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Synthesis and structure–activity relationship of new 1,5-dialkyl-1,5-benzodiazepines as cholecystokinin-2 receptor antagonists

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ABSTRACT

This article deals with the synthesis and the activities of some 1,5-dialkyl-3-arylureido-1,5-benzodiazepin-2,4-diones which were prepared as potential CCK2 antagonists, with the intention to find a possible follow up of our lead compound GV150013, showing an improved pharmacokinetic profile. The phenyl ring at N-5 was replaced with more hydrophilic substituents, like alkyl groups bearing basic functions. In some cases, the resolution of the racemic key intermediates 3-amino-benzodiazepines was also accomplished. Among the compounds synthesized and characterised so far in this class, the 5-morpholinoethyl derivative 54, was selected as potential follow up of GV150013 and submitted for further evaluation.

1. Introduction

The brain neurotransmitter cholecystokinin (CCK) is a 33 aminoacids peptide present in both the gastrointestinal tract and the central nervous system. Two receptor subtypes have been identified: the CCK1 receptors, which are present mainly in the periphery (tissues and nervous system) and the CCK2 receptors, which are largely distributed in the central nervous system.[1] The CCK1 receptor subtypes are involved both in the control of the gall bladder and in the digestive enzyme secretion, while the CCK2 subtypes are mainly responsible for neurotransmission and neuromodulation. In particular, during the last years, it has been demonstrated that CCK2 antagonists are targets for anxiety and panic.[2–7] Many different classes of compounds have been reported as CCK2 antagonists[8]: 1,4-benzodiazepines[3] and 1,3,4-benzotriazepines[9], dipeptoids[10], quinazolines[11], pyrazolidinones[12], quinazolinones.[13] As part of our search for new CCK2 antagonists endowed with anxiolytic activity, we have already reported the synthesis of a number of 1-alkyl-5-aryl-1,5-benzodiazepines bearing either arylureido[14–16] or (aryloxycarbonyl)amino groups at the C-3 position[17] (Fig. 1, compound of general formula **I** and **II**, respectively). It was noticed that the substituent at the N-1 position played an important role in achieving a good selectivity for the type 2

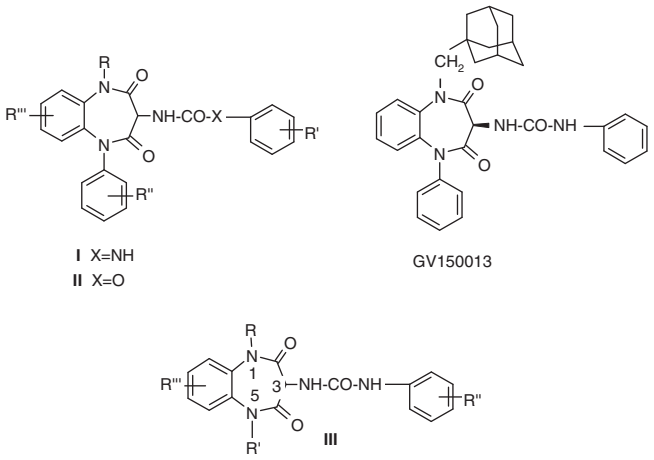

Figure 1. Structures of 1,5-benzodiazepines.

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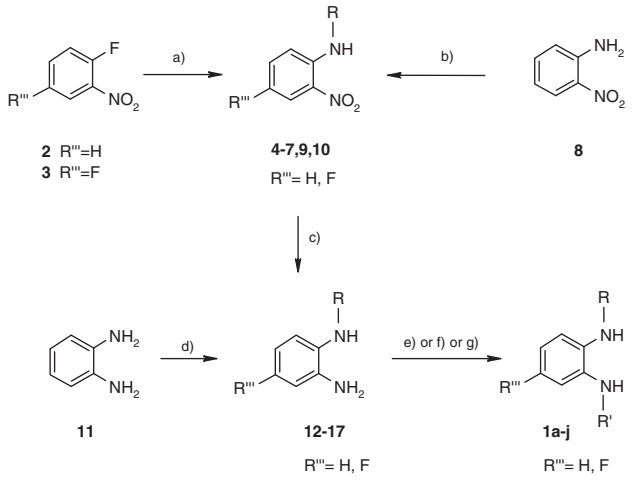
receptors; in particular, the structure–activity studies performed suggested that a bulky and lipophilic group at N-1 was important for achieving good selectivity.[18,19] On this basis, GV150013 (Fig. 1) was accepted into exploratory development (pK_i = 9.02; selectivity ratio 2/1 = 1175; duration of action in mice 6–8 h; solubility less than 0.20 mg/mL and oral bioavailability less than 10% and recommended for the treatment of panic attacks, anxiety and pain.[20,21]

