 nM inhibitor in the sigma-2 ligand displacement assay, and has no signficant activity against sigma-1 (> 10 μM). This new series of sigma-2 ligands was also shown to be highly drug-like, with some members having thermodynamic solubility > 2.4 mM in PBS buffer, and with substantial microsomal stability. This work demonstrates that this class of compounds is suitable for further development as unique chemical tools that can be used to study role of sigma-2 in cancer and CNS disorders. In addition, work to further improve potency of this compound class and define its effects in *in vitro* and *in vivo* disease models may support its development as a novel class of therapeutics for a range of diseases.

Experimental Section

Pharmacophore Modeling

To build the ligand-based pharmacophore model, we considered all 20 compounds presented in this study. We used the Pharmacophore Alignment and Scoring Engine (PHASE) tool implemented in the Schrödinger suite to build the model. The first step in the Phase run is to add the 3D structures of the compounds along with their activity. We added the 20 ligands prepared using the lig-prep panel of the Schrödinger suite. Next, we generated conformers for all the ligands using the conformation generation tool. In the third step, we classified the compounds as active and inactive. We considered the active compounds to be those with a $pK_i > 6.0$. The active set contained 14 compounds and the inactive set contained 6 compounds (7a,7b,7h,7u,7s,7r).

To construct the hypotheses, we considered 15 compounds consisting of 12 from the active set and 3 from the inactive set. Running the Phase module, we then generated 10 hypotheses using these 15 compounds. We then analyzed the different hypotheses and tried to predict the other 5 compounds not included in the model building set. Out of the 10 pharmacophore models (hypotheses), one with a 5-point pharmacophoric feature gave the best prediction of the 5 test compounds. This model has 3 hydrophobic features (HHH), one positive ionizable group (P), and one ring aromatic (R) feature (Figure 2).

Solubility

A selection of 5 of the most potent and/or selective ligands were chosen for microsomal stability and solubility analyses. From stock solutions in DMSO, concentrations of 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.9,0.9 μM solutions in acetonitrile were prepared in a 96-well plate while keeping the DMSO concentration constant at 0.5 %. Individual standard solubility curves for each compound were generated from the UV-absorption measured on a Synergy HTX plate reader. 1 mg of each compound in 1 mL of PBS (pH = 7.4) was stirred at 500 rpm for 16 hours at room temperature. After this time, the solution was allowed to equilibrate for 30 minutes. The solution was then filtered through 0.2 μm filter and the UV-absorption of the filtrate was measured. The solubility was extrapolated from the standard solubility curve previously generated for each compound.

Mouse Liver Microsomal (MLM) Stability

In a 96-well plate, 100 μL of Mouse Liver microsomes (0.4 mg/mL, Corning, cat# 452702) with co-factor (NADPH) in Phosphate Buffer (0.5 M) was added to 10 μM of the compounds. Verapamil-HCl was run as the control. At time = 0 min (T_0), the reactions were immediately quenched and mixed with 2x the volume of ice-cold acetonitrile. The other reactions were incubated at 37 °C with shaking for 60 min. At time 60 min (T_{60}), the reactions were stopped by mixing with 2x volume of ice-cold acetonitrile. The samples were centrifuged to remove the precipitated protein and the supernatants were analyzed by Acquity-H UPLC/MS in SRM mode to quantitate the remaining parent compound at both T_0 and T_{60}. All reactions were run in duplicate and the results are the mean of the runs. The percent of the parent compound remaining was calculated from the formula:

% parent compound remaining = (concentration at 60 min / concentration at 0 min) * 100

Sigma Receptor Assays

Sigma-1 and sigma-2 assays were conducted by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271–2013-00017-C (NIMH PDSP). For experimental details please refer to the PDSP web site https://pdsp.unc.edu/ims/investigator/web/.

Chemistry

Unless otherwise noted, all materials for the synthetic chemistry portion were obtained from commercial suppliers (e.g. Combi-Blocks, CombiPhos, Fisher Scientific, Sigma-Aldrich, VWR) and used without further purification. The ^{1}H-and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer using $CDCl_3$ or CD_3OD as the solvent. Chemical shifts are expressed in ppm (δ scale) and referenced to residual protonated solvent. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Thin layer chromatography (TLC) was performed on glass backed Merck silica gel 60 F_{254} plates, column chromatography was performed using KP-SIL silica gel (Biotage, USA), and flash column chromatography was performed on Biotage prepacked columns using the automated flash chromatography system Biotage Isolera One. The purities of all final compounds were

> 95% as determined by UPLC analysis unless otherwise indicated. High Resolution Mass analysis was performed on an Agilent 6210A LC-TOF.

Preparative HPLC Purification

All compounds that are designated as being purified by preparative HPLC were purified according to the same method, as described here. Purification was carried out using a Gilson GX-271 preparative scale reverse phase HPLC system with Gilson model 159 UV-VIS detector and Phenonemex Kinetex 5 μm, C18, 100 Å, 50 mm × 30 mm column. Compounds were eluted using a gradient mobile phase of A:B (90:10 to 0:100) over 5 min at a flow rate of 50.0 mL/min, where solvent A was H_2O (with 0.1% formic acid) and solvent B was CH_3CN (with 0.1% formic acid). The product fractions were combined and dried using a Genevac EZ-2 Centrifugal Evaporator.

General procedure A: synthesis of amides

To a solution carboxylic acid (1.0 equiv.) in DCM (0.1M) was added EDC (1.1 equiv.) and HOBT (1.0 equiv.). The reaction mixture was stirred at RT for 30 min and then amine (1.0 equiv.) and TEA (1.1 equiv.) were added. The mixture was then stirred at RT for 8 h, diluted with DCM and washed with H_2O and brine. The organic extract was dried over with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a residue which was purified by a short silica gel plug (10% MeOH in DCM) to give the amide.

General procedure B: removal of ketal protecting group

Ketals (1.0 equiv.) were dissolved in THF (0.8M) then 3N HCl (5.0 equiv.) was added to the mixture. The resulting solution was heated at 50 °C for 2 h. The solvents were evaporated and EtOAc was added. The organic layer was washed with saturated $NaHCO_3$ and H_2O. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give the crude product which was purified by a short silica gel plug using 0–10% MeOH in DCM to give the product.

General procedure C: reductive amination

A solution of the ketone (1 equiv.) and acetic acid (1.5 equiv.) in DCE (1.5 mL) was stirred at room temperature. The amine (2 equiv.) was added, followed by $NaBH(OAc)_3$. The mixture was stirred at room temperature overnight, quenched with saturate $NaHCO_3$ aqueous solution (3 mL), and extracted with DCM (3 mL × 3). The organic layers were combined, dried over Na_2SO_4, concentrated and further purified by and further purified by preparative HPLC according to the general procedure to provide the desired product as FA salt or free amine.

