Bioorganic & Medicinal Chemistry 18 (2010) 1822–1833

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

A concise synthesis of 1,4-dihydro-[1,4]diazepine-5,7-dione, a novel 7-TM receptor ligand core structure with melanocortin receptor agonist activity

Jerzy R. Szewczyk [a,*], Chris P. Laudeman [d], Doug M. Sammond [a], Manon Villeneuve [c], Douglas J. Minick [c], Mary K. Grizzle [b], Alejandro J. Daniels [b], John L. Andrews [b], Diane M. Ignar [b]

[a] Department of Medicinal Chemistry, Five Moore Drive, PO Box 13398, Research Triangle Park, NC 27709-3398, United States
[b] Department of Metabolic Diseases, Five Moore Drive, PO Box 13398, Research Triangle Park, NC 27709-3398, United States
[c] Department of Analytical Chemistry GlaxoSmithKline, Five Moore Drive, PO Box 13398, Research Triangle Park, NC 27709-3398, United States
[d] Seachaid Pharmaceuticals, Inc. 801 Capitola Drive, Ste. 5 Durham, NC 27713, United States

ARTICLE INFO

Article history:
Received 9 June 2008
Revised 16 January 2010
Accepted 20 January 2010
Available online 25 January 2010

Keywords:
G protein-coupled receptors
Agonist
MC4R
MC1R
Tetrahydrodiazepine-5,7-dione
Food intake

ABSTRACT

Finding small non-peptide molecules for G protein-coupled receptors (GPCR) whose endogenous ligands are peptides, is a very important task for medicinal chemists. Over the years, compounds mimicking peptide structures have been discovered, and scaffolds emulating peptide backbones have been designed. In our work on GPCR ligands, including cholecystokinin receptor-1 (CCKR-1) agonists, we have employed benzodiazepines as a core structure. Looking for ways to reduce molecular weight and possibly improve physical properties of GPCR ligands, we embarked on the search for molecules providing similar scaffolds to the benzodiazepine with lower molecular weight. One of our target core structures was 1,4-dihydro-[1,4]diazepine-5,7-dione. There was not, however, a known synthetic route to such molecules. Here we report the discovery of a simple and concise method for synthesis of 2-[6-(1*H*-indazol-3-ylmethyl)-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-[1,4]diazepin-1-yl]-*N*-isopropyl-*N*-phenyl-acetamide as an example of a compound containing the tetrahydrodiazepine-5,7-dione core. Compounds from this series were tested in numerous GPCR assays and demonstrated activity at melanocortin 1 and 4 receptors (MC1R and MC4R). Selected compounds from this series were tested in vivo in Peptide YY (PYY)-induced food intake. Compounds dosed by intracerebroventricular and oral routes reduced PYY-induced food intake and this effect was reversed by the cyclic peptide MC4R antagonist SHU9119.

1. Introduction

Identifying small molecule non-peptide agonists for receptors for which a peptide is the endogenous ligand, has been a very important undertaking for medicinal chemists. For many years, morphine and related opioids were the only examples of non-peptide ligands for peptide receptors. Discovery in the last decade of non-peptide agonists for angiotensin II,[1] growth hormone secretagogues[2–4] cholecystokinin receptors[5] and melanocortin receptors[6,7] has changed the landscape.

At GSK, we have employed benzodiazepine as a core for our CCK R-1 agonists.[8–10] In the search for an alternative template providing a similar arrangement of substituents to benzodiazepine, we turned our interest to tetrahydrodiazepines-5,7-diones. Unfortunately compounds with this core structure have not been described in the chemical literature. Retrosynthetic analysis led us to propose the following (Scheme 1) approach.

2. Results and discussion

2.1. Chemistry

We decided to utilize the reaction between an aldehyde diacetal and an amide as a critical step for seven-membered ring closure. A similar approach was also used by others in the synthesis of six membered rings.[11,12]

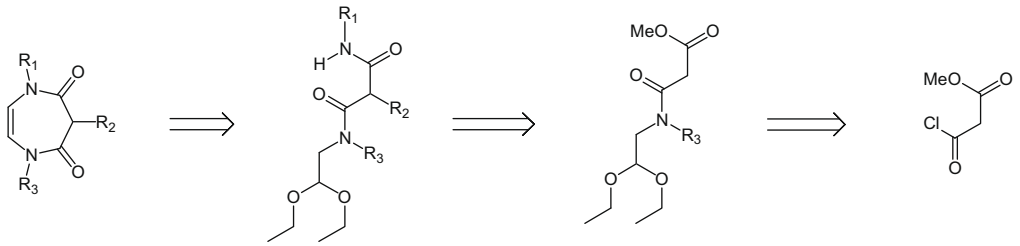
Compound **7** was prepared via a short synthesis beginning with the reaction of 2-(*N*-anilino)acetaldehyde diethyl acetal **1** with methyl malonylchloride **2** in the presence of base, to give amide **3** in 75% yield. Subsequent ester hydrolysis afforded the malonic mono-amide, mono-carboxylic acid **4** in 98% yield (Scheme 2).

Separately, 2-bromo-*N*-isopropyl-*N*-phenyl-acetamide was prepared by a previously described method.[10] Aminolysis of the 2-bromoacetamide **5** afforded the N-substituted glycine amide **6** in 88% yield (Scheme 3).

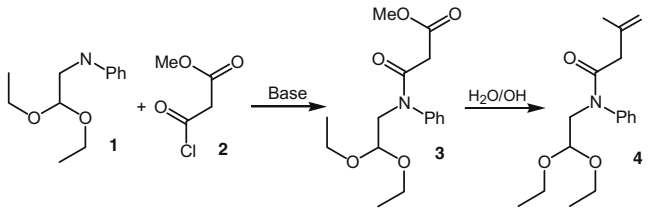
Coupling of the malonic mono-amide mono-carboxylic acid **4** with the N-substituted glycine amide **6**, in the presence of 1 equiv of EDC, yielded compound **7** in 85% yield. Subsequent

* Corresponding author. Tel.: +1 919 408 0512.
E-mail address: kjszewczyk@verizon.net (J.R. Szewczyk).

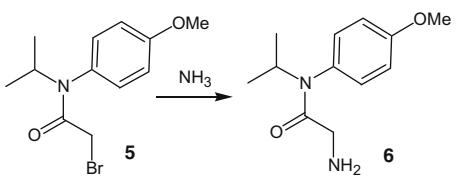
0968-0896/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2010.01.049



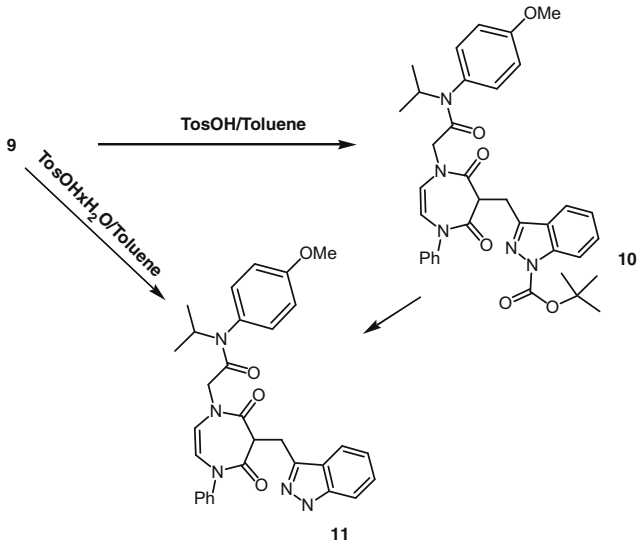
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 5.

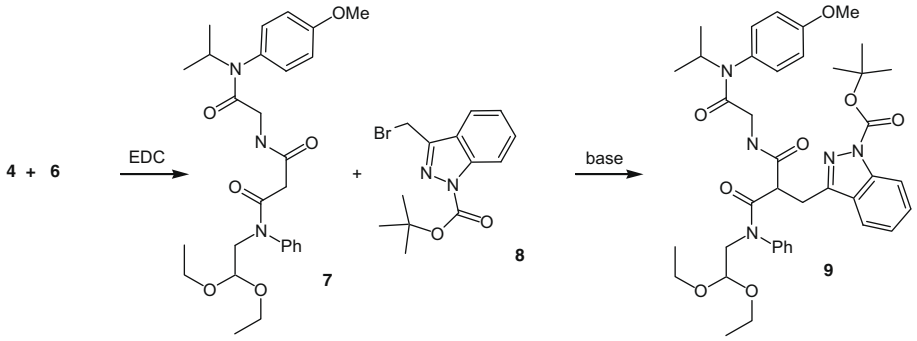
alkylation of the compound **7** with *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate **8** (prepared as described previously)[9] provided a racemic mixture of compound **9** in 78% yield (Scheme 4).

Treatment of compound **9** with anhydrous *p*-toluenesulfonic acid in toluene led to the acid-catalyzed reaction of the acetal and the amide resulting in cyclization to give the 2,4-dioxo-1,5-diazepine compound **10** in 93% yield. Treatment of compound **10** with trifluoroacetic acid in methylene chloride afforded diazepine **11** in 95% yield. Alternatively treatment of compound **9** with *p*-toluenesulfonic acid hydrate in toluene provided final product **11** (69% yield) in a one step conversion (Scheme 5).

2.2. SAR

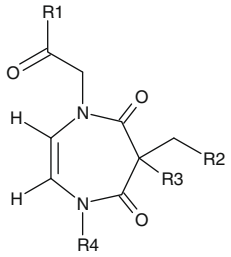
Compound **11** was tested in numerous GPCR assays and showed agonist activity at MC1R and MC4R. Since it is important to have receptor-selective compounds, we embarked on development of SAR around this series to obtain MC1R- and MC4R-selective compounds. We used an identical synthetic route to synthesize all racemic compounds listed in Table 1 (compounds **11–35**). Enantiomers **11-(*S*)** and **11-(*R*)** were isolated by chiral preparative HPLC of the respective racemic mixtures. Relative to α-MSH, compounds tended to be both more potent and efficacious at MC1R, thus the greater challenge was to find molecules that are selective for MC4R. Further, SAR for MC4R was very narrow, and even small changes caused loss of agonist activity. However, some trends did emerge.