The substitution of the N-5 phenyl ring with alkyl group was also attempted and the compounds so obtained retained the affinity and selectivity for the CCK2 receptor.[22–24] Thus, in order to improve the physicochemical properties of GV150013, further studies were devoted to the identification of a possible follow up endowed with a better pharmacokinetic profile. Our activities were directed towards the synthesis of compounds of general formula **III** (Fig. 1), bearing hydrophilic substituents (in particular, aminoalkyl substituents) at the N-5 position, while maintaining at N-1 those alkyl groups that had given the best results in the *N*-5 phenyl series[19] (like, e.g., isopentyl, cyclohexylmethyl and 1-adamantylmethyl). Further modulation of the activity was attempted with different substituents on the ureidic aromatic ring. Thus, this article deals with the studies regarding the synthesis and the pharmacological evaluation of this latter type of 1,5-benzodiazepines, where a water solubilizing group at N-5 was introduced to improve the pharmacokinetic profile; at the same time, the combination of other substituents would maintain the characteristics of a good CCK2 receptor antagonist such as GV150013 in term of affinity, selectivity and duration of action.

2. Chemistry

Two main approaches were explored for the synthesis of racemic ureido compounds. In the first one, both the substituents at N-1 and N-5 positions were introduced at the beginning of the synthetic pathway; thus, the N,N'-dialkyl-1,2-phenylenediamines **1** (Scheme 1) were identified as the most important key intermediates, and their synthesis was accomplished according to different sequences, as outlined in the Scheme 1.

Reaction of either 2-fluoronitrobenzene **2** or 2,4-difluoronitrobenzene **3** with the alkyl amine in tetrahydrofuran, gave the



Figures legends

legend	R	R'	R'''
a	3-methyl-1-butyl	2-(N-morpholino)ethyl	H
b	cyclohexylmethyl	2-diethylaminoethyl	H
c	cyclohexylmethyl	2-(N-morpholino)ethyl	H
d	3-methyl-1-butyl	(4-methoxyphenyl)methyl	H
e	(1-adamantyl)methyl	2-(N-morpholino)methyl	H
f	(1-adamantyl)methyl	2-(1-pyrrolidino)ethyl	H
g	1-adamantanecarbonyl	2-(N-morpholino)ethyl	F
h	(1-adamantyl)methyl	2-(N-morpholino)ethyl	F
i	1-adamantanecarbonyl	(4-methoxyphenyl)methyl	H
j	(1-adamantyl)methyl	(4-methoxyphenyl)methyl	H

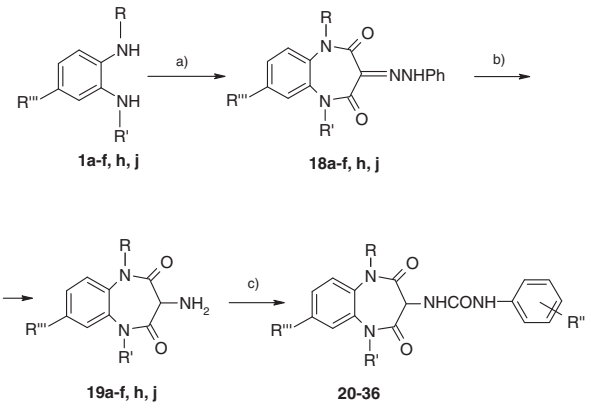
Scheme 1. Reagents and conditions: (a) R-NH_2., THF; (b) 1) RCOCl, TEA, acetone; (2) BH_3Me_2S, toluene; (c) $Na_2S_2O_4$, K_2CO_3, EtOH, H_2O; (d) RX, NaI, DMF; (e) R'X, NaI, DMF or R'X, NaI, K_2CO_3, xylene; (f) R'CHO, $Na(CN)BH_3$, AcOH, MeOH; (g) (1) R'COCl, TEA, THF; (2) vitride, toluene or BH_3Me_2S, THF.