Ethyl 2-(8-hydroxy-1,4-dioxaspiro[4.5]dec-7-en-7-yl)-2-oxoacetate (2).—To a solution of 1,4-dioxaspiro[4.5]decan-8-one **1** (7.81 g, 50 mmol) in anhydrous THF (75 mL) cooled to −78 °C under a N_2 atmosphere was added LDA (27.5 mL, 55 mmol). After stirring for 15 minutes, diethyl oxalate (7.47 mL, 55 mmol) was added in portions over 10 min. The reaction was gradually warmed to RT and stirred for 16 h. The reaction mixture was quenched with 1N HCl and the resulting mixture was extracted with EtOAc, washed with

brine and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (0–100% EtOAc in Hexanes) to yield the Ethyl 2-(8-hydroxy-1,4-dioxaspiro[4.5]dec-7-en-7-yl)-2-oxoacetate **2** (8.7 g, 68%) as a thick yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.35 (s, 1H), 4.33 (q, *J* = 7.22 Hz, 2H), 3.97 – 4.02 (m, 4 H), 2.74 (s, 2 H), 2.68 (t, *J* = 6.87 Hz, 2H), 1.91 (t, *J* = 6.87 Hz, 2H), 1.37 (t, *J* = 7.17 Hz, 3H). LCMS (ESI) *m/z*: $[M+H]^+$ 257.16.

Ethyl 1'-propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxylate (3).—Propylhydrazine dihydrochloride (2 g, 13.6 mmol) was added to a solution of ethyl 2-oxo-2-(8-oxo-1,4-dioxaspiro[4.5]decan-7-yl)acetate **2** (3.49 g, 13.6 mmol) and K_2CO_3 (3.76 g, 27.2 mmol) in EtOH (84 mL). The reaction mixture was stirred at RT for 4 h and then concentrated *in vacuo*. The residue was re-dissolved in EtOAc and H_2O and the layers separated. The aqueous layer was extracted with EtOAc and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue obtained was purified by flash silica gel chromatography (0–60% EtOAc in Hexanes) to give ethyl 1'-propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxylate **3** (3.3 g, 82 %) as a thick oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.37 (q, *J* = 7.22 Hz, 2H), 3.97 – 4.08 (m, 6H), 2.98 (s, 2H), 2.78 (t, *J* = 6.56 Hz, 2H), 1.98 (t, *J* = 6.56 Hz, 2H), 1.86 (m, 2H), 1.34 – 1.40 (m, 3H), 0.92 (t, *J* = 7.48 Hz, 3H). LCMS (ESI) *m/z*: $[M + H]^+$ 295.41.

1'-Propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxylic acid (4).—To a mixture of ethyl 1'-propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxylate **3** (3.3 g, 11.21 mmol) in EtOH (50 mL) was added a 2M solution of NaOH (50 mL) and the reaction mixture stirred at RT for 4 h. The EtOH was evaporated and the aqueous mixture was washed with diethyl ether. The pH of the aqueous layer was adjusted to 5–6. The aqueous layer was then extracted with EtOAc (×3). The combined organic extract was dried using anhydrous Na_2SO4, filtered and concentrated under reduced pressure to yield 1'-propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxylic acid **4** (2.5 g, 84 %) as an off-white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 3.99 – 4.06 (m, 6H), 2.90 (s, 2H), 2.81 (t, *J* = 6.56 Hz, 2H), 1.94 – 2.00 (m, 2H), 1.79 – 1.90 (m, 2H), 0.92 (t, *J* = 7.32 Hz, 3H). LCMS (ESI) *m/z*: $[M + H]^+$ 267.37. LCMS (ESI) *m/z*: $[M + H]^+$ 267.37.

N,N-dimethyl-1'-propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxamide (5a).—Prepared according to General Procedure A using carboxylic acid **4** (0.3 g, 1.13 mmol) to afford N,N-dimethyl-1'-propyl-1',4',6',7'-tetrahydrospiro[[1,3]dioxolane-2,5'-indazole]-3'-carboxamide **5a** (145 mg, 44 %) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.97 – 4.07 (m, 4H), 3.92 (t, *J* = 7.32 Hz, 2H), 3.32 (br. s, 3H), 3.05 (br. s, 3H), 2.93 (s, 2H), 2.77 (t, *J* = 6.56 Hz, 2H), 1.99 (t, *J* = 6.56 Hz, 2H), 1.83 (m, *J* = 7.32 Hz, 2H), 0.93 (t, *J* = 7.32 Hz, 3H). LCMS (ESI) *m/z*: $[M + H]^+$ 294.30.

Piperidin-1-yl(1-propyl-1,4,6,7-tetrahydrospiro[indazole-5,2'-[1,3]dioxolan]-3-yl)methanone (5b).—Prepared according to General Procedure A using carboxylic acid **4**

(200 mg, 0.751 mmol) and piperidine (77mg, 0.901 mmol) to afford **5b** 168 mg, 67 %) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.01 (dddd, *J* = 13.9, 8.3, 6.6, 4.0 Hz, 4H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.82 (s, 2H), 3.67 (s, 2H), 2.90 (s, 2H), 2.76 (t, *J* = 6.6 Hz, 2H), 1.99 (t, *J* = 6.6 Hz, 2H), 1.83 (h, *J* = 7.4 Hz, 2H), 1.71 – 1.51 (m, 6H), 0.93 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.5, 142.5, 137.3, 116.7, 108.8, 64.8, 51.0, 48.3, 43.4, 32.2, 31.4, 26.9, 25.0, 23.6, 19.7, 11.4. LCMS (ESI) *m/z*: $[M + H]^+$ 334.31.

N,N-dimethyl-5-oxo-1-propyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxamide (6a).—Prepared according to General Procedure B using **5a** (320 mg, 1.1 mmol) to afford N,N-dimethyl-5-oxo-1-propyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxamide **6a** (230 mg, 85 %) as yellow waxy solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90 (td, *J* = 7.0, 1.1 Hz, 2H), 3.77 – 3.63 (m, 4H), 2.70 – 2.65 (m, 3H), 2.53 – 2.38 (m, 3H), 1.80 (q, *J* = 7.3 Hz, 2H), 1.55 (d, *J* = 7.8 Hz, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.5, 163.9, 142.6, 138.0, 116.5, 77.2, 77.2, 77.0, 76.7, 60.8, 55.4, 50.7, 25.8, 24.8, 23.5, 21.1, 11.2. LCMS (ESI) *m/z*: $[M + H]^+$ 250.24.

3-(piperidine-1-carbonyl)-1-propyl-1,4,6,7-tetrahydro-5H-indazol-5-one (6b).—Prepared according to General Procedure B using **5b** (240 mg 0.702 mmol) to afford **6b** (177 mg, 85 %) as a yellow solid ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.06 – 3.94 (m, 3H), 3.90 (t, *J* = 7.3 Hz, 1H), 3.82 (s, 2H), 3.65 (s, 2H), 2.89 (s, 1H), 2.75 (t, *J* = 6.6 Hz, 1H), 2.68 (t, *J* = 7.0 Hz, 1H), 1.98 (t, *J* = 6.6 Hz, 1H), 1.82 (hept, *J* = 7.6 Hz, 2H), 1.71 – 1.49 (m, 6H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 208.6, 163.5, 142.4, 137.1, 116.5, 108.7, 64.6, 50.9, 32.0, 31.3, 24.8, 23.5, 23.4, 19.6, 11.2. LCMS (ESI) *m/z*: $[M + H]^+$ 290.31.