We have found that the acetanilide moiety at N-1 could be modified in a number of ways: *para* substitution of the phenyl ring with small groups (OMe, OCF3, OH, F) enhanced activity on both receptors, but the large polar group morpholine led to a loss of



Scheme 4.

Table 1
SAR table for MC4R and MC1R agonist activity in reporter gene assays



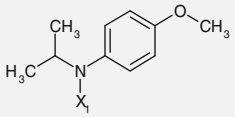
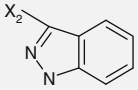
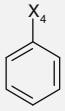
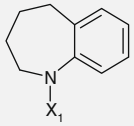
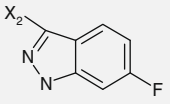
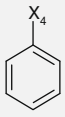
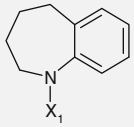
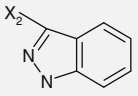
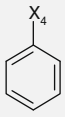
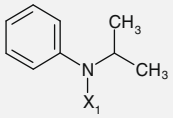
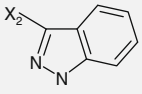
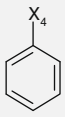
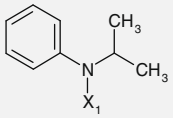
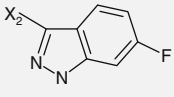
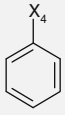
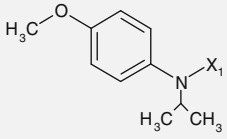
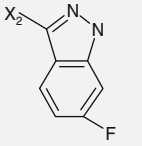
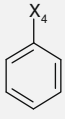
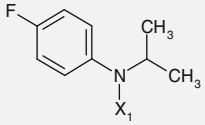
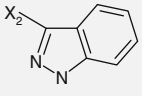
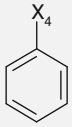
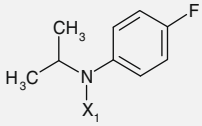
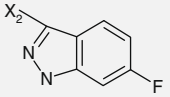
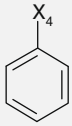
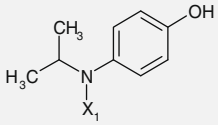
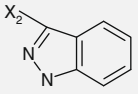
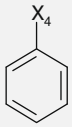
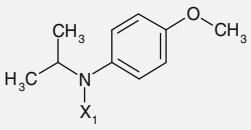
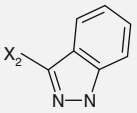
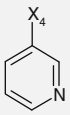
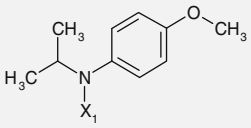
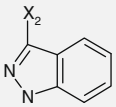
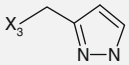
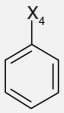
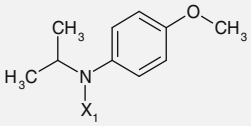
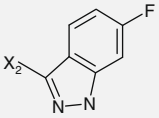
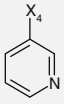
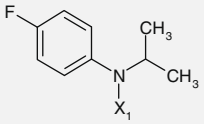
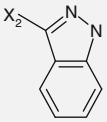
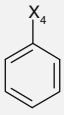
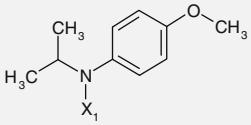
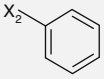
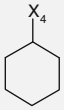
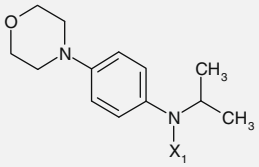
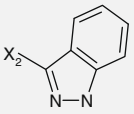
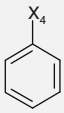
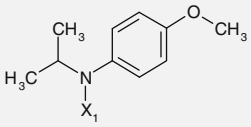
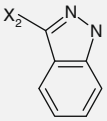
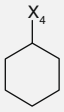
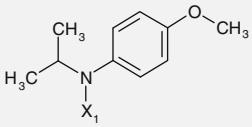
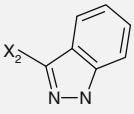
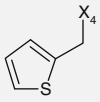
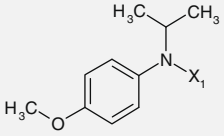
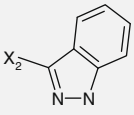
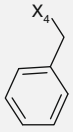
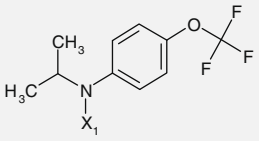
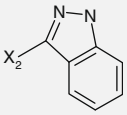
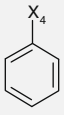
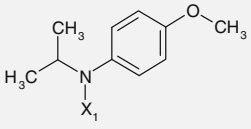
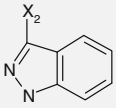
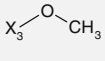
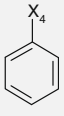
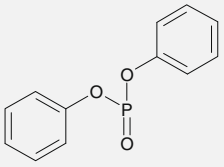
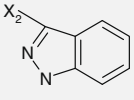
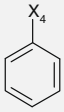
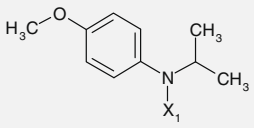
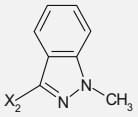
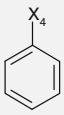
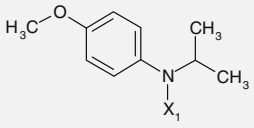
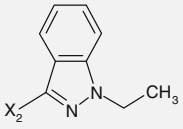
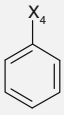
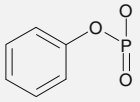
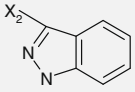
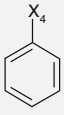
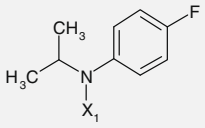
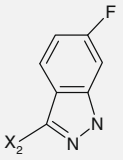
#	R1	R2	R3	R4	MC4R		MC1R	
					pEC_{50}	% of alpha-MSH	pEC_{50}	% of alpha-MSH
11			**H**		**6.15**	**45**	7.53	68
11-(*S*)	Enantiomer *S* of compound **11**				**4.50**	**14***	5.81	28
11-(*R*)	Enantiomer *R* of compound **11**				**6.47**	**53**	7.80	82
12			**H**		**6.36**	**16**	8.80	61
13			**H**		**6.49**	**17**	8.80	61
13-(*S*)	Enantiomer *S* of compound **13**				**6.10**	**20**	7.21	76
13-(*R*)	Enantiomer *R* of compound **13**				**7.00**	**27**	8.80	86
14			**H**		**5.49**	**22**	6.15	42
14-(*S*)	Enantiomer *S* of compound **14**				**4.50**	**3***	4.50	9*
14-(*R*)	Enantiomer *R* of compound **14**				**5.97**	**26**	6.53	54
15			**H**		**6.27**	**19**	6.73	51
15-(*S*)	Enantiomer *S* of compound **15**				**4.50**	**0***	4.50	2*
15-(*R*)	Enantiomer *R* of compound **15**				**5.90**	**23**	7.45	79
16			**H**		**6.45**	**39**	8.80	83
17			**H**		**6.55**	**22**	7.30	54
18			**H**		**6.75**	**19**	7.85	53
19			**H**		**6.00**	**30**	6.26	54
19-a	1st Enantiomer of compound **19**				**6.00**	**44**	6.80	79
19-b	2nd Enantiomer of compound **19**				**4.50**	**5***	7.28	16

Table 1 (*continued*)

#	R1	R2	R3	R4	MC4R		MC1R	
					pEC_{50}	% of alpha-MSH	pEC_{50}	% of alpha-MSH
20			**H**		**5.54**	**31**	6.75	60
21					**5.98**	**16**	6.40	30
22			**H**		**6.18**	**31**	7.90	65
23					**7.35**	**52**	5.53	33
24			**H**		**4.50**	**0***	4.50	0*
25			**H**		**4.50**	**3***	6.08	22
26			**H**		**4.50**	**19***	4.50	25*
27			**H**		**4.50**	**10***	5.93	43
28			**H**		**4.50**	**6***	6.10	59
29			**H**		**6.82**	**78**	7.65	91
30					**6.78**	**74**	5.86	58
31			**H**		**4.50**	**5***	4.50	1*

(*continued on next page*)

Table 1 (*continued*)

#	R1	R2	R3	R4	MC4R		MC1R	
					pEC_{50}	% of alpha-MSH	pEC_{50}	% of alpha-MSH
32			**H**		**4.50**	**5***	4.50	6*
33			**H**		**4.50**	**5***	4.50	2*
34			**H**		**4.50**	**4***	4.50	3*
35			**H**		**4.50**	**5***	5.43	62

4.5 Denotes that EC_{50} is higher than 30 μM.
* Denotes efficacy at 10 μM.

MC4R activity while only slightly diminishing MC1R activity. Modification from the isopropyl phenyl amide to 2,3,4,5-tetrahydro-1*H*-benzazepine did not dramatically affect MC4R activity but led to compounds such as number **12** with high potency on MC1R. With $pEC_{50} = 8.8$ on MC1R, these compounds demonstrated >100-fold selectivity over MC4R. An isosteric phosphonate group in place of the anilide moiety at N-1 rendered compounds inactive on both receptors. We found even less tolerance for changes at N-4.