2-nitro intermediates **4**–**7**. Then, reduction with sodium hydrosulphite and alkylation gave compounds of type **1**.[16] The latter reaction was conducted in three different ways: 1) by using an alkyl halide in either dimethylformamide or xylene[25] in the presence of potassium carbonate; 2) by coupling the *N*-alkylphenylenediamines (**12**–**17**, Scheme 1) with a carbonyl chloride followed by reduction of the amido group to the corresponding amine; 3) via reductive amination by using sodium cyanoborohydride in methanol/acetic acid mixture.[26] The phenylenediamine intermediates **12**–**17** were easily obtained also starting either from commercially available 2-nitroaniline **8** (by reaction with an appropriate carbonyl chloride followed by two reductions, the first one with borane, the next with sodium hydrosulphite) or by direct alkylation of the 1,2-phenylenediamine **11** (Scheme 1).

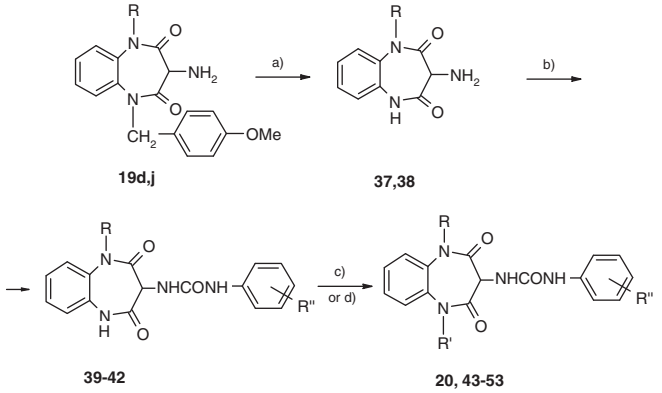
Starting from intermediates **1**, the target 1,5-benzodiazepines **20**–**36** were synthesized according to the route outlined in Scheme 2. Intermediates **1** were condensed with 2-phenylhydrazonomalonyl dichloride[27,28] in tetrahydrofuran or ethyl acetate to give intermediates of type **18**. Reduction of the latter with zinc and acetic acid followed by reaction with the appropriate aryl isocyanate led to the final ureido compounds.

In order to introduce the N-5 substituents at a later stage of the synthesis, the *N*-1-alkyl-3-aminobenzodiazepines **37** and **38** (Scheme 3) were identified as useful and advanced key intermediates, which could be functionalized with various aryl isocyanates at the C-3 amino group and with different alkyl substituents at the 5 position. More specifically, this sequence was applied to both the 1-isopentyl and 1-adamantylmethyl substituents at the N-1 position (Scheme 3) which in the previous series have proven beneficial in contributing to both high affinity and high selectivity for the CCK2 receptors.[19] As outlined in the Scheme 3, compounds **37** and **38** were obtained from the corresponding intermediates **19d**, **j**, bearing the *p*-methoxybenzyl substituent as a N-5 protective group. The deprotection of **19d**, **j** with cerium ammonium nitrate gave the desired intermediate **37** and **38** in 29% and 84% yields respectively. The next reaction with the appropriate isocyanates gave intermediates **39**–**42**. The latter were alkylated with either sodium hydride and alkyl halides in DMF at 70 °C for 5 h or under milder conditions using the alkyl halide in the presence of potassium carbonate in acetone/water at reflux overnight[29,30] to give the final compounds **43**–**53** (it is noteworth that the latter method gave better yields than the first one, 70–90% vs 50% with NaH).

The stereogenic center at the C-3 position on the benzodiazepine nucleus represents an additional site for the modulation of receptor discrimination. It had already been demonstrated in the *N*-5-aryl series that a different stereochemistry at C-3 could result in a different 2/1 selectivity.[18] On the other hand, the chiral



Scheme 2. Reagents and conditions: (a) PhNHN=C(COCl)$_2$, THF; (b) Zn/AcOH; (c) ArNCO, CH_3CN.



Scheme 3. Reagents and conditions: (a) CAN, CH_3CN, H_2O; (b) ArNCO, CH_3CN; (c) R'X, NaH, DMF; (d) R'X, K_2CO_3, acetone, H_2O.