5-((4-Fluorobenzyl)amino)-N,N-dimethyl-1-propyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxamide (7a).—Prepared according to General Procedure C using **6a** (150 mg, 0.6 mmol) to afford **7a** (114 mg, 53 %) as light pink solid (FA salt). ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 – 7.51 (m, 2H), 7.08 – 7.17 (m, 2H), 4.05 (s, 2H), 4.00 (t, *J* = 7.02 Hz, 2H), 3.26 (s, 3H), 3.10 – 3.24 (m, 2H), 3.08 (s, 3H), 2.82 – 2.92 (m, 1H), 2.64 – 2.74 (m, 1H), 2.52 (dd, *J* = 15.56, 9.46 Hz, 1H), 2.22 – 2.33 (m, 1H), 1.95 (s, 3H), 1.80 – 1.87 (m, 3H), 0.88 – 0.93 (m, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 167.0, 163.4, 143.3, 140.0, 132.6, 132.5, 116.9, 116.7, 116.1, 55.1, 51.9, 50.3, 39.6, 36.1, 28.0, 27.1, 24.5, 23.0, 20.7, 11.5. HRMS *m/z* calcd. for $C_{20}H_{27}FN_4O$ $[M + H]^+$ 359.4689; found: 359.4670.

5-((4-chlorobenzyl)amino)-N,N-dimethyl-1-propyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxamide (7b).—Prepared according to General Procedure C using **6a** (30 mg, 0.120 mmol) to afford **7b** (27 mg, 60 %) as white solid (FA salt). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (s, 1H, FA), 7.38 (d, *J* = 7.9 Hz, 2H), 7.25 (d, *J* = 8.1 Hz, 2H), 4.11 (d, *J* = 12.6 Hz, 1H), 4.01 – 3.85 (m, 3H), 3.31 (s, 3H), 3.27 – 3.15 (m, 2H), 3.02 (s, 3H), 2.76 (dd, *J* = 15.1, 10.0 Hz, 1H), 2.63 (dd, *J* = 16.5, 5.2 Hz, 1H), 2.54 – 2.42 (m, 1H), 2.09 – 1.94 (m, 1H), 1.79 (h, *J* = 7.4 Hz, 2H), 1.66 (dp, *J* = 12.1, 6.1 Hz, 1H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.1, 164.7, 141.9, 137.47\, 134.9, 131.9, 130.6, 129.0, 114.9, 53.7, 51.0, 47.9, 39.2, 35.9, 25.7, 24.5, 23.5, 19.8, 11.2. HRMS *m/z* calcd. for $C_{20}H_{27}ClN_4O$ $[M + H]^+$ 375.1946; found: 375.1945.

5-((2,4-dimethoxybenzyl)amino)-N,N-dimethyl-1-propyl-4,5,6,7-tetrahydro-1H-indazole-3-carboxamide (7c).—Prepared according to General Procedure C using **6a** (30 mg, 0.120 mmol), to afford **7c** (23 mg, 47 %) as white solid (FA salt). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H, FA), 7.25 (d, *J* = 9.7 Hz, 1H), 6.59 – 6.14 (m, 2H), 4.15 (d, *J* = 12.4 Hz, 1H), 4.01 (d, *J* = 12.5 Hz, 1H), 3.89 (td, *J* = 6.9, 2.9 Hz, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.30 (s, 5H), 3.04 (s, 3H), 2.83 (dd, *J* = 15.2, 9.2 Hz, 1H), 2.70 (d, *J* = 16.2 Hz, 1H), 2.54 (t, *J* = 11.2 Hz, 1H), 2.26 – 2.13 (m, 1H), 2.02 – 1.73 (m, 3H), 0.90 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.9, 164.8, 162.0, 159.0, 142.2, 137.4, 132.8, 114.7, 112.2, 104.6, 98.4, 55.5, 55.5, 53.2, 50.9, 43.8, 39.1, 35.9, 25.7, 24.6, 23.4, 19.5, 11.2. HRMS *m/z* calcd. for $C_{22}H_{32}N_4O_3$ $[M + H]^+$: 401.2547; found: 401.2533.

(5-((3-Hydroxyphenethyl)amino)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)(piperidin-1-yl)methanone (7d).—Prepared according to General Procedure C from **6b** to afford **7d** as colorless oil (57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (s, 2H, FA), 7.00 (t, J = 7.8 Hz, 1H), 6.70 (s, 1H), 6.65 (dd, J = 8.1, 2.1 Hz, 1H), 6.56 (d, J = 7.5 Hz, 1H), 3.80 (dt, J = 20.9, 6.6 Hz, 4H), 3.58 (t, J = 5.2 Hz, 2H), 3.35 (q, J = 10.2, 9.5 Hz, 1H), 3.18 (ddt, J = 32.9, 15.1, 6.5 Hz, 3H), 2.86 (dq, J = 14.1, 7.5, 6.5 Hz, 2H), 2.78 – 2.62 (m, 2H), 2.55 (ddd, J = 16.2, 9.8, 5.5 Hz, 1H), 2.32 (d, J = 12.4 Hz, 1H), 1.93 (dd, J = 13.2, 7.4 Hz, 1H), 1.73 (h, J = 7.3 Hz, 2H), 1.59 (ddq, J = 24.1, 12.6, 6.2 Hz, 6H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 163.3, 157.6, 141.6, 137.7, 137.5, 129.8, 119.6, 115.9, 114.5, 114.2, 54.3, 53.4, 50.8, 48.4, 46.4, 43.6, 32.3, 26.6, 25.7, 25.4, 24.5, 24.3, 23.3, 19.5, 11.1. HRMS *m/z* calcd. for $C_{24}H_{34}N_4O_2$ $[M + H]^+$: 411.2755; found: 411.2752.

4-(((3-(Piperidine-1-carbonyl)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-5-yl)amino)methyl)benzenesulfonamide (7e).—Prepared according to General Procedure C from **6b** to afford **7e** as colorless oil (39%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.22 (s, 2H, FA), 7.91 (d, J = 8.1 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 4.23 (d, J = 13.3 Hz, 1H), 4.15 (d, J = 13.3 Hz, 1H), 3.98 – 3.88 (m, 2H), 3.81 (q, J = 5.7 Hz, 2H), 3.68 (t, J = 5.3 Hz, 2H), 3.33 – 3.22 (m, 1H), 3.17 (dd, J = 15.3, 5.1 Hz, 1H), 2.81 (ddd, J = 16.2, 5.8, 3.2 Hz, 1H), 2.67 (dd, J = 15.3, 9.4 Hz, 2H), 2.36 – 2.27 (m, 1H), 2.02 – 1.87 (m, 1H), 1.82 (q, J = 7.3 Hz, 2H), 1.74 – 1.56 (m, 6H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 165.6, 163.6, 143.2, 141.7, 137.6, 129.9, 126.69\, 114.2, 53.2, 50.8, 48.4, 48.2, 43.4, 26.6, 26.3, 25.6, 24.8, 24.4, 23.2, 19.4, 10.9. HRMS *m/z* calcd. for $C_{23}H_{33}N_5O_3S$ $[M + H]^+$ 460.2377; found: 460.2332.