A simple phenyl group is preferred at N-4. While substantial changes are tolerated for MC1R, only a pyridyl group preserved some MC4R activity. Replacing phenyl with cyclohexyl resulted in a total loss of activity at both MC4R and MC1R. Changing to

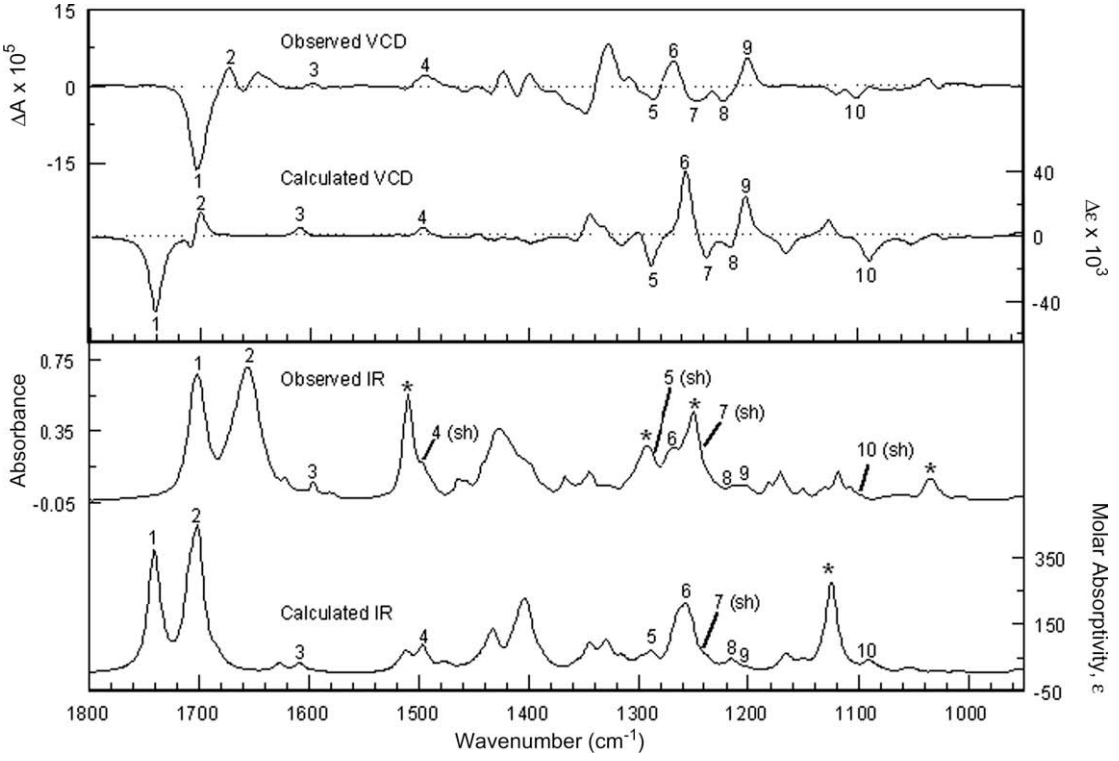


Figure 1. VCD (upper panel) and IR spectra (lower panel) observed for compound **11-(*R*)** are compared with corresponding spectra calculated for the reduced-structure model (Fig. 2). The 10 VCD 'marker' bands were used to make the configurational assignments in this study. Absorption bands identified by (*) in the experimental IR data are due to vibrational modes of the functional groups omitted from the model. The band near 1125 cm^{-1} in the calculated IR spectrum is due to the *N*,*N*-dimethyl group (C–N stretch) in the model.

benzyl and to a lesser extent 2-thienylmethyl, seen in compounds **27** and **28**, retained some MC1R activity but abolished all MC4R activity. Truncation to methyl at N-4 gave a compound with weak activity on only MC1R. While modifications at N-1 and N-4 provided compounds selective for MC1, changes at C-6 led to compounds with enhanced selectivity for MC4R over MC1R.

The indazole at C-6 was found to be necessary for activity. So, for the purposes of this study, substituents at C-6 were limited to primarily 1*H*-indazol-3-ylmethyl and its 6-fluoro analog. The presence of the 6-fluoro substituent on the indazole group tended to increase potency at both MC4R and MC1R. This was especially the case when the *p*-methoxy anilide at N-1 was incorporated along with the 6-fluoro indazole at C-6 which provided compound **16** with pEC_{50} = 8.80 at MC1R. However, introduction of a methoxy group at C-6 along with the 1*H*-indazol-3-ylmethyl moiety led to compounds with 10–50-fold selectivity for MC4R. The greatest enhancement of MC4R activity was seen when the *p*-fluoro anilide was utilized rather than the *p*-methoxy anilide at N-1. With the addition of the methoxy group at C-6, seen in compound **23**, this provided a sixfold increase in potency for MC4R while diminishing activity at MC1R nearly 60-fold. Introduction of a larger group, 1*H*-pyrazol-3-ylmethyl, led to decreased activity at both receptors. The greatest impact of this change was on MC1R with over 10-fold decrease in potency relative to α-MSH. To explore the effects of the chirality at C-6, several racemic mixtures were resolved to give the stereoisomers of the following compounds **11, 13, 14, 15,** and **19**. In most cases only one enantiomer was found to be active on MC4R, and the same enantiomer was more potent at MC1R. A notable exception is compound **19b** which had no measurable activity on MC4R but was found to have pEC_{50} = 7.3 on MC1R.

2.3. Vibrational circular dichroism calculation

In order to advance our understanding of the SAR of these compounds, we sought to determine the absolute configurations of compounds **11-(*S*)** and **11-(*R*)**, the enantiomers of compound **11** (Scheme 5). The absolute configurations of these molecules were assigned by ab initio vibrational circular dichroism (VCD) using the reduced-structure model shown in Figure 2. Reduced-structure modeling has been used successfully in VCD studies of larger molecules,[13b,14] and was used here to allow VCD calculations to be carried out at a higher level of theory, increasing the reliability of the simulated VCD spectrum. The VCD and IR spectra calculated for the reduced-structure model are compared in Figure 1 with experimental data observed for compound **11-(*R*)**. In the upper panel of the Figure 1, the calculated VCD spectrum is in very good qualitative agreement with experimental, indicating good characterization of the conformational state surrounding the chiral center and assuring reliable stereochemical assignments. As expected, the overall qualitative agreement between IR spectra is not as good due to replacement of the 4-methoxyphenyl and isopropyl groups in with methyl groups in the model.

A set of 10 bands are labeled in all spectra shown in Figure 1. These 'marker' bands are due to vibrational modes of functional groups in common with both the model and compound **11-(*R*)** and were first identified in the calculated IR and VCD spectra, then assigned in experimental data. In the upper panel of this figure, the signs of the marker bands are the same in the VCD spectra of compound **11-(*R*)** and the model, indicating that this molecule has the same absolute configuration as the model. In the comparison of compound **11-(*S*)** and the model (not shown), the 10 VCD marker bands were oppositely signed, indicating that the stereocenter in this molecule is the mirror image of the model. Based on these results, compound **11-(*S*)** was assigned as the (6S)-enantiomer and compound **11-(*R*)** as the (6*R*)-enantiomer (Fig. 3).

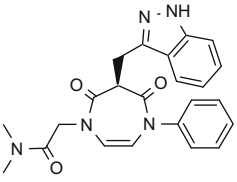


Figure 2. Reduced structured model: 2-[(6*R*)-(1*H*-Indazol-3-ylmethyl)-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-[1,4]diazepin-1-yl]-*N*,*N*-dimethyl-acetamide.

2.4. In vivo activity

11-(*R*) (10 mg/kg) was orally administered to Sprague Dawley rats as a suspension in 50% PEG/25% EtOH/25% H_2O and plasma samples were taken to generate a pharmacokinetic profile (Table 2). Compound 37 had low oral bioavailability and exposure and a short half-life.

In order to test the efficacy and MC4R-specificity of compound **11** [racemic mixture containing **11-(*R*)**], 1 nmol SHU9119, an cyclic peptide MC4R antagonist (Ac-Nle-c[Asp-His-*D*Nal(2′)-Arg-Trp-Lys]-NH_2), was administered by intracerebroventricular (ICV) injection to lean rats 30 min before ICV administration of vehicle or 100 pmol compound **11**. Later (15 min), peptide YY (PYY), an orexigenic agent, was administered ICV and food intake was monitored for 3 h. Compound **11** significantly inhibited PYY-induced food intake during the course of the study and this effect was completely reversed by SHU9119 (Fig. 4).

Oral administration of 5 or 25 mg/kg of compound **11-(*R*)** to sated lean rats antagonized the orexigenic effect of subsequent ICV administration of 0.23 nmol PYY (Fig. 5). In the vehicle group, PYY administration induced food intake during the light cycle period when rats are normally inactive with respect to food intake. Both doses of compound **11-(*R*)** inhibited PYY-induced food intake to the same extent. The natural cycle of nocturnal feeding began at 6 PM when lights were turned off and continued until lights were turned on at 6 AM. During this period, the 25 mg/kg dose of compound **11-(*R*)** continued to fully suppress food intake.

In contrast to these observations, administration of 25 mg/kg **11-(*R*)** to lean rats only mildly suppressed food intake (~20%) when given just prior to the onset of nocturnal cycle (data not shown). The mechanism underlying this discrepancy between efficacy observed with or without PYY treatment is not understood.

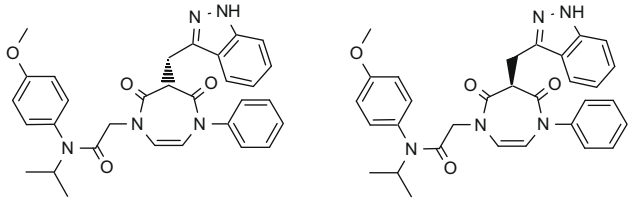


Figure 3.

Table 2
PK properties of **11-(R)**

Dose		C_{max} (ng/mL)	T_{max} (h)	AUC_{inf} (h nf/mL)	Bioavail (%)	$t_{1/2}$ (h)
mg/kg	Route					
10	PO	123	0.67	260	6.2	1.5

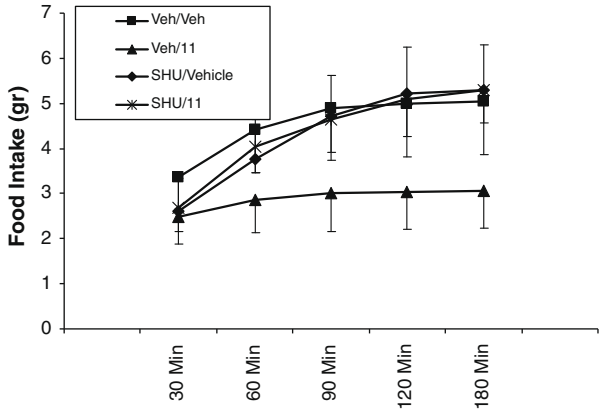


Figure 4. Vehicle (saline) or 1 nmol SHU9119 was administered 30 min before vehicle (2% DMSO/saline) or 100 pmol of compound **11**. Later (15 min), 0.23 nmol PYY was administered to all rats. Treatments were administered ICV. $N = 7–8$ per group. Compound **11** significantly suppressed PYY-induced food consumption at all time points from 60 min to end of study ($p < 0.05$).