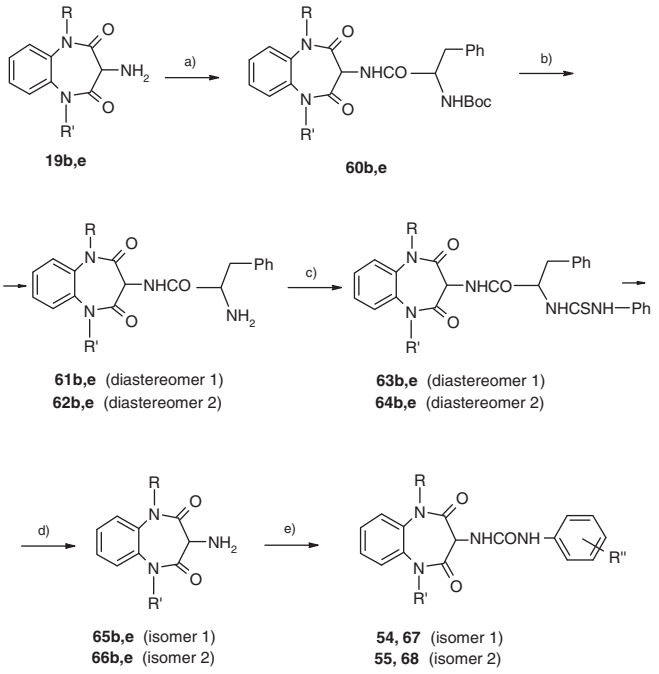
discrimination may be dependent on the nature of the substituents at different positions, as proven by the results reported by Hagishita et al. on symmetrical *N*,*N*-dialkyl-1,5-benzodiazepines.[19] Therefore, we undertook the evaluation for potential chiral discrimination within the current series. While in the initial phase of this work the final chiral compounds were isolated by preparative HPLC (compounds **54**–**59**) we also considered the identification of a suitable chemical method for the preparation of the target compounds as single enantiomers. Initially, we focused our interest on the resolution of the compounds with the best in vitro profiles; in particular, the 1-(1-adamantyl)methyl-5-[2-(*N*-morpholino)ethyl]-benzodiazepine. On the basis of the previous experience gained with the *N*-5-phenyl series,[18] the selective crystallization of diastereomeric salts of the 3-amino derivatives with optically active acids (eg.camphorsulphonic acid) was unsuccessfully attempted, being the basic substituent (eg morpholine) the main reacting site in the salification step. Starting again from 3-amino intermediates, the formation of chromatographically separable diastereomers was attempted by the addition of either i) a chiral carbonate or chloroformate;[31,32] or ii) a carbohydrate,[33] but no significant results were obtained. Finally, the resolution into the target enantiomers (**54**, **55**, **67**, **68**) was obtained using the two following methods:

Method a (applied to the racemic amines **19b** and **19e**) involves the use of L-phenylalanine, as reported for the resolution of some 1,4-benzodiazepines,[34,35] to give the corresponding diastereomeric phenylalanyl amides, which upon separation and removal of the phenylalanyl moiety via Edman degradation provided the desired amines (Scheme 4).[36]

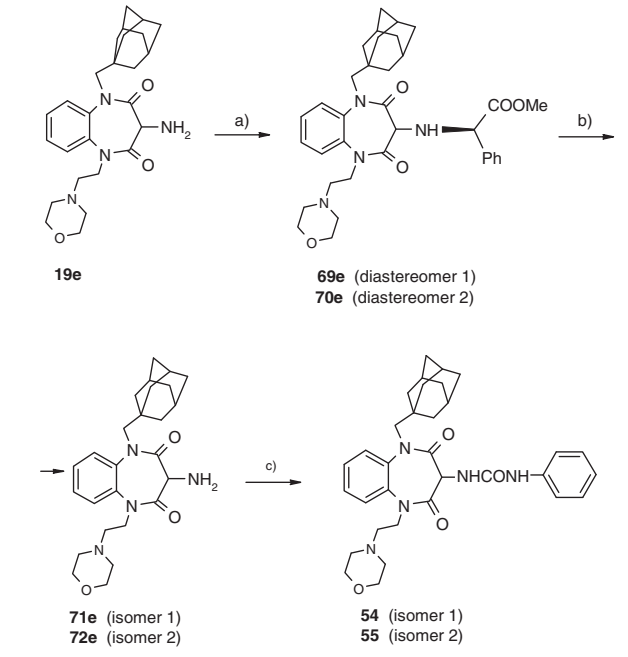
Method b involves the synthesis of intermediate tosylmandelates and has been successfully applied to various substrates.[37,38] We here report its application for the resoluton of **19e** only. Thus, the tosylate of the chiral mandelate was reacted with racemic 3-aminobenzodiazepines **19e** to obtain the diastereomeric mixture of **69e** and **70e** (Scheme 5). The chromatographic separation of the two diastereomers followed by hydrogenolysis to remove the chiral auxiliary led to the desired enantiomeric amines **71e** and **72e**, which were in turn transformed into the corresponding ureas by reaction with the proper aryl isocyanates. This new and simple method was very successfully used, giving the final isomeric urea derivatives in better yields than the previously mentioned method.[38]