Piperidin-1-yl(1-propyl-5-((2-(pyridin-4-yl)ethyl)amino)-4,5,6,7-tetrahydro-1H-indazol-3-yl)methanone (7f).—Prepared according to General Procedure C from **6b** to afford **7f** as a white solid (57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 – 8.58 (m, 2 H, FA), 7.11 – 7.22 (m, 2 H), 3.90 (td, *J*=7.17, 1.53 Hz, 2 H), 3.78 (br. s., 2 H), 3.67 (br. s., 2 H), 3.42 – 3.53 (m, 1 H), 3.03 – 3.09 (m, 4 H), 2.94 – 3.00 (m, 3 H), 2.79 – 2.88 (m, 2 H), 2.63 – 2.72 (m, 1 H), 2.53 – 2.62 (m, 1 H), 2.41 (dd, *J*=15.56, 8.54 Hz, 1 H), 2.05 (s, 3 H), 1.80 (q, *J*=7.32 Hz, 2 H), 1.71 – 1.77 (m, 1 H), 1.63 – 1.69 (m, 3 H), 1.56 (br. s., 2 H), 1.21 (t, *J*=7.02 Hz, 1 H), 0.90 (t, *J*=7.48 Hz, 3 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.9, 163.9, 149.7, 149.0, 142.5, 137.9, 124.3, 115.9, 53.8, 50.8, 48.3, 47.1, 43.4, 35.5, 28.8, 27.7, 24.9, 23.6, 21.5, 19.9, 11.3. HRMS *m/z* calcd. for $C_{23}H_{34}N_5O$ $[M + H]^+$ 396.2758; found: 396.2760.

Piperidin-1-yl(5-((4-(piperidin-1-yl)benzyl)amino)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)methanone (7g).—Prepared according to General Procedure C from **6b** to afford **7g** as a colorless oil (65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (s, 2H, FA), 7.33 – 7.15 (m, 2H), 6.81 (d, J = 8.6 Hz, 2H), 4.03 (d, J = 13.0 Hz, 1H), 3.93 (d, J = 13.1 Hz, 1H), 3.89 – 3.72 (m, 4H), 3.62 (q, J = 6.1 Hz, 2H), 3.29 – 3.15 (m, 2H), 3.09 (t, J = 5.4 Hz, 4H), 2.80 (dd, J = 14.8, 9.1 Hz, 1H), 2.69 (ddd, J = 16.5, 5.8, 3.0 Hz, 1H), 2.52 (ddd, J = 16.6, 10.7, 5.9 Hz, 1H), 2.23 (dd, J = 12.1, 5.8 Hz, 1H), 1.97 – 1.85 (m, 1H), 1.76 (h, J = 7.3 Hz, 2H), 1.70 – 1.46 (m, 12H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 163.3, 152.3, 141.9, 137.3, 131.0, 120.2, 115.9, 114.2, 52.3, 50.8, 49.7, 48.3, 47.8, 43.4, 26.7, 25.7, 25.5, 24.6, 24.2, 24.1, 23.3, 19.5, 11.1. HRMS *m/z* calcd. for $C_{28}H_{41}N_5O$ $[M + H]^+$ 464.3384; found: 464.3400.

Piperidin-1-yl(5-((4-(piperidin-1-yl)phenyl)amino)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)methanone (7h).—Prepared according to General Procedure C from **6b** to afford **7h** as a colorless oil (37%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (d, J = 32.0 Hz, 1H, FA), 7.09 (d, J = 8.3 Hz, 2H), 6.57 (d, J = 8.3 Hz, 2H), 3.92 (t, J = 7.2 Hz, 2H), 3.84 – 3.52 (m, 5H), 3.27 – 2.92 (m, 6H), 2.59 (dq, J = 56.7, 7.0 Hz, 3H), 2.20 – 2.00 (m, 1H), 1.84 (dp, J = 29.4, 7.3, 6.3 Hz, 7H), 1.62 (dddd, J = 29.1, 24.0, 12.2, 5.8 Hz, 10H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7, 163.8, 144.4, 142.6, 138.8, 137.6, 122.1, 120.8, 116.5, 115.4, 114.0, 77.2, 77.0, 76.7, 54.7, 50.7, 50.0, 49.8, 48.9, 48.2, 43.3, 28.3, 27.5, 26.8, 25.7, 25.4, 24.7, 24.6, 23.4, 22.9, 19.2, 11.2. HRMS *m/z* calcd. for $C_{27}H_{39}N_5O$ $[M + H]^+$ 450.3222; found: 450.3225.

5-((4-Phenoxyphenyl)amino)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)(piperidin-1-yl)methanone (7i).—Prepared according to General Procedure C from **6b** to afford **7i** as a colorless oil (32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.20 (m, 2H), 6.98 (tt, J = 7.2, 1.2 Hz, 1H), 6.94 – 6.89 (m, 2H), 6.89 – 6.83 (m, 2H), 6.66 – 6.53 (m, 2H), 3.92 (t, J = 7.2 Hz, 2H), 3.84 – 3.70 (m, 3H), 3.66 (d, J = 6.3 Hz, 2H), 3.13 (dd, J = 15.9, 5.0 Hz, 1H), 2.78 – 2.60 (m, 2H), 2.56 (dd, J = 15.9, 7.5 Hz, 1H), 2.22 – 2.06 (m, 1H), 1.94 – 1.76 (m, 4H), 1.70 – 1.46 (m, 6H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.7, 159.0, 147.5, 143.7, 142.7, 137.6, 129.4, 121.9, 121.3, 117.1, 115.7, 114.2, 50.8, 50.7, 49.1, 48.1, 43.2, 28.5, 27.8, 26.8, 25.7, 24.7, 23.5, 19.3, 11.2. HRMS *m/z* calcd. for $C_{28}H_{34}N_4O_2$ $[M + H]^+$ 459.2755; found: 459.2764.