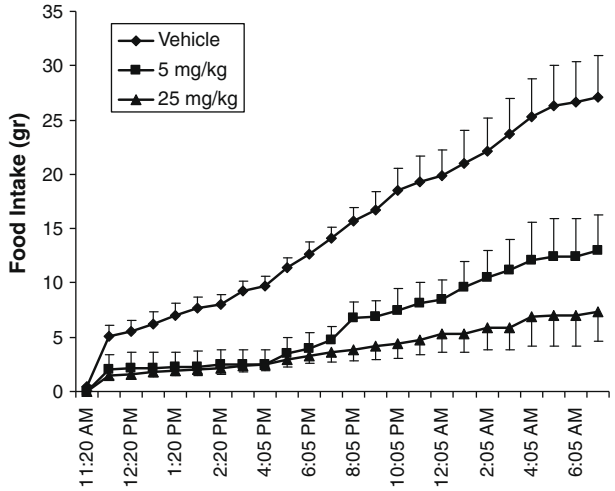


Figure 5. Vehicle (50% PEG500, 25% EtOH, 25% water) or compound **11-(*R*)** were administered by oral gavage at 10:30 AM. 0.23 nmol of PYY was administered ICV to all rats at 11:00 AM. The dark cycle started at 6 PM and ended at 6 AM. $N = 5$/group. Both 5 and 25 mg/kg of compound **11-(*R*)** significantly suppressed food consumption during the entire experiment ($p < 0.05$).

One possibility could be that the presence of PYY during the daylight hours reduces the sensitivity of the system to orexigenic factors during the dark cycle rendering the MC4R agonist more effective than in the basal state.

3. Conclusions

MC4R is expressed in areas of the brain thought to be involved in control of food consumption. Inactivation of receptor activity through mutation results in obesity in rodents and humans, thus, agonists of MC4R are being sought as therapeutics for obesity. In addition to expression in melanocytes, MC1R is also expressed on immune cells such as macrophages and is thought to mediate the anti-inflammatory effect of MSH.

We report in this manuscript, the development of a new and concise method for the synthesis of tri-substituted diazepinediones, a novel class of compounds with agonist activity at MC1R and MC4R. While this particular scaffold consistently provided compounds more potent at MC1R, we established that addition of a methoxy group at C-6 imparts significant MC4R selectivity. Further, the chirality at C-6 was found to strongly influence both potency and efficacy. This finding of the necessary geometry at this position will inform future SAR elucidation, and all reported findings can now be combined in the hope of finding more potent and selective agonists for MC4R. It is clear that additional work is necessary to fully determine the scope of SAR for diazepinediones, and future work will include in vivo evaluation of compounds in this class.

4. Experimental

4.1. Receptor pharmacology

Reporter gene assays for melanocortin receptors were performed as described previously.[15] CHO-6xCRE-luc+ reporter cell lines expressing human MC1R, MC3R, MC4R, and MC5R and host cell line were harvested with trypsin 48 h prior to assay and plated at 4000 cells/well in DMEM/F12 with 10% FBS in 96 well plates. Prior (16 h) to the assay, the medium was replaced with 90 μl/well of serum-free DMEM/F12. At the time of the assay the compounds were added in a 10 μl volume and plates were incubated for 4 h at 37 °C in a cell culture incubator. Medium was then aspirated and 50 μL of 1:1 mixture of LucLite® and dPBS/1 mM $CaCl_2$/1 mM $MgCl_2$ was added. Plates were sealed and subjected to dark adaptation at room temperature for 10 min before luciferase activity was quantitated on a TopCount™ microplate scintillation counter (Packard) using 3 s/well count time. Compound activity was expressed as a percentage of the fold stimulation of NDP-αMSH (melanocyte-stimulating hormone) control for each receptor subtype. The control value is the average of duplicate wells treated with 1×10^{-7} M NDP-αMSH.

4.2. Food consumption experiments

Lean Sprague-Dawley rats weighing approximately 400 g were fitted with indwelling cannula in the third ventricle (Charles River Laboratories, Raleigh, NC). Food consumption was monitored every half hour in cages fitted with accuscan systems (AccuScan Instruments Inc., DietMax Food Consumption System, Columbus, OH). Rats were acclimated to the chambers and powdered standard laboratory chow (Purina Chow 5001 Richmond, IN). Animals were handled prior to study by removing from chamber, placement in cage where injections occur, removing headcap and manipulating cannula.

In Figure 4, the vehicle for 11 was sterile saline containing 2% DMSO. PYY (Quality Controlled Biochemicals, Hopkinton, MA) and SHU9119 were dissolved in sterile water for ICV delivery. Volumes of injection were 1 μl for PYY and SHU9119 and 2 μl for 11. In Figure 5, the vehicle for oral administration of compound **11-(*R*)** was 50% PEG500/25%ETOH/25% water, PYY was delivered ICV in sterile saline water.

All procedures were performed in compliance with the Animal Welfare Act, USDA regulations and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee. Animals were housed at 72 F and 50% relative humidity with a 12-h light and dark cycle.

4.3. Analytical chemistry

VCD and IR spectra were acquired in the mid-infrared region (2000–950 cm^{-1}) using a Chiral*ir*™ VCD spectrometer (Bomem/BioTools) equipped with a dual photoelastic modulator (dual PEM). Spectra were acquired at 4 cm^{-1} resolution with a scan

speed of approx. 50 scans per minute. The unpolarized IR spectrum and accompanying VCD spectrum were acquired for 12 and 180 min, respectively. Samples were dissolved in $CDCl_3$ at a concentration of 58 mg/mL and spectral measurements made using a sealed transmission cell with BaF_2 windows, a 0.1-mm pathlength and an internal volume of approximately 75 μl. The measured VCD spectra (VCD_{obs}) were corrected for baseline artifacts by subtracting the enantiomer spectra from each other:

$$1/2\{[VCD_{obs}(-)] - [VCD_{obs}(+)]\} \text{ and } 1/2\{[VCD_{obs}(+)] - [VCD_{obs}(-)]\}$$

Due to the relatively large size of these optical isomers, the reduced-structure model in Figure 2 was used instead of a full structure model. Geometry optimizations, harmonic vibrational frequencies, and VCD/IR intensities were calculated with the B3LYP density functional using the 6-311G(d,p) basis set. Calculations were carried out with the GAUSSIAN '03 (G03) program package.[16] For comparison with experimental, calculated vibrational frequencies were scaled using a uniform scaling factor of 0.98, and calculated IR and VCD line intensities converted to Lorentzian bands with 6 cm^{-1} half-width-at-half-height. Composite VCD and IR spectra were generated using the fractional populations of five model conformers estimated by Boltzmann statistics.[13]

4.4. Chemistry

4.4.1. Methyl 3-[(*N*-2,2-diethoxyethyl)-*N*-phenyl]amino-3-oxopropanoate (3)

A solution of 1.1 g (8.1 mmol) of methyl malonyl chloride (**2**) in chloroform (10 ml) was added to a solution of 1.67 g (8 mmol) of 2-(*N*-anilino)acetaldehyde diethyl acetal (**1**) and 0.79 g of pyridine in chloroform (20 ml) at 0 °C. The reaction mixture was stirred for 2 h, evaporated and the residue purified by column chromatography on silica gel (hexane 50%/ethyl acetate 50%), to afford 2 g (80%) of the titled product (**3**). ^{1}H NMR (300 MHz, $CDCl_3$): 7.38 (m, 3H); 7.26 (m, 2H), 4.82 (t, *J* = 5.6 Hz, 1H), 3.80 (d, *J* = 5.6 Hz, 2H), 3.67 (s, 3H), 3.60 (m, 4H), 3.20 (s, 2H), 1.16 (t, *J* = 7 Hz, 6H).

4.4.2. 3-[(*N*-2,2-Diethoxyethyl)-*N*-phenyl]amino-3-oxopropanoic acid (4)

To a solution of 2.0 g (6.5 mmol) of methyl 3-[*N*-(2,2-diethoxyethyl)-*N*-phenyl]amino-3-oxopropanoate (**3**) in THF (10 ml) and water (5 ml), 3.4 ml of 1 N aqueous solution of NaOH was added and resultant mixture was stirred overnight at room temperature. THF was removed in vacuo and 1 N aqueous solution of $NaHSO_4$ (3.5 ml) was added. The product was extracted with ethyl acetate (2 × 50 ml). The organic layers were combined, dried with anhydrous $MgSO_4$ and the solvent removed in vacuo to afford 1.87 g (98%) of product (**4**). ^{1}H NMR (300 MHz, $CDCl_3$) 7.38 (m, 3H), 7.26 (m, 2H), 4.82 (t, *J* = 5.6 Hz, 1H), 3.80 (d, *J* = 5.6 Hz, 2H), 3.67 (s, 3H), 3.60 (m, 4H), 3.20 (s, 2H), 1.16 (t, *J* = 7 Hz, 6H),

4.4.3. N^1-Isopropyl-N^1-(4-methoxyphenyl)glycinamide (6)

2.86 g (10 mmol) of 2-Bromo-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide (**5**) was dissolved in methanol saturated with ammonia at 0 °C and left for 3 days at room temperature in a sealed flask. Methanol and ammonia were removed in vacuo and the residue was dissolved in chloroform (100 ml) and washed with water (2 × 50 ml). The organic layer was separated and dried with anhydrous $MgSO_4$ and concentrated in vacuo to afford 1.95 g (88%) of compound (**6**). ^{1}H NMR (300 MHz, $CDCl_3$) 6.96 (m, 4H), 4.99 (sept., *J* = 6.6 Hz, 1H), 3.84 (s, 3H), 2.97 (s, 2H), 1.58 (s, 2H), 1.05 (d, *J* = 6.6 Hz, 6H).