3. Biology

The biological evaluation of the new compounds was performed according to the following screening sequence and adopting well



Scheme 4. Reagents and conditions: (a) $PhCH_2CH(NHBoc)COOH$, DCC, EDC, AcOEt; (b) (1) TFA, CH_2Cl_2; (2) NH_3, AcOEt; (c) PhNCS, CH_2Cl_2; (d) TFA; (e) ArNCO, CH_3CN.



Scheme 5. Reagents and conditions: (a) PhCH(OTos)COOMe, DIPEA, THF (b) H_2, $Pd(OH)_2/C$, MeOH; (c) ArNCO, CH_3CN.

described methods: (a) displacement binding assay to evaluate the affinity for the CCK2 and CCK1 receptor subtypes;[39,40] (b) in vivo black-white box test in Mice to evaluate the anxiolytic activity;[21] (c) bioavailability studies.

4. Results and discussion

Initially, the 1,5-dialkyl-benzodiazepines were synthesized with the aim of investigating the role of the *N*-5 phenyl and consequently spread out the area of interest. Moreover, if an alkyl was tolerated, it could have been further functionalized with basic groups, thus responding to the emerging expectations of the project for a more soluble and bioavailable compound with respect to our initial lead GV150013. It was soon realised that the *N*-5-phenyl could have been replaced by a proper alkyl group while retaining both the affinity and the selectivity obtained in the previous series. The results obtained in our investigations are reported in the table below. For the sake of simplicity, the compounds are listed and discussed according to the substituent at the N-1 position, which were in turn selected during our previous study related to the *N*-1-alkyl-*N*-5-phenyl series.[18,19]

4.1. In vitro data

4.1.1. 1-Isopentyl substituted compounds (Table 1)

Compounds bearing morpholinoethyl, dimethylaminoethyl or diethylaminoethyl substituents at the N-5 position proved to have both good affinity and selectivity. Furthermore, these compounds are quite soluble in acidic conditions and some are also soluble in water. Good binding values were obtained with both the 5-dimethylaminoethyl **44** and 5-diethylaminoethyl **45** derivatives; the selectivity of the former was further increased by the introduction of a 4-methoxy group at the C-3 side chain, even though this caused a decrease of the affinity for the CCK2 receptor (**46**). A minor affinity for the CCK2 receptor was also observed with the piperidinoethyl derivative **43**, whose selectivity is similar to that of the morpholino compound **20**.

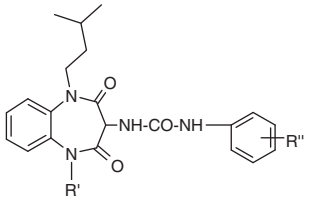
On the basis of its interesting physicochemical characteristics, the morpholinoethyl derivative **20** (selectivity 234; solubility 0.46 mg/mL at pH 2.4) was selected as a starting point for an optimization study aimed at obtaining compounds endowed with an increased selectivity, while retaining or increasing both the solubility and the CCK2 affinity. At first, deeper studies on the modulation of the activity via modification of the ureidic aromatic ring at the C-3 position were undertaken. Monosubstituted phenyl derivatives proved to be interesting compounds (see compounds **21**–**25** and **47**) in terms of both affinity and selectivity with the exception of compound **26**, whose affinity for both receptors dropped significantly (the selectivity maybe underestimated indeed). On the contrary, substituents at the 2 position of the aromatic ring, even in combination with other groups at different positions, caused a decrease in affinity for the CCK2 receptor (see **27**, **28**, **29**, **30**). Finally, the objective was partially reached with the para hydroxy derivative **48** (selectivity 1122; pK_i 8.13; solubility 0.45 mg/ml at pH 2.4).

Only a few examples of cyclohexylmethyl derivatives were synthesized in order to study the behaviour of molecules having an N-1 substituent with intermediate steric hindrance and lipophilicity with respect to those of the isopentyl and the adamantylmethyl groups. In particular, derivatives bearing either a diethylaminoethyl or a morpholinoethyl substituent at N-5 were tested (**31** and **32**, respectively). Compound **31** showed quite interesting binding values, while the morpholine derivative **32** proved to be less selective.