1-(3-((3-(Piperidine-1-carbonyl)-1-propyl-4,5,6,7-tetrahydro-1H-indazol-5-yl)amino)propyl)pyrrolidin-2-one (7j).—Prepared according to General Procedure C from **6b** to afford **7j** as a waxy yellow solid (45 %). ^{1}H NMR (500 MHz, CD_3OD) δ 3.98 (t, *J*=7.02 Hz, 2 H), 3.67 (br. s., 4 H), 3.45 – 3.50 (m, 2 H), 3.36 (t, *J*=6.87 Hz, 2 H), 2.86 – 2.94 (m, 2 H), 2.77 – 2.84 (m, 1H), 2.64 – 2.68 (m, 2 H), 2.38 (t, *J*=8.09 Hz, 2 H), 2.27 – 2.34 (m, 1 H), 2.09 – 2.17 (m, 1 H), 2.03 – 2.09 (m, 2 H), 1.80 – 1.86 (m, 2 H), 1.75 – 1.80 (m, 2 H), 1.69 – 1.75 (m, 3 H), 1.62 – 1.69 (m, 3 H), 1.58 (br. s., 2 H), 0.90 (t, *J*=7.48 Hz, 3 H). ^{13}C NMR (126 MHz, CD_3OD) δ 177.9, 166.0, 143.5, 140.5, 116.4, 55.1, 51.6, 45.0, 44.5, 41.4, 32.1, 29.5, 28.2, 27.9, 27.0, 25.7, 24.5, 20.8, 19.0, 11.4. HRMS *m/z* calcd. for $C_{23}H_{38}N_5O_2$ $[M + H]^+$ 416.3020; found: 416.3025.

Piperidin-1-yl(1-propyl-5-(((tetrahydro-2*H*-pyran-4-yl)methyl)amino)-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)methanone (7k).—Prepared according to General Procedure C from **6b** to afford **7k** as a colorless oil (45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.98 – 3.72 (m, 6H), 3.62 (dt, J = 16.9, 5.9 Hz, 2H), 3.40 – 3.10 (m, 4H), 2.90 (dd, J = 12.1, 7.5 Hz, 1H), 2.85 – 2.68 (m, 3H), 2.62 (dt, J = 10.7, 5.5 Hz, 1H), 2.48 – 2.29 (m, 1H), 2.08 – 1.87 (m, 2H), 1.86 – 1.48 (m, 10H), 1.29 (dtd, J = 12.9, 7.2, 3.5 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.3, 163.2, 142.0, 137.3, 114.6, 67.2, 67.1, 54.8, 50.9, 48.2, 43.4, 32.8, 30.6, 26.7, 25.9, 25.7, 24.6, 24.4, 23.3, 19.9, 11.1. HRMS *m/z* calcd. for $C_{22}H_{36}N_4O_2$ $[M + H]^+$ 398.2911; found: 398.2924.

(5-(3,4-dihydroisoquinolin-2(1H)-yl)-1-propyl-4,5,6,7-tetrahydro-1H-indazol-3-yl)(piperidin-1-yl)methanone (7l).—Prepared according to General Procedure C from **6b** to afford **7l** as a colorless oil (45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 – 7.14 (m, 3 H), 7.00 – 7.05 (m, 1 H), 3.89 – 3.94 (m,2 H), 3.77 (br. s., 2 H), 3.69 (br. s., 2 H), 3.04 (dd, *J*=15.26, 4.58 Hz, 1 H), 2.89 – 2.96 (m, 4 H), 2.75 – 2.83 (m, 1 H), 2.59 – 2.70 (m, 2 H), 2.18 – 2.28 (m, 1H), 1.79 – 1.91 (m, 3 H), 1.60 – 1.70 (m, 7 H), 1.57 (br. s., 2 H), 0.92 (t, *J*=7.48 Hz, 3 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.1, 142.7, 138.2, 134.6, 128.8, 126.9, 126.1, 125.6, 116.9, 60.5, 52.3, 50.9, 47.1, 29.8, 26.6, 24.9, 23.6, 22.7, 21.3, 11.3. HRMS *m/z* calcd. for $C_{25}H_{34}N_4O$ $[M + H]^+$ 407.2805; found: 407.2811.

(5-((4-fluorobenzyl)(methyl)amino)-1-propyl-4,5,6,7-tetrahydro-1H-indazol-3-yl)(piperidin-1-yl)methanone (7m).—Prepared according to General Procedure C from **6b** to afford **7m** as a colorless oil (45%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (dd, *J*=8.54, 5.49 Hz, 2 H), 6.95 – 7.01 (m, 2 H), 3.91 (t, *J*=7.17 Hz, 2 H), 3.78 (br. s., 2 H), 3.69 (br. s., 2 H), 3.57 – 3.66 (m, 2 H), 2.88 – 2.96 (m, 2 H), 2.73 – 2.79 (m, 1 H), 2.55 – 2.69 (m, 2 H), 2.24 (s, 3 H), 2.11 (dd, *J*=12.51,3.36 Hz, 1 H), 1.79 – 1.83 (m, 2 H), 1.66 (br. s., 4 H), 1.57 (br. s., 2 H), 0.91 (t, *J*=7.32 Hz, 3 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.0, 142.7, 138.2, 130.2, 130.1, 117.2, 115.2, 115.0, 59.8, 57.3, 50.8, 48.3, 43.3, 37.6, 27.0, 26.4, 25.9, 24.9, 23.6, 21.8, 21.5, 11.3. HRMS *m/z* calcd. for $C_{24}H_{33}FN_4O$ $[M + H]^+$ 413.2711; found: 413.2713.

(5-(4-methylpiperazin-1-yl)-1-propyl-4,5,6,7-tetrahydro-1H-indazol-3-yl)(piperidin-1-yl)methanone (7n).—Prepared according to General Procedure C from **6b** to afford **7n** as a colorless oil (45%). ^{1}H NMR (500 MHz, $CDCl_3$) 3.87 – 3.95(m, 2 H), 3.72 (br. s., 2 H), 3.67 (br. s., 2 H), 2.93 (dd, *J*=15.41, 4.73 Hz, 1 H), 2.65 – 2.77 (m, 5 H), 2.40 – 2.62 (m, 4 H), 2.29 (s, 3 H), 2.16 (dt, *J*=9.84,2.25 Hz, 1 H), 1.75 – 1.94 (m, 4 H), 1.59 – 1.75 (m, 6 H),1.55 (br. s., 2 H), 0.87 – 0.96 (m, 3 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.1, 142.8, 138.1, 116.6, 60.9, 55.6, 50.8, 46.1, 25.9, 24.9, 23.6, 23.5, 21.2, 11.3. HRMS *m/z* calcd. for $C_{21}H_{36}N_5O$ $[M + H]^+$ 374.2914; found 374.2918.