4.4.4. *N*-(2,2-Diethoxyethyl)-*N*-phenylamino 2-[*N*'-isopropyl-(*N*'-4-methoxyphenylamino)-2-oxoethyl] Malonamide (7)

A solution of 0.6 g (2 mmol) of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide (**6**), 0.53 g (2 mmol) of 3-[(*N*-2,2-diethoxyethyl)-*N*-phenyl]amino-3-oxopropanoic acid (**4**) and 0.31 g of HOBT in DMF (5 ml) was cooled to 0 °C and 0.47 g of EDC was added in one portion. The reaction mixture was stirred overnight and poured into ice water and were extracted with ethyl acetate (3 × 15 ml). The combined organic layers were dried with anhydrous $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel (1% MeOH/99% $CHCl_3$), providing 900 mg (89%) of compound (**7**). ^{1}H NMR (300 MHz, $CDCl_3$) 8.21 (m, 1H), 7.28 (m, 6H), 6.99 (m, 2H), 6.88 (m, 2H), 4.95 (sept., *J* = 7 Hz, 1H), 4.80 (t, *J* = 5.6 Hz, 1H), 3.81 (s, 3H), 3.78 (m, 2H), 3.54 (m, 6H), 3.03 (s, 2H), 1.13 (t, *J* = 7 Hz, 6H), 1.03 (d, *J* = 7 Hz, 6H).

4.4.5. *N*-(2,2-Diethoxyethyl)-*N*-phenylamino 2-[*N*'-Isopropyl-(*N*'-4-methoxyphenylamino)-2-oxoethyl] 2-(1-*tert*-butyloxycarbonyl-1*H*-indazol-3-ylmethylene)malonamide (9)

To a solution of 680 mg (1.36 mmol) of *N*-(2,2-diethoxyethyl)-*N*-phenylamino 2-[*N*'-isopropyl-(*N*'-4-methoxyphenylamino)-2-oxoethyl] malonamide (**7**) in dry DMF (5 ml), 2.74 ml of 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene was added at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and a solution of 423 mg (1.36 mmol) of *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate (**8**) in dry DMF (2 ml) was added dropwise. The reaction mixture was stirred overnight at room temperature, poured into water and this mixture was extracted with ethyl acetate (2 × 15 ml). The organic phase was separated and dried. The solvent was removed in vacuo and the residue was purified by column chromatography on silica gel (MeOH 1%/$CHCl_3$ 99%) to afford 700 mg (71% yield) of product. ^{1}H NMR (400 MHz, $CDCl_3$) 8.05 (d, *J* = 8 Hz, 1H), 7.64 (d, *J* = 8 Hz, 1H), 7.46 (m, 1H), 7.12 (m, 11H), 4.95 (sept., *J* = 7 Hz, 1H), 4.60 (t, *J* = 5.6 Hz, 1H), 3.88 (m, 1H), 3.81 (s, 3H), 3.68 (m, 3H), 3.52 (m, 5H), 3.24 (m, 2H), 1.70 (s, 9H), 1.03 (m, 12H).

4.4.6. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-N-(4-methoxyphenyl)acetamide (10)

A solution of 200 mg (0.27 mmol) of *N*-(2,2-diethoxyethyl)-*N*-phenylamino 2-[*N*'-isopropyl-(*N*'-4-methoxyphenylamino)-2-oxoethyl] 2-(1-*tert*-butyloxycarbonyl-1*H*-indazol-3-ylmethylene) malonamide (**9**) and *p*-toluenesulfonic acid (anhydrous, 20 mg) in dry toluene (80 ml) was placed in an oil bath at 70 °C and stirred for 30 min. Toluene was removed in vacuo and the residue was purified by column chromatography on silica gel (hexane 50%/ethyl acetate 50%) to afford 170 mg (98% yield) of product (**10**). ^{1}H NMR (400 MHz, $CDCl_3$) 8.03 (d, *J* = 8.4 Hz, 1H). 7.85 (d, *J* = 8 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.31 (m, 6H), 7.11 (m, 2H), 6.93 (m, 2H), 6.00 (s, 2H), 4.95 (sept., *J* = 6.7 Hz, 1H), 4.35 (d–d, *J* = 8.9 Hz, *J* = 4.8 Hz, 1H), 3.93 (m, 2H), 3.85 (d–d, *J* = 16.6 Hz, 8.9 Hz, 1H), 3.82 (s, 3H), 3.58 (d–d, *J* = 16.6 Hz, *J* = 4.8 Hz, 1H), 1.65 (s, 9H), 1.04 (d, *J* = 6.7 Hz, 6H).

4.4.7. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide (11)

170 mg (0.27 mmol) of 2-[2,4-dioxo-3-(1-*tert*-butyloxycarbonyl-1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide (**10**) was dissolved in $CHCl_3$ (10 ml) and TFA (5 ml) was added. The reaction mixture was stirred for 6 h and the solvents were removed in vacuo. The crude product was purified by RP HPLC Dynamax (C-8) (10 ml/min (50% acetonitrile, 50% water (0.1% TFA).135 mg (94% yield) of product (**11**) was obtained as a white solid. HPLC Column:

Dynamax C-8 2 ml/min, 30–70% Acetonitrile in aqueous TFA (0.1% v/v) over 20 min, t_R = 17.1 min. ^{1}H NMR (400 MHz, $CDCl_3$) 7.93 (d, J = 8.3 Hz, 1H), 7.59 (m, 2H), 7.11 (m,2H), 7.31 (m, 6H), 6.93 (m,2H), 6.00 (s, 2H), 4.92 (sept., J = 6.3 Hz, 1H), 4.15 (m, 1H), 3.92 (m, 4H), 3.82 (s, 3H), 1.04 (d, J = 6.3 Hz, 6H).

MS (FAB) $[M+H]^+$ = 538. Elemental Anal. Calcd: C, 69.26; H, 5.81; N, 13.03. Found: C, 69.12; H, 5.78; N, 12.99.

4.4.8. 3-(6-Fluoro-1*H*-indazol-3-ylmethylene)-1-[2-oxoethyl-2-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)]-5-phenyl-1*H*-1,5-diazepine-2,4-dione (Compound 12)

This compound was prepared as compound **11** using 2-oxo-2-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)ethanamine instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide and *tert*-butyl 3-(bromomethyl)-6-fluoro-1*H*-indazole-1-carboxylate instead of of *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate. ^{1}H NMR (300 MHz, DMSO-d_6) 7.83–7.91 (m, 1H); 7.22–7.50 (m, 10H); 6.95–7.01 (m, 1H); 6.28 (d, J = 6.8 Hz, 1H); 6.22 (d, J = 6.8, 1H); 4.13–4.50 (m, 3H); 3.71–3.95 (m, 1H); 3.36–3.59 (m, 2H); 2.55–2.93 (, 3H); 1.89–2.01 (m, 1H); 1.67–1.78 (m, 2H); 1.24–1.39 (m, 1H) MS (ESI): M+H = 538.

4.4.9. 3-(1*H*-Indazol-3-ylmethylene)-1-[2-oxoethyl-2-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)]-5-phenyl-1*H*-1,5-diazepine-2,4-dione (Compound 13)

This compound was prepared as compound **11** using 2-oxo-2-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)ethanamine instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide. 2-oxo-2-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)ethanamine. ^{1}H NMR (400 MHz, CDCl3) 7.72–7.79 (m, 1H); 7.17–7.36 (m, 11H); 7.04–7.11 (m, 1H); 5.90–6.11 (m, 2H); 4.61–4.72 (m 1H); 3.66–4.31 (m, 4H); 3.47–3.65 (m, 1H); 2.85–3.02 (m, 1H); 2.60–2.73 (m, 2H); 1.72–2.01 (m. 3H); 1.28–1.43 (m, 1H) MS (ESI): M+H = 520.

4.4.10. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-phenylacetamide (Compound 14)

This compound was prepared as compound **11** using N^1-isopropyl-N^1-phenylglycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide. ^{1}H NMR (400 MHz, $CDCl_3$): 7.82 (d, J = 9 Hz, 1H); 7.09–7.47 (m, 13H); 6.03 (d, J = 6.2, 1H); 5.98 (d, J = 6.2 Hz, 1H); 4.98 (q, J = 6.8 Hz, 1H); 4.26 (dd, J = 7.8 Hz, J = 2.0 Hz, 1H); 3.97 (d, J = 16.5 Hz, 1H); 3.88 (d, J = 16.5, 1H); 3.78–3.86 (m, 1H); 3.63 (dd, J = 16.3 Hz, J = 5.5 Hz, 1H); 1.05–1.92 (m, 6H). MS (FAB) $[M+H]^+$ = 508.

4.4.11. 2-[2,4-Dioxo-3-(6-fluoro-1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-phenylacetamide (Compound 15)

This compound was prepared as compound **11** using N^1-isopropyl-N^1-phenylglycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl) glycinamide and *tert*-butyl 3-(bromomethyl)-6-fluoro-1*H*-indazole-1-carboxylate instead of of *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate. ^{1}H NMR (300 MHz, $CDCl_3$): 7.71 (dd, J = 8.8 Hz, J = 5.3 Hz, 1H); 7.18–7.50 (m, 10H); 6.98 (dd, J = 9.3 Hz, J = 1.9 Hz, 1H); 6.87 (td, J = 9.1 Hz, J = 2.0 Hz, 1H); 6.05 (d, J = 6.6 Hz, 1H); 5.95 (d, J = 6.6 Hz, 1H); 5.01 (q, J = 6.7 Hz, 1H); 3.74–4.28 (m, 5H); 3.46–3.56 (m, 1H); 1.09 (m, 6H). (MS (FAB) $[M+H]^+$ = 526.

4.4.12. 2-[2,4-Dioxo-3-(6-fluoro-1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide (Compound 16)

This compound was prepared as compound **11** *tert*-butyl 3-(bromomethyl)-6-fluoro-1*H*-indazole-1-carboxylate instead of *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate. ^{1}H NMR (400 MHz, $CDCl_3$) 7.58–7.64 (m, 1H); 7.18–7.34 (m, 5H); 7.13–7.18 (m, 1H); 7.04–7.08 (m, 1H); 6.02 (d, J = 6.2 Hz, 1H); 5.84–5.89 (m, 1H); 4.97 (q, J = 6.8 Hz, 1H); 4.23 (dd, J = 8.6 Hz, J = 5.0 Hz, 1H); 4.05–4.09 (m, 1H); 3.80(s, 3H); 3.70–3.82 (m, 2H); 3.36–3.44 (m, 1H); 1.05)(d, J = 6.6 Hz, 6H). MS (ESI): M+H = 556.