4.1.2. 1-(1-Adamantyl)methyl substituted compounds (Table 2)

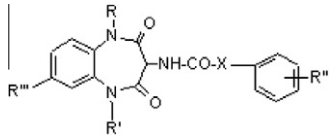
Considering the dramatic importance of the 1-adamantylmethyl group in the 5-aryl series,[18] which resulted in the most active and selective compounds, many basic derivatives synthesized belong to this series. Several of them showed an interesting in vitro profile, but unfortunately only few could be selected for in vivo evaluation. Compound **50** showed good selectivity and also a good value of solubility in acidic conditions (0.85 mg/ml). Its homologue **51** showed even a better selectivity. Interesting selectivity values were also obtained with the pyrrolidinoethyl substituted compound **35**, but it was not progressed into in vivo evaluation because of the better results obtained with the morpholinoethyl substituted compounds.

Table 1
Binding data of 1-isopentyl derivatives



Compound	R′	R″	pK_i CCK-2	pK_i CCK-1	Selectivity 2/1
20	CH_2CH_2-(*N*-morpholine)	H	8.54 ± 0.01	6.17 ± 0.09	234
21	CH_2CH_2-(*N*-morpholine)	4-Cl	8.71 ± 0.04	5.99 ± 0.06	525
22	CH_2CH_2-(*N*-morpholine)	3-Me	9.11 ± 0.14	6.56 ± 0.01	372
23	CH_2CH_2-(*N*-morpholine)	4-CF_3	8.44 ± 0.08	5.83 ± 0.05	407
24	CH_2CH_2-(*N*-morpholine)	4-Br	8.44 ± 0.08	5.97 ± 0.03	295
25	CH_2CH_2-(*N*-morpholine)	4-F	8.40 ± 0.10	5.84 ± 0.03	363
26	CH_2CH_2-(*N*-morpholine)	4-OEt	7.17 ± 0.02	<5.0	148
27	CH_2CH_2-(*N*-morpholine)	4-Br-2-Me	7.85 ± 0.06	6.27 ± 0.03	38
28	CH_2CH_2-(*N*-morpholine)	2-Me-4-OMe	7.54 ± 0.07	5.18 ± 0.09	229
29	CH_2CH_2-(*N*-morpholine)	2-iPr	6.89 ± 0.10	<5.0	78
30	CH_2CH_2-(*N*-morpholine)	2-Cl, 6-Me	6.93 ± 0.03	5.29 ± 0.07	44
47	CH_2CH_2-(*N*-morpholine)	4-OMe	8.10 ± 0.02	5.29 ± 0.08	646
48	CH_2CH_2-(*N*-morpholine)	4-OH	8.13 ± 0.01	5.08 ± 0.04	1122
43	CH_2CH_2-(*N*-piperidine)	H	8.17 ± 0.06	5.69 ± 0.02	302
44	CH_2CH_2-NMe_2	H	8.36 ± 0.05	5.48 ± 0.04	759
46	CH_2CH_2-NMe_2	4-OMe	7.95 ± 0.15	4.76 ± 0.02	1549
45	CH_2CH_2-NEt_2	H	8.80 ± 0.04	5.39 ± 0.02	2570

Table 2
Binding data of others derivatives



Compound	R	R′	R″	R‴	pK_i CCK-2	pK_i CCK-1	Selectivity 2/1
31	Cyclohexylmethyl	CH_2CH_2-NEt_2	H	H	8.99 ± 0.08	5.82 ± 0.09	1479
32	Cyclohexylmethyl	CH_2CH_2-(*N*-morpholine)	H	H	8.56 ± 0.07	6.46 ± 0.05	126
33	Cyclohexylmethyl	CH_2CH_2-(*N*-morpholine)	3-Me	H	9.26 ± 0.08	6.76 ± 0.05	316
34	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	H	H	9.24 ± 0.11	5.69 ± 0.03	3548
36	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	H	7-F	8.36 ± 0.07	5.58 ± 0.01	603
53	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	4-F	H	8.66 ± 0.05	5.85 ± 0.08	646
35		CH_2CH_2-(*N*-pyrrolidine)	H	H	8.98 ± 0.06	5.02 ± 0.07	9120
50	(1-Adamantyl)methyl	CH_2CH_2-NMe_2	H	H	9.00 ± 0.11	5.49 ± 0.02	3236
51	(1-Adamantyl)methyl	$CH_2CH_2CH_2$-NMe_2	H	H	8.44 ± 0.13	4.69 ± 0.05	5623
52	(1-Adamantyl)methyl	CH_2CH_2-NEt_2	4-F	H	8.71 ± 0.05	5.78 ± 0.04	851