2-(2-((3-(Piperidine-1-carbonyl)-1-propyl-4,5,6,7-tetrahydro-1H-indazol-5-yl)amino)ethyl)guanidine (7o)—Prepared according to General Procedure C from **6b** to afford **7o** as a colorless glassy solid (95%). ^{1}H NMR (500 MHz, CD_3OD) δ 4.07 (t, J = 6.9 Hz, 2H), 3.83 – 3.59 (m, 7H), 3.41 (d, J = 6.4 Hz, 2H), 3.23 (dd, J = 15.4, 4.7 Hz, 1H), 3.00 (d, J = 16.7 Hz, 1H), 2.92 – 2.70 (m, 2H), 2.47 (d, J = 12.6 Hz, 1H), 2.11 (dd, J = 12.0, 6.0 Hz, 1H), 1.87 (q, J = 7.2 Hz, 2H), 1.75 (q, J = 5.6 Hz, 2H), 1.70 – 1.55 (m, 6H), 0.94 (t, J =

7.1 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 157.4, 140.8, 113.1, 65.5, 54.6, 50.5, 43.8, 37.7, 33.4, 24.8, 24.0, 23.7, 22.8, 18.8, 14.0, 9.9. HRMS *m/z* calcd. for $C_{19}H_{34}N_7O$ $[M + H]^+$ 376.2819; found 376.2823.

(5-((3-(Methylamino)propyl)amino)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)(piperidin-1-yl)methanone (7p).—Prepared according to General Procedure C from **6b** to afford **7p** as a colorless glassy solid (88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.87 (td, J = 7.0, 2.2 Hz, 1H), 3.75 (dq, J = 10.7, 6.8, 5.9 Hz, 1H), 3.69 – 3.53 (m, 1H), 3.07 (d, J = 23.9 Hz, 1H), 2.78 (dd, J = 17.7, 7.0 Hz, 1H), 2.65 (q, J = 6.7, 4.3 Hz, 1H), 2.45 – 2.30 (m, 1H), 1.78 (p, J = 7.3 Hz, 1H), 1.70 – 1.49 (m, 2H), 0.89 (q, J = 7.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.6, 141.9, 137.5, 113.7, 55.0, 51.0, 48.5, 46.9, 43.7, 42.6, 33.5, 26.7, 25.8, 25.3, 24.7, 24.3, 23.4, 19.5, 11.2. HRMS *m/z* calcd. for $C_{20}H_{36}N_5O$ $[M + H]^+$ 362.2914; found 362.2913.

(5-(4-(4-Nitrophenyl)piperazin-1-yl)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)(piperidin-1-yl)methanone (7q).—Prepared according to General Procedure C from **6b** to afford **7q** as a colorless oil (41%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 2H, FA), 8.16 – 8.03 (m, 2H), 6.92 – 6.69 (m, 2H), 4.00 – 3.72 (m, 4H), 3.62 (dt, J = 25.0, 5.3 Hz, 6H), 3.34 – 3.19 (m, 1H), 3.12 (ddq, J = 21.5, 11.0, 5.1 Hz, 5H), 2.87 – 2.54 (m, 3H), 2.46 – 2.26 (m, 1H), 1.94 – 1.71 (m, 3H), 1.71 – 1.46 (m, 6H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 163.3, 154.1, 142.1, 139.4, 137.5, 125.9, 115.4, 113.4, 61.2, 50.9, 48.3, 47.8, 45.9, 43.5, 26.7, 25.7, 25.1, 24.6, 23.4, 21.6, 20.7, 11.1. HRMS *m/z* calcd. for $C_{26}H_{36}N_6O_3$ $[M + H]^+$ 481.2922; found: 481.2934.

4-((4-(3-(Piperidine-1-carbonyl)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-5-yl)piperazin-1-yl)methyl)benzonitrile (7r).—Prepared according to General Procedure C from **6b** to afford **7r** as a colorless oil (32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 2H), 7.61 (d, J = 7.9 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 3.88 (tt, J = 9.2, 4.6 Hz, 4H), 3.72 – 3.53 (m, 4H), 3.48 – 3.34 (m, 1H), 3.13 (dq, J = 26.5, 11.2, 8.4 Hz, 5H), 2.84 – 2.57 (m, 7H), 2.50 – 2.37 (m, 1H), 1.98 (s, 1H), 1.80 (dq, J = 21.9, 7.3, 6.8 Hz, 3H), 1.72 – 1.44 (m, 6H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 163.1, 142.9, 142.2, 137.4, 132.3, 129.4, 118.7, 115.1, 111.4, 61.4, 61.0, 50.9, 50.3, 48.2, 43.4, 26.8, 25.8, 24.9, 24.7, 23.4, 21.0, 20.7, 11.1. HRMS *m/z* calcd. for $C_{28}H_{38}N_6O$ $[M + H]^+$ 475.3193; found: 475.3181.

Piperidin-1-yl(5-((2-(piperidin-1-yl)ethyl)amino)-1-propyl-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)methanone (7s).—Prepared according to General Procedure C from **6b** to afford **7s** as a colorless oil (49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 4H, FA), 8.39 (s, 2H), 3.88 (t, J = 7.2 Hz, 2H), 3.78 (t, J = 5.4 Hz, 2H), 3.63 (d, J = 5.7 Hz, 2H), 3.36 – 3.14 (m, 4H), 3.14 – 2.95 (m, 2H), 2.86 (d, J = 10.6 Hz, 4H), 2.78 – 2.49 (m, 3H), 2.31 – 2.17 (m, 1H), 2.05 – 1.89 (m, 1H), 1.85 – 1.36 (m, 14H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 163.4, 142.2, 137.4, 114.5, 77.3, 77.0, 76.8, 54.6, 53.8, 53.7, 50.8, 48.2, 43.3, 40.5, 26.7, 25.7, 24.9, 24.7, 23.5, 23.3, 22.5, 19.3, 11.1. HRMS *m/z* calcd. for $C_{23}H_{39}N_5O$ $[M + H]^+$ 402.3227; found: 402.3232.

Piperidin-1-yl(1-propyl-5-(4-(pyridin-4-yl)piperidin-1-yl)-4,5,6,7-tetrahydro-1*H*-indazol-3-yl)methanone (7t)—Prepared according to General Procedure C from **6b** to afford **7t** as a colorless oil (34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 2H), 7.24 (d, J = 5.3 Hz, 2H), 3.94 (tt, J = 7.9, 4.1 Hz, 4H), 3.71 (t, J = 5.2 Hz, 2H), 3.57 (dddd, J = 24.6, 12.6, 5.6, 2.8 Hz, 3H), 3.21 (dd, J = 15.1, 4.8 Hz, 1H), 3.09 (td, J = 12.3, 2.8 Hz, 1H), 2.95 – 2.62 (m, 5H), 2.55 (ddd, J = 12.6, 5.3, 2.5 Hz, 1H), 2.32 (tt, J = 12.8, 9.2 Hz, 2H), 2.11 – 1.99 (m, 2H), 1.94 (dq, J = 11.9, 6.2 Hz, 1H), 1.84 (h, J = 7.3 Hz, 2H), 1.77 – 1.52 (m, 6H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 163.0, 153.0, 149.4, 142.2, 137.4, 122.4, 115.1, 77.3, 77.0, 76.8, 61.4, 51.1, 50.9, 48.2, 45.7, 43.4, 40.4, 29.8, 29.5, 26.8, 25.8, 25.1, 24.7, 23.3, 20.8, 20.7, 11.1. HRMS *m/z* calcd. for $C_{26}H_{37}N_5O$ $[M + H]^+$ 436.3012; found: 436.3015.