4.4.13. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (Compound 17)

This compound was prepared as compound **11** using N^1-isopropyl-*N*'-(4-fluorophenyl)glycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide. ^{1}H NMR (400 MHz, DMSO-d_6): 12.58 (s, 1H); 7.75 (d, J = 8.0 Hz, 1H); 7.22–7.44 (m, 11H); 7.05 (t, J = 7.5 Hz, 1H); 6.16 (br s, 1H); 4.75 (q, J = 6.6 Hz, 1H); 4.14 (t, J = 7.0 Hz, 1H); 3.98 (d, J = 6.3 Hz, 1H); 3.75 (d, J = 6.3 Hz, 1H); 3.30–3.54 (m, 3H); 0.94 (d, J = 6.8 Hz, 6H). MS (FAB) $[M+H]^+$ = 526.

4.4.14. 2-[2,4-Dioxo-3-(6-fluoro-1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (Compound 18)

This compound was prepared as compound **11** using N^1-isopropyl-*N*'-(4-fluorophenyl)glycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide and *tert*-butyl 3-(bromomethyl)-6-fluoro-1*H*-indazole-1-carboxylate instead of *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate. ^{1}H NMR (400 MHz, DMSO-d_6) 12.65 (s,1H); 7.77–7.82 (m, 1H); 7.16–7.4 (m, 9H); 6.86–6.96 (m, 1H); 6.16 (br s, 1H); 4.75 (m, 1H); 3.96–4.13 (m, 1H); 3.75 (d, J = 16.2 Hz, 1H); 3.24–3.50 (m, 1H); 3.29 (s, 3H); 0.94 (m, 6H). MS (ESI): M+H = 544.

4.4.15 (2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3.4.5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-(4-hydroxyphenyl)-*N*-isopropylacetamide (Compound 19)

To the solution of 2-[2,4-dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide (1 g, 1.86 mM) in 50 ml of dichloromethane was cooled to 0 °C and 10-fold excess of BBr_3 was added. The reaction mixture was stirred for 3 h at room temp, cooled to 0 °C and methanol was added dropwise. Organic solvents were evaporated under reduced pressure and residue was purified using column chromatography on silica gel using mixture of hexane–ethyl acetate (1:3) as an eluent providing 700 mg (72% yield)of pure product. ^{1}H NMR (400 MHz, $CDCl_3$) 7.76 (d, J = 7.8 Hz, 1H); 7.16–7.30 (m, 8H); 7.02–7.08 (m, 1H); 6.66–6.95 (m, 3H); 5.82–5.89 (m, 2H); 4.87 (q, J = 6.8 Hz, 1H); 3.63–3.96 (m, 4H); 0.97 (two d, J = 6.6 Hz, 6H). MS (FAB) $[M+H]^+$ = 524.

4.4.16. 2-[6-(1*H*-Indazol-3-ylmethyl)-5,7-dioxo-4-(3-pyridinyl)-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide (Compound 20)

This compound was prepared as compound **11** using *H*-[2,2-bis(ethyloxy)ethyl]-3-pyridinamine instead of 2-(*N*-anilino)acetaldehyde diethyl acetal. ^{1}H NMR (400 MHz, $CDCl_3$) 10.40 (br s, 1H), 8.61 (m, 1H), 8.53 (m, 1H), 7.82 (m, 1H), 7.751 (m, 1H), 7.44–7.32 (m, 3H), 7.24–7.12 (m, 3H), 7.02–6.96 (m, 2H), 6.14 (d, J = 6.3 Hz,1H), 5.98 (d, J = 6.3 Hz,1H), 5.00 (sept., J = 6.3 Hz, 1H), 4.38–4.32 (m, 1H), 4.00 (d, J = 8.2 Hz, 1H), 3.87 (s, 3H), 3.92–3.82 (m, 1H), 3.66–3.56 (m, 1H), 1.10 (d, J = 6.3 Hz, 6H), MS (FAB) $[M+H]^+$ = 539.

4.4.17. 2-[6-(6-Fluoro-1*H*-indazol-3-ylmethylene)-5,7-dioxo-4-(3-pyridinyl)-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide (Compound 22)

This compound was prepared as compound **11** using *N*-[2,2-bis(ethyloxy)ethyl]-3-pyridinamine instead of 2-(*N*-anilino)acetaldehyde diethyl acetal and *tert*-butyl 3-(bromomethyl)-6-fluoro-

1*H*-indazole-1-carboxylate instead of *tert*-butyl 3-(bromomethylene)-1*H*-indazole-1-carboxylate. ^{1}H NMR (400 MHz, $CDCl_3$) 10.40 (br s, 1H), 8.61 (m, 1H), 8.53 (m, 1H), 7.82 (m, 1H), 7.751 (m,1H), 7.30–7.26 (m, 1H), 7.24–7.12 (m, 3H), 7.02–6.96 (m,3H), 6.14 (d, J = 6.3 Hz,1H), 5.98 (d, J = 6.3 Hz,1H), 5.00 (sept., J = 6.3 Hz, 1H), 4.38–4.32 (m, 1H), 4.00 (d, J = 8.2 Hz, 1H), 3.87 (s, 3H), 3.92–3.82 (m, 1H), 3.66–3.56 (m, 1H), 1.10 (d, J = 6.3 Hz, 6H), MS (FAB) $[M+H]^+$ = 557.

4.4.18. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-3-methoxy-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-(4-fluorophenyl) *N*-isopropylacetamide (Compound 23)

This compound was prepared as compound **11** using N^1-isopropyl-*N*'-(4-fluorophenyl)glycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide and methyl 3-chloro-2-(methyloxy)-3-oxopropanoate instead of methyl malonyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$) 7.96 (d, J = 8.4 Hz, 1H); 7.49–7.58 (m, 2H); 7.32–7.36 (m, 2H); 7.15–7.28 (m, 8H); 5.95 (d, J = 6.6 Hz, 1H); 5.92 (d, J = 6.6 Hz, 1H); 4.98 (q, J = 7.2 Hz, 1H); 4.04–4.21 (m, 3H); 3.74 (d, J = 6.6 Hz, 1H); 3.47 (s, 3H); 1.07 (m, 6H) MS (FAB) $[M+H]^+$ = 556.

4.4.19. 2-[4-Cyclohexyl-5,7-dioxo-6-(phenylmethyl)-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide (Compound 24)

Compound **24** was prepared by a previously published solid supported synthesis.[17] ^{1}H NMR (300 MHz, $CDCl_3$) 7.32–7.08 (m, 9H), 6.99 (s, 1H), 6.96 (s, 1H), 5.95 (d, J = 6.5 Hz, 1H), 5.90 (d, J = 6.5 Hz, 1H), 4.97 (quint, J = 6.7 Hz, 1H), 4.40 (m, 1H), 4.05 (d, J = 16.5 Hz, 1H), 3.87 (s, 3H), 3.70 (d, J = 16.5 Hz, 1H), 3.53–3.30 (m, 3H), 1.90–1.60 (m, 7H), 1.54–1.20 (m, 5H), 1.09 (d, J = 6.7 Hz, 3H), 1.08 (d, J = 6.7 Hz, 3H). MS (ES+) $[M+H]^+$ = 504.

4.4.20. 2-[6-(1*H*-Indazol-3-ylmethyl)-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(4-morpholinyl)phenyl]acetamide (Compound 25)

This compound was prepared as compound **11** using N^1-(1-methylethyl)-N^1-[4-(4-morpholinyl)phenyl]glycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide. ^{1}H NMR (400 MHz, $CDCl_3$) 7.71 (d, J = 8.0 Hz, 1H); 7.35–7.19 (m, 6H); 7.12–7.01 (m, 3H); 6.91–6.83 (m, 3H); 6.10 (d, J = 7.4 Hz, 1H); 5.88 (d, J = 7.4 Hz, 1′H); 4.96 (q, J = 7.2 Hz, 1H); 4.27–4.22 (m, 1H); 4.03 (d, J = 16.4 Hz, 1H); 3.88–3.75 (m, 6H); 3.5 (dd, J = 16.4 Hz, J = 5.1 Hz, 1H); 3.16 (m, 4H); 1.04 (m, 6H).

4.4.21. 2-[4-Cyclohexyl-6-(1*H*-indazol-3-ylmethyl)-5,7-dioxo-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide trifluoroacetate (Compound 26)

This compound was prepared as compound **24** using 1,1-dimethylethyl 3-(bromomethyl)-1*H*-indazole-1-carboxylate instead of benzyl bromide. ^{1}H NMR (300 MHz, $CDCl_3$) 7.87 (d, J = 7.5 Hz, 1H), 7.48 (m, 2H), 7.20 (m, 1H), 7.08 (m, 2H), 6.91 (m, 2H), 4.92 (quint, J = 6.7 Hz, 1H), 4.35 (m, 1H), 4.04–3.91 (m, 2H), 3.81 (s, 3H), 3.79–3.66 (m, 3H), 1.84–1.58 (m, 7H), 1.51–1.17 (m, 5H), 1.02 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.8 Hz, 3H). MS (ES+) $[M+H]^+$ = 544.

4.4.22. 2-[6-(1*H*-Indazol-3-ylmethyl)-5,7-dioxo-4-(2-thienyl methyl)-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide (Compound 27)

This compound was prepared as compound **11** using 2,2-bis(ethyloxy)-*N*-(2-thienylmethyl)ethanamine instead of 2-(*N*-anilino)acetaldehyde diethyl acetal. ^{1}H NMR (300 MHz, $CDCl_3$) 7.90 (d, J = 8.1 Hz, 1H), 7.51–7.38 (m, 2H), 7.24–7.14 (m, 3H), 7.09–7.02 (m, 1H), 7.00–6.90 (m, 4H), 5.98 (dd, J = 6.3 Hz, 14.7 Hz, 2H), 4.99–4.88 (m, 3H), 4.17–4.06 (m, 2H), 3.91–3.82 (m, 2H), 3.86 (s, 3H), 3.75–3.65 (M, 2H), 1.07 (d, J = 6.7 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H). MS (ES+) $[M+H]^+$ = 558.