Focusing attention on the 5-morpholinoethyl series, compound **34** was quickly identified as a high selective compound with nanomolar affinity for the CCK2 receptor. Moreover its solubility was 0.230 mg/mL. Two close analogues of compound **34** were prepared, namely the *p*-fluoro derivative **53** (solubility 0.02 mg/mL) and the 7-fluoroderivative **36** (solubility 0.36 mg/mL). All these compounds exhibited very interesting binding values.

4.1.3. Enantiomers: binding assays (Table 3)

A few compounds were selected for the optical resolution into their enantiomers. Also in this class, as in the 5-aryl series, the two isomers showed a different behaviour. In the cyclohexylmethyl series, only one compound **31** was selected for the optical resolution; in this case, none of the enantiomers proved overall better than the racemic mixture (compound **68** showed higher affinity for CCK2 but lower selectivity). As far as the *N*-1-(1-adamantyl) methyl-5-[2-(*N*-morpholino)ethyl] derivatives are concerned, the isomers of the parent compound **34** (**54** and **55**) had a very different behaviour; in particular, the (−) isomer **54** was more potent towards the CCK2 receptor than the racemate, while maintaining the same affinity for the CCK1 receptor, thus resulting nearly 3 times more selective.

Compound **56**, the (−) isomer of the 7-fluoro derivative **36** is 1.5 times more selective than the corresponding racemate, while **58**, the (−) isomer of **53**, is very potent on the CCK2 receptors with a noteworthy increase in selectivity (4.5 times more than the corresponding racemate). On the other hand, the corresponding (+) isomers, **57** and **59**, showed much reduced selectivity. However, compound **53** and its (−) isomer **58** proved to be poorly soluble (0.02 mg/mL) and were not considered for further studies.

4.2. In vivo tests and pharmacokinetic evaluation

The most soluble and active compounds were tested in the black and white box model in mice and their pharmacological profile was evaluated. The black/white box model was used for the evaluation of both potency and duration of action of the compounds (Table 4). The compounds were administered orally to the animals 60 min before the experiment.

In the isopentyl series, a short duration of action was observed; in particular compound **48** exhibited a very short duration of action, probably shorter than the time of pretreatment of the animals. Better results were obtained with the adamantylmethyl series, which lasted longer than 4 h, thus suggesting that the duration of action is linked to the lipophilicity of the molecules (the isopentyl **48**, which is the less lipophilic amongst all the compounds tested, has *c* log *P* = 4.9, while for the adamantylmethyl derivatives, the *c* log *P* values are higher than 6). Considering all the data reported in Table 4, it appears that in order to have a duration of action longer than 4 h, the required *c* log *P* should be higher than 6.2. From these and previous data, it is also quite evident that compound **54** seems to be the best one in this series and its activity can be compared with that of GV150013 (mouse black/white box model: ED_{50} (μg/Kg po) 0.05 and 0.002 for GV150013 and **54** respectively).[18]

The more interesting compounds were tested both in dogs and mice as methocel suspension in order to evaluate their pharmacokinetic parameters (Table 5). In particular the bioavailability was considered as a discriminant factor for the choice of the possible follow up of GV150013.

Bioavailability of compounds **20** and **44** was acceptable, but their duration of action was shorter than 4 h and therefore they were not considered, whilest amongst the others, compound **54** emerged as the best. Further observations led to a more precise definition of a correlation between lipophilicity, bioavailability and duration of action of this type of benzodiazepines; the optimal value of *c* log *P* should be comprised in the the range 6.2 and 6.8, in order to have the best compromise between bioavailability and duration of action. Compound **54** meets this criterion and presents a good balance between solubility (increased with respect to that of GV150013) and duration of action. Its pharmacological and pharmacokinetic properties compared well to those of the lead compound GV150013, and make compound **54** a potential follow up of GV150013.