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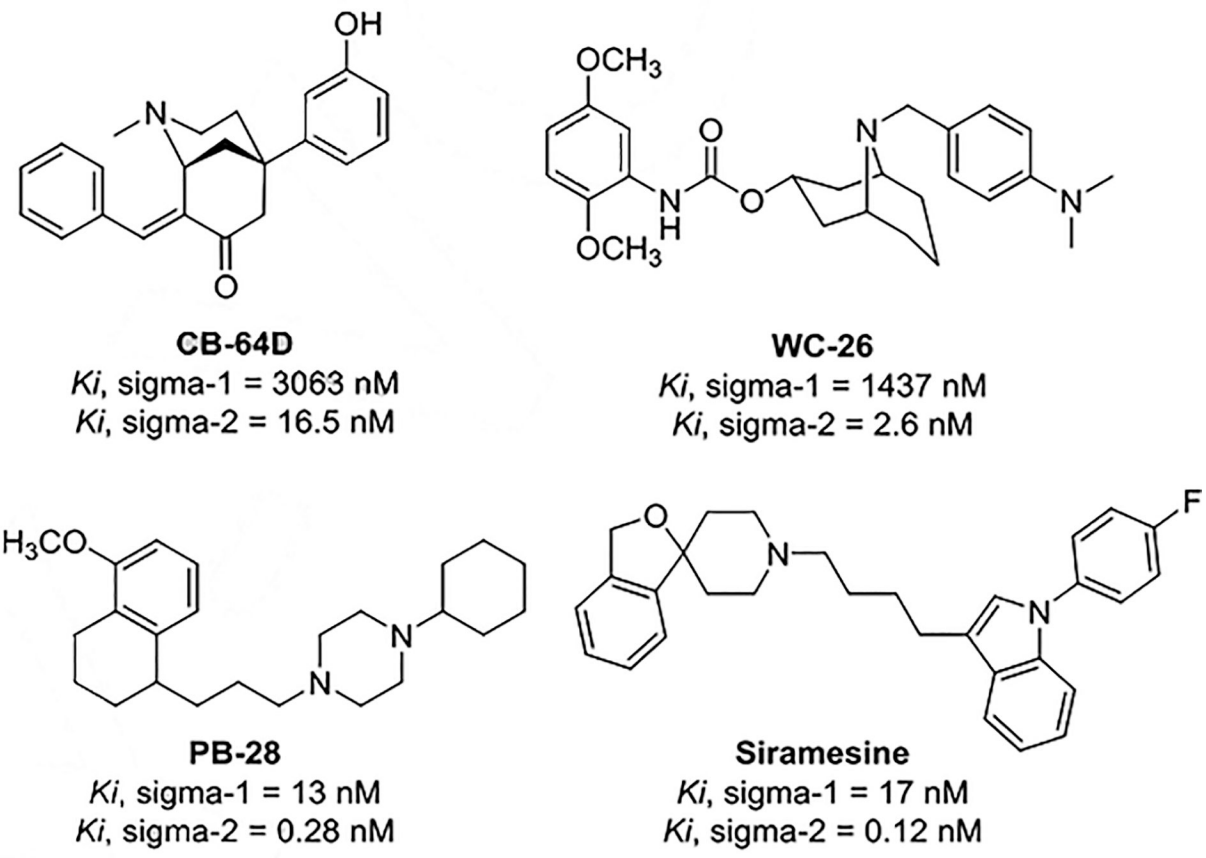


Figure 1.
Examples of known sigma-2 selective ligands.

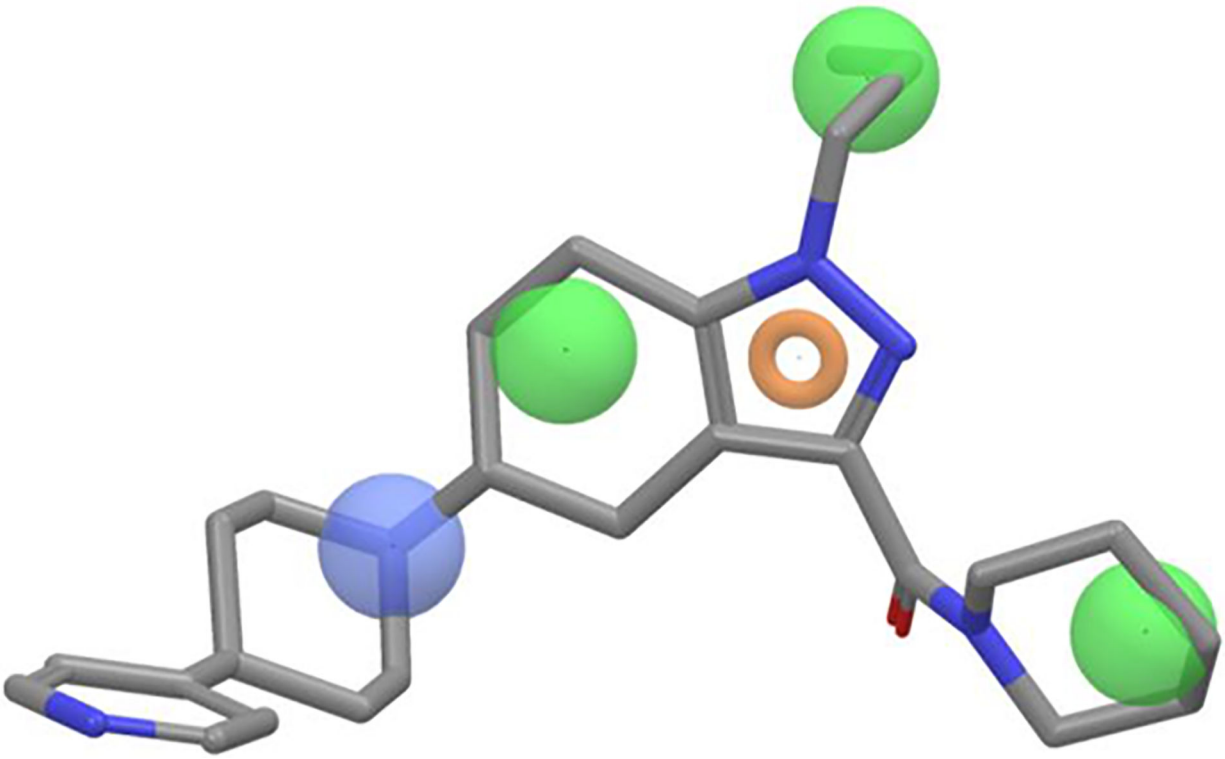
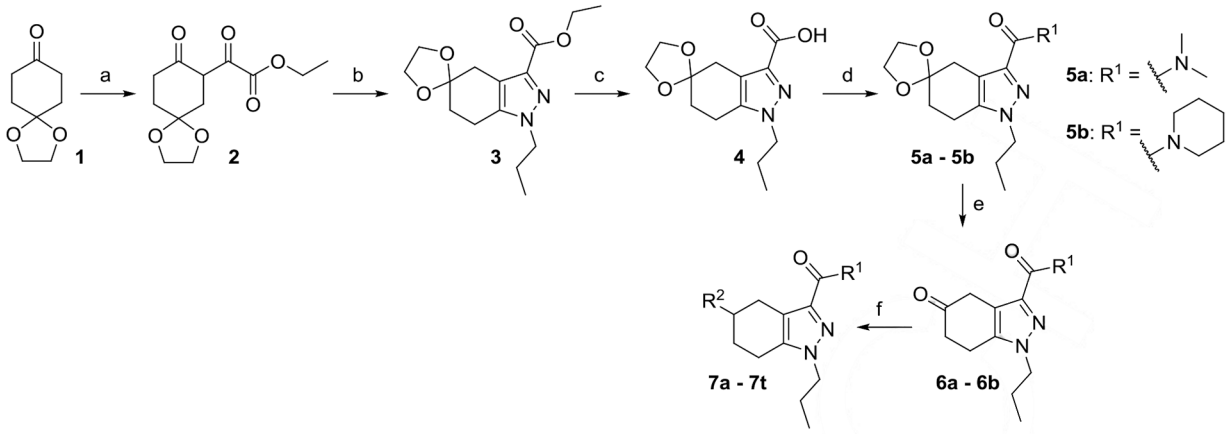