4.4.23. 2-[6-(1*H*-Indazol-3-ylmethyl)-5,7-dioxo-4-(phenylmethyl)-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide (Compound 28)

This compound was prepared as compound **11** using 2,2-bis(ethyloxy)-*N*-(phenylmethyl)ethanamine instead of 2-(*N*-anilino)acetaldehyde diethyl acetal. ^{1}H NMR (300 MHz, $CDCl_3$) 7.91 (d, J = 8.1 Hz, 1H), 7.54–7.41 (m, 2H), 7.35–7.04 (m, 9H), 6.99–6.92 (m, 3H), 5.93 (d, J = 6.5 Hz, 1H), 5.86 (d, J = 6.5 Hz, 1H), 5.02–4.90 (m, 1H), 4.78 (br s, 2H), 4.23–4.14 (m, 1H), 4.05 (s, 0.5H), 4.00 (s, 0.5H), 3.91–3.78 (m, 3H), 3.86 (s, 3H), 3.74 (m, 1H), 1.09–1.04 (m, 6H). MS (ES+) $[M+H]^+$ = 552.

4.4.24. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(2-methyl-4-trifluoromethoxy)phenyl)acetamide (Compound 29)

This compound was prepared as compound **11** using N^1-isopropyl-N^1-(4-trifluoromethoxyphenyl)glycinamide instead of N^1-isopropyl-N^1-(4-methoxyphenyl)glycinamide. ^{1}H NMR (400 MHz, CD_3OD) 7.91 (1H, d, J = 8.06 Hz), 7.35 (10H, m), 7.21 (1H, m), 7.09 (1H, dd, J = 7.51 Hz), 6.11 (2H, m), 4.69 (1H, qq, J = 6.59 Hz), 4.22 (1H, m), 4.07 (2H, m), 3.71 (2H, m), 3.56 (1H, m), 2.39 (3H, d, J = 4.03 Hz), 1.99 (3H, s), 1.22 (6H, m). MS (EI) $[M+H]^+$ = 606.

4.4.25. 2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-3-methoxy-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-(4-methoxyphenyl) *N*-isopropylacetamide (Compound 30)

This compound was prepared as compound **11** using methyl 3-chloro-2-(methyloxy)-3-oxopropanoate instead of methyl malonyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$) 7.96 (d, J = 8.4 Hz, 1H); 7.49–7.08 (m, 8H); 6.96–6.76 (m, 4H); 5.96 (d, J = 6.6 Hz, 1H); 5.89 (d, J = 6.6 Hz, 1H); 4.98 (q, J = 7.2 Hz, 1H); 4.04–4.21 (m, 3H); 3.74 (d, J = 6.6 Hz, 1H); 3.87 (s, 3H), 3.47 (s, 3H); 1.07 (m, 6H) MS (FAB) $[M+H]^+$ = 568.

4.4.26. Diphenyl {[6-(1*H*-indazol-3-ylmethyl)-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]methyl} phosphonate (Compound 31)

Under nitrogen atmosphere and anhydrous conditions, a mixture of 6.12 g (40.5 mmol) of benzyl carbamate, 5.14 g (50.3 mmol) of acetic anhydride, and 1.22 g (40.6 mmol) of paraformaldehyde in 4 mL of acetic acid was heated at 65 °C for 3 h. The resulting solution was treated with 12.7 g (40.8 mmol) of triphenyl phosphite and heated at 115–120 °C for 2 h. The mixture was concd under high vacuum then a portion of the material was taken up in a small volume of diethyl ether and chilled overnight to give precipitate that was isolated by filtration, washed well with diethyl ether, and dried under high vacuum to give 2.44 g of diphenyl [({[(phenylmethyl)oxy]carbonyl}amino)methyl]phosphonate as a bright white solid. Of this sample, 88 mg (0.22 mmol) was taken up in glacial acetic acid and treated with 1 mL of 30% HBr/acetic acid and stirred for 2 h at room temperature. The solution was concentrated in vacuo and the resulting sample in a few drops of acetic acid was added to diethyl ether to induce precipitation. After standing overnight, the ethereal supernatant was decanted and the solids were washed thoroughly with diethyl ether under nitrogen atmosphere. The resulting solids were dried under high vacuum to give diphenyl (aminomethyl)phosphonate hydrobromide as a finely divided light tan powder that was immediately utilized in the subsequent reaction. See note in following paragraph.

A solution of 108 mg (0.20 mmol) of methyl 3-[[2,2-bis(ethyloxy)ethyl](phenyl)amino]-2-(1*H*-indazol-3-ylmethyl)-3-oxopropanoate, prepared in an the same way as Compound 9, in THF (5 mL) and water (2 mL) at 0 °C was treated with 0.205 mL of 1.00 M NaOH. The solution was stirred at room temperature for 4 h then concd in vacuo to remove THF. The aqueous suspension was lyophillized to give white solid powder which was taken up in anhydrous DMF (4 mL) and treated with 29 mg (0.21 mmol) of HOBT, 61 mg (0.21 mmol) of EDCI, and 26 mg (0.20 mmol) DIEA. The solution was stirred for 30 min at room temperature then treated with 0.22 mmol of diphenyl (aminomethyl)phosphonate hydrobromide [Can omit above prep and note here 'prepared as previously reported. . .Ref: Oleksyszyn, J.; Subotkowska, L. Synthesis Communications, vol. 11, p 906 (1980)] in 1.5 mL of DMF. The resulting solution was stirred at room temperature overnight then concd in vacuo, applied to silica gel, and purified by column chromatography eluting with 50–60% EtOAc/hexane to give 82 mg (0.106 mmol) of intermediate diphenyl ({[3-[[2,2-bis(ethyloxy)ethyl](phenyl)amino]-2-(1*H*-indazol-3-ylmethyl)-3-oxopropanoyl]amino}methyl)phosphonate. This intermediate was taken up in dry toluene and treated with anhydrous toluenesulfonic acid (0.12 mmol) in toluene. A small proportion of the toluene was evaporated to ensure dryness then the solution was heated at 70 °C for 1 h. The reaction mixture was applied to a 2 g silica gel cartridge and products were eluted with 80% EtOAc/hexane to give Boc protected intermediate and 4 mg of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) 8.15 (d, J = 8.7 Hz, 1H), 7.85 (br d, J = 8.0 Hz, 2H), 7.71 (m, 2H), 7.35–6.98 (m, 16H), 6.12 (dd, J = 39, J = 6.3, 2H), 4.57 (m, 1H), 3.98 (m, 2H). MS (ES+) $[M+H]^+$ = 579.

4.4.27. *N*-(1-methylethyl)-2-{6-[(1-methyl-1*H*-indazol-3-yl)methyl]-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl}-*N*-[4-(methyloxy)phenyl]acetamide (Compound 32)

2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)-acetamide (64 mg; 0.1 mmol) was dissolved in DMF and cooled to °C. 0.6 M solution of $Na(NSiMe_2)_3$ was added dropwise, and mixture was stirred 30 min at 0 °C, and iodomethane (14 mg) was added in DMF. Reaction mixture was stirred for 2 h at ethyl acetate and washed with 1 M NaHSO4 and satd sodium bicarbonate. The organic layer was dried and solvent removed under reduced pressure. Crude product was purified by HPLC on C-8 RP acetonitrile/water (0.1% TFA) to provide 60 mg of desired material as a white solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) 7.86 (d, J = 8.4 Hz, 1H); 7.49–7.08 (m, 8H); 6.96–6.83 (m, 2H); 6.05–5.93 (m, 2H); 4.98–4.88 (m, 1H); 4.20–4.15 (m, 1H); 3.96 (s, 3H), 3.92 (m, 4H), 3.82 (s, 3H); 1.04 (d, J = 6.3 Hz, 6H) MS (FAB) $[M+H]^+$ = 552.

4.4.28. 2-{6-[(1-Ethyl-1*H*-indazol-3-yl)methyl]-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl}-*N*-(1-methylethyl)-*N*-[4-(methyloxy)phenyl]acetamide (Compound 33)

2-[2,4-Dioxo-3-(1*H*-indazol-3-ylmethylene)-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide 64 mg (0.1 mM) was dissolved in DMF and cooled to °C. 0.6 M solution of $Na(NSiMe_2)_3$ was added dropwise and mixture was stirred 30 min at 0 °C and iodoethane (16 mg) was added in DMF. Reaction mixture was stirred for 2 h at rt and solvent removed under reduced pressure. Residue was dissolved in ethyl acetate and washed with 1 M NaHSO4 and satd sodium bicarbonate. Organic layer was dried and solvent removed under reduced pressure. Crude product was purified by HPLC on C-8 RP using acetonitrile/water(0.1% TFA) providing 60 mg of desired material as a white solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$) 7.86 (d, J = 8.4 Hz, 1H); 7.49–7.08 (m, 8H); 6.96–6.83 (m, 2H); 6.05–5.93 (m, 2H); 4.98–4.88 (m, 1H); 4.32(q, J = 7.0 Hz 2H), 4.20–4.15 (m, 1H); 3.98–3.82 (m, 4H),3.82 (s, 3H); 3.35–3.58 (m, 1H); 1.43 (t, J = 7.0 Hz, 3H); 1.04 (d, J = 6.3 Hz, 6H) MS (FAB) $[M+H]^+$ = 566.

4.4.29. Phenyl hydrogen {[6-(1*H*-indazol-3-ylmethyl)-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl]methyl}phosphonate (Compound 34)

14 mg (0.021 mmol) of 1,1-dimethylethyl 3-[(1-{[hydroxy(phenyloxy)phosphoryl]methyl}-5,7-dioxo-4-phenyl-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-6-yl)methyl]-1*H*-indazole-1-carboxylate, prepared as an intermediate of comopound **31**, in 1 mL of dichloromethane at 0 °C was treated with 0.5 mL of trifluoroacetic acid. The solution was stirred for 3 h then concd in vacuo. The residue was twice taken up in dichloromethane and conc. in vacuo. The sample was taken up in 1,4-dioxane and treated with 0.2 mL of 1.00 M NaOH. The resulting solution was stirred at room temperature overnight. The reaction was treated with excess acetic acid and conc. in vacuo to give crystalline solid which was purified by preparative HPLC on RP-C8 eluting with acetonitrile/water (0.1% TFA). Fractions of interest were lyophilized to give 3 mg of the TFA salt of the title compound as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$+D_2O) 7.82 (m, 1H), 7.44–6.99 (m, 15H), 6.58 (br s, 1H), 5.96 (br s, 1H), 4.07 (m, 1H), 3.80–3.55 (m, 2H). MS (ES+) $[M+H]^+$ = 503.

4.4.30. 2-{6-[(6-Fluoro-1*H*-indazol-3-yl)methyl]-4-methyl-5,7-dioxo-4,5,6,7-tetrahydro-1*H*-1,4-diazepin-1-yl}-*N*-(4-fluorophenyl)-*N*-(1-methylethyl)acetamide (Compound 35)

This compound was prepared as compound **11** using 2,2-bis(ethyloxy)-*N*-methylethanamine instead of 2-(*N*-anilino)acetaldehyde diethyl acetal. ^{1}H NMR (400 MHz, $CDCl_3$) 7.78(d,d, 1H); 7.249–7.08 (m, 4H); 7.02–6.98 (m, 1H); -6.93–6.86 (m, 1H); 5.91 (d, 1H); 5.87 (d, 1H); 4.98–4.88 (m, 1H); 4.03–3.92 (m, 2H); 3.78–3.68 (m, 2H),3.3.63–3.57 (m, 1H); 3.12 (s, 3H); 1.04 (d, J = 6.3 Hz, 6H) MS (FAB) $[M+H]^+$ = 582.

Racemic compounds were separated on a Prochrom Preparative Supercritical Fluid Chromatograph Super C20 as summarized below.

4.4.30.1. Resolution of Compound 11. *Anal. Column* Chiralcel OD (250 × 4.6 mm).

70% CO_2/30% MeOH (10% Chloroform), 2 ml/min, 40C, 210 Bars.
Enantiomer #1: 3.0 min [Compound **11-(*S*)**].
Enantiomer #2: 5.5 min [Compound **11-(*R*)**].
Prep. Column Chiralcel OD,(250 × 20 mm).
50 g/min CO_2, 19.3 ml/min MeOH (10% Chloroform), 40C, 210 Bars.
Enantiomer #1: 6.0 min [Compound **11-(*S*)**].
Enantiomer #2: 13.1 min [Compound **11-(*R*)**].

4.4.30.2. Resolution of Compound 14. *Anal. Column* Chiralcel OJ (250 × 4.6 mm).

88% CO_2/12% EtOH, 2 ml/min, 40C, 210 Bars, 13.9 and 18.5 min.
Enantiomer #1: 13.9 min [Compound **14-(*S*)**].
Enantiomer #2: 18.5 min [Compound **14-(*R*)**].
Prep. Column Chiralcel OJ (250 × 20 mm).
45 g/min CO2, 9.9 ml/min EtOH, 40C, 210 Bars.
Enantiomer #1: 13.0 min [Compound **14-(*S*)**].
Enantiomer #2: 17.8 min [Compound **14-(*R*)**].

4.4.30.3. Resolution of Compound 15. *Anal. Column* Chiralcel OJ (250 × 4.6 mm).

88% CO2/12% EtOH, 2 ml/min, 40C, 210 Bars.
Enantiomer #1: 10.7 min [Compound **15-(*S*)**].
Enantiomer #2: 14.0 min [Compound **15-(*R*)**].
Prep. Column Chiralcel OJ (250 × 20 mm).

45 g/min CO2, 9.9 ml/min EtOH, 40C, 210 Bars.
Enantiomer #1: 10.5 min [Compound **15-(*S*)**].
Enantiomer #2: 14.5 min [Compound **15-(*R*)**].

4.4.30.4. Resolution of Compound 13. *Anal. Column* Chiralcel OD (250 × 4.6 mm), 7.
0% CO2/30% MeOH (10% Chloroform), 2 ml/min, 40C, 210 Bars.
Enantiomer #1: 8.2 min [Compound **13-(*S*)**].
Enantiomer #2: 14.4 min [Compound **13-(*R*)**].
Prep. Column Chiralcel OD (250 × 20 mm).
45 g/min CO2, 16 ml/min MeOH (10% Chloroform), 40C, 210 Bars.
Enantiomer #1: 8.5 min [Compound **13-(*S*)**].
Enantiomer #2: 11.5 min [Compound 1**13-(*R*)**].

4.4.30.5. Resolution of 19. *Anal. Column* Chiralpak AS (250 × 4.6 mm).
85% CO2/15% EtOH, 2 ml/min, 40C, 210 Bars.
Enantiomer #1: 18.4 min (Compound **19-a**).
Enantiomer #2: 27.1 min (Compound **19-b**).
Prep. Column Chiralcel AS (250 × 20 mm).
42 g/min CO2, 8 ml/min EtOH, 40C, 210 Bars.
Enantiomer #1: 12.3 min (Compound **19-a**).
Enantiomer #2: 17.6 min (Compound **19-b**).

References and notes

1. Wan, Y.; Wallinder, C.; Plouffe, B.; Beaudry, H.; Mahalingam, A. K.; Wu, X.; Johansson, B. t.; Holm, M.; Botoros, M.; Karlen, A.; Pettersson, A.; Nyberg, F.; Faendriks, L.; Gallo-Payet, N.; Hallberg, A.; Alterman, M. *J. Med. Chem.* **2004**, *47*, 5995.
2. Ye, Z.; Gao, Y.; Bakshi, R. K.; Chen, M. H.; Rohrer, S. P.; Feighner, S. D.; Pong, S. S.; Howard, A. D.; Blake, A.; Birzin, E. T.; Locco, L.; Parmar, R. M.; Chan, W. W.; Schaeffer, J. M.; Smith, R. G.; Patchett, A. A.; Nargund, R. P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 5.
3. Nargund, R. P.; Barakat, K. H.; Cheng, K.; Chan, W. W.-S.; Butler, B. R.; Smith, R. G.; Patchett, A. A. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1265.
4. Lugar, W.; Clay, M. P.; Lindstrom, T. D.; Woodson, A. L.; Smiley, D.; Heiman, M. L.; Dodge, J. A. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5873.
5. Szewczyk, J. R.; Laudeman, C. *Curr. Top. Med. Chem.* **2003**, *3*, 837.
6. Bednarek, M. A.; Fong, T. M. *Expert Opin. Ther. Patents* **2004**, *14*, 327.
7. Sebhat, I.; Ye, Z.; Bednarek, M.; Weinberg, D.; Nargund, R.; Fong, T. M. *Annu. Rep. Med. Chem.* **2003**, *38*, 31.
8. Aquino, C. J.; Armour, D. R.; Berman, J. M.; Birkemo, L. S.; Carr, R. A. E.; Croom, D. K.; Dezube, M.; Dougherty, R. W., Jr.; Ervin, G. N., et al *J. Med. Chem.* **1996**, *39*, 562.
9. Henke, B. R.; Aquino, C. J.; Birkemo, L. S.; Croom, D. K.; Dougherty, R. W., Jr.; Ervin, G. N.; Grizzle, M. K.; Hirst, G. C.; James, M. l. K.; Johnson, M. F.; Queen, K. L.; Sherrill, R. G.; Sugg, E. h. E.; Suh, E. M.; Szewczyk, J. W.; Unwalla, R. J.; Yingling, J.; Willson, T. M. *J. Med. Chem.* **1997**, *40*, 2706.
10. Dezube, M.; Hirst, G. C.; Willson, T. M.; Sherrill, R. G.; Sugg, E. E.; Szewczyk, J. R Patent EP 4026[9611940].
11. Todd, M. H.; Ndubaku, C.; Bartlett, P. A. *J. Org. Chem.* **2002**, *67*, 3985.
12. Guo, C.; Reich, S.; Showalter, R.; Villafranca, E.; Dong, L. *Tetrahedron Lett.* **2000**, *41*, 5307.
13. (a) McCann, J. L.; Rauk, A.; Wieser, H. A conformational study of (1*S*,2*S*,5*S*)-(+)-menthol using vibrational circular dichroism spectroscopy *Can. J. Chem.* **1998**, *76*, 274–283; (b) Freedman, T. B.; Cao, X.; Dukor, R. K.; Nafie, L. A. Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism *Chirality* **2003**, *15*, 743–758.
14. (a) Freedman, T. B.; Cao, X.; Phillips, L. M.; Cheng, P. T. W.; Dalterio, R.; Shu, Y.; Zhang, H.; Zhao, N.; Shukia, R. B.; Tymiak, A.; Gozo, S. K.; Nafie, L. A.; Gougoutas, J. Z. Determination of the absolute configuration and solution conformation of a novel disubstituted pyrrolidine acid a by vibrational circular dichroism *Chirality* **2006**, *18*, 746–753; (b) Minick, D. J.; Copley, R. C.; Szewczyk, J. R.; Rutkowske, R. D.; Miller, L. A. *Chirality* **2007**, *19*, 731.
15. Goetz, A. S.; Andrews, J. L.; Littleton, T. R. Ignar DM development of a facile method for high throughput screening with reporter gene assays *J. Biomol. Screening* **2000**, *5*, 377.
16. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M.A.; Cheeseman, J.R.; Montgomery, Jr. J.A.; Vreven, T.; Kudin, K.N.; Burant, J.C.; Millam, J.M.; Iyengar, S.S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G.A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J.E.; Hratchian, H.P.; Cross, J.B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.E.; Yazyev, O.; Austin, A.J.; Cammi, R.; Pomelli, C.; Ochterski, J.W.; Ayala, P.Y.; Morokuma, K.; Voth, G.A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. GAUSSIAN *03, Revision C.02*, Gaussian, Inc.: Wallingford CT, 2004.
17. Laudeman, C. P.; Szewczyk, J. R.; Andrews, J. L.; Barvian, K. K.; Carpenter, A. J.; Drewry, D. H.; Fernandez, E. P.; Gerritz, S.; Ignar, D. M.; Minick, D. J.; Salovich, J. M.; Speake, J. D. A Facile Solid Phase Synthesis of Diazepinediones; A Novel Class of Compounds with MC4R Activity, 30th National Medicinal Chemistry Symposium, 2006, Poster 123.