Figure 2.

Molecular modeling of potent sigma-2 ligands. The common-features based pharmacophore model was generated based on the sigma-2 binding SAR. The pharmacophore consists of thee hydrophobic groups (green spheres), one positive ionizable group (blue sphere), and one ring aromatic feature (orange circle). The 5-point pharmacophore model is shown overlaid with compound **7t**.

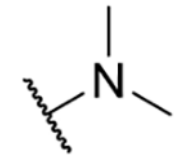
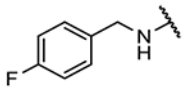
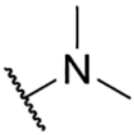
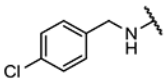
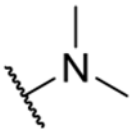
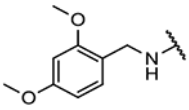
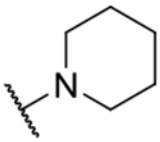
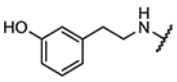
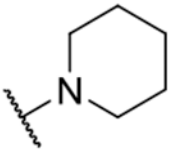
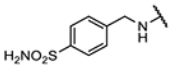


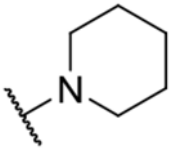
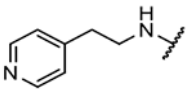
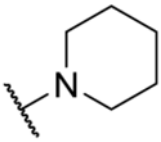
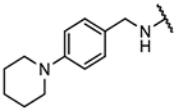
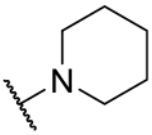
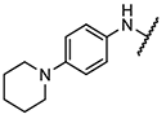
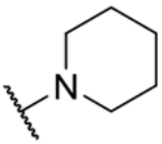
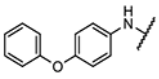
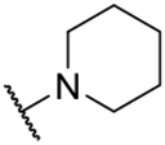
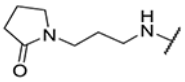
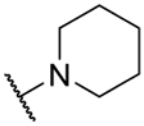
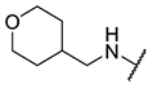
Scheme 1.

Reagents and conditions: a) diethyloxalate, LDA, THF, −78°C to rt, 16 h, 68%; b) propyl hydrazine, K_2CO_3, EtOH, rt, 3 h, 82%; c) NaOH, EtOH, rt, 4 h, 84%; d) R^1NH, EDC, HOBt, TEA, DCM, rt, 8 h, 44% for **5a** and 67% for **5b**; e) 3N HCl, THF, 50 °C, 2 h, 73% for **6a** and 85% for **6b**; f) R^2NH_2, AcOH, 1,2-DCE, 30 min, then $NaBH(OAc)_3$ overnight 32–95%..

Table 1.

Sigma Binding Affinity of Dimethyl and Piperidine Amides

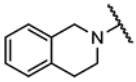
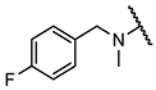
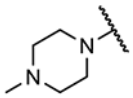
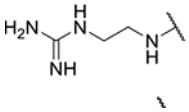
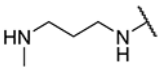
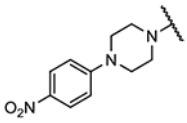
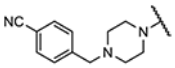
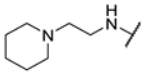
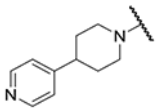
			$pK_i \pm SEM$ (M) K_i (nM)	
Cpd	**R^1**	**R^2**	**Sigma-1[a]**	**Sigma-2[a]**
7a			5.8 ± 0.05 1576	5.7 ± 0.06 2193
7b			<5	6.0 ±0.1 1019
7c			<5	6.1 ±0.09 839
7d			6.1 ±0.1 872	6.2 ±0.1 612
7e			<5	6.4 ±0.1 439

Cpd	R^1	R^2	$pK_i \pm SEM$ (M) Sigma-1[a]	K_i (nM) Sigma-2[a]
7f			<5	<5
7g			<5	6.8 ±0.1 169
7h			<5	6.1 ±0.1 787
7i			<5	6.5 ±0.1 310
7j			<5	6.2 ± 0.07 673
7k			<5	6.1 ±0.1 710

[a] Errors are reported as the Standard Error of the Mean (SEM) and are based on triplicate measurement in the binding assay.

Table 2.

Sigma Binding Affinity of Substituted Amines

Cpd	R2	pKi ± SEM (M) Ki (nM) Sigma-1[a]	Sigma-2[a]
7l		<5	7.5 ±0.1 31
7m		<5	7.7 ±0.1 19
7n		6.7 ±0.1 192	7.5 ±0.1 34
7o		<5	5.7 ±0.1 2156
7p		<5	5.7 ±0.1 1987
7q		5.8 ±0.1 1591	6.3 ±0.1 526
7r		<5	6.0 ±0.1 1099
7s		<5	6.4 ±0.1 434
7t		<5	7.8 ±0.1 16

[a] Errors are reported as the Standard Error of the Mean (SEM) and are based on triplicate measurement in the binding assay.

Table 3.

In vitro microsomal stability and solubility

Compound	Microsomal Stability[a]	Solubility (μM)[b]
7a	21 ± 0.2	1783
7g	47 ± 0.3	463
7l	17 ±3.2	713
7m	0	2423
7s	54 ± 0.9	1743

[a] Mouse liver microsomes, % remaining after 60-minute incubation at 37 °C (reactions were run in duplicate).

[b] Solubility measured in PBS buffer at pH=7.4.