Table 4
In vivo values

Compound	ED_{50} (μg/kg po)	Duration of action (h)	*c* log *P*
20	0.011	2–4	5.12
34	0.001	4–6	6.41
44	0.041	2–4	5.24
45	0.0006	Not tested	6.13
48	Not significant effects up to 300		
50	0.004	4–6	6.53
54	0.002	4–6	6.41
56	0.080	4–6	6.77

Table 5
Pharmacokinetic parameters of the more interesting compounds

compound	Animal	Clearance (mL/min)	Volume (L/kg)	$t_{1/2}$ (h)	Oral bioavail. (%)	Solubility (μg/mL)
20	Dog	14	1.0	0.9	36	450
	Rat	34	1.1	0.38	18	
34	Dog	34	4.8	1.6	9	230
	Rat	12	1.0	1.1	13	
44	Dog	23	2.5	1.3	40	850
	Rat	37	3.1	1.0	20	
50	Rat	28	2.7	1.1	5	850
54	Rat	12	1.1	1.1	10	230
56	Rat	23	2.2	1.1	5	360

5. Conclusions

The objective of the work here described was to find out a compound with in vitro characteristics similar to those reported for the lead CCK2 antagonist GV150013 associated with improved solubility and pharmacokinetic parameters. The difficulties in combining all these characteristics became very evident in the basic series of compounds, where the increased bioavailability implied a reduction of duration of action.

Even though exact SARs were not defined, some general conclusions can be drawn. Compounds with an isopentyl chain at N-1 had interesting binding values, good solubility and bioavailability, but did not have the expected behaviour in vivo (short duration of action), particularly when compared with the adamantyl counterparts. With an adamantylmethyl group at the N-1 position, interesting results were obtained, particularly with the morpholinoethyl derivative in its enantiomeric form (compound **54**). Its affinity, selectivity and duration of action were combined with good solubility and acceptable bioavailability to give a compound that could be a suitable candidate as a follow up for GV150013. Nevertheless, more studies are necessary in order to find an even better combination between the basic groups at the 5 position and the substituents at the C-3 aromatic ring. This would also give us more insight into the medicinal chemistry of this class of benzodiazepines.

6. Experimental

Melting points (mp) were determined on a Büchi Mp apparatus and are uncorrected. All temperatures refer to °C. Infrared spectra (IR) were measured in chloroform-d1 solutions, unless otherwise specified, on a Brucker ISF48 FT-IR instrument. Proton Magnetic Resonance (^{1}H-NMR) spectra were recorded either on a Varian

Table 3
Binding data of the isomers

Compound	R	R′	R″	R‴	Isomer	pK_i CCK-2	pK_i CCK-1	Selectivity 2/1
54	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	H	H	(−)	9.64 ± 0.03	5.69 ± 0.04	8913
55	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	H	H	(+)	8.03 ± 0.07	5.80 ± 0.02	170
56	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	H	7-F	(−)	8.52 ± 0.00	5.51 ± 0.01	1023
57	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	H	7-F	(+)	7.31 ± 0.13	5.65 ± 0.07	46
58	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	4-F	H	(−)	9.45 ± 0.01	5.99 ± 0.01	2884
59	(1-Adamantyl)methyl	CH_2CH_2-(*N*-morpholine)	4-F	H	(+)	7.85 ± 0.01	5.59 ± 0.10	182
67	Cyclohexylmethyl	CH_2CH_2-NEt_2	H	H	1	8.23 ± 0.13	5.57 ± 0.05	457
68	Cyclohexylmethyl	CH_2CH_2-NEt_2	H	H	2	9.40 ± 0.05	6.33 ± 0.06	1175

300 MHz or 400 MHz spectrometers as solutions in chloroform-d1, unless otherwise specified. Chemical shifts are reported in ppm downfield using residual $CHCl_3$ as internal reference. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. FAB positive mass spectra (MS) were taken on a VG4-triple quadrupole Fison instrument. Optical rotations were determined at 20 °C with a Jasco DIP 360 instrument (l = 10 cm, cell volume = 1 mL, λ = 589 nm). Flash silica gel chromatography was carried out over Silica gel 230–400 mesh supplied by Merck AG Darmstadt, Germany. TLC refers to thin layer chromatography on 0.25 mm Silica gel plates (60F-254 Merck) and visualized with UV light. Solutions were dried over anhydrous sodium sulphate. Methylene chloride was redistilled over calcium hydride and tetrahydrofuran was redistilled over sodium. The following abbreviations are used in the text. AcOEt = ethyl acetate, DCM = methylene chloride, DMF = N,*N*′-dimethylformamide, Et2O = diethyl ether, IPA = isopropanol, MeOH = methanol, P = petroleum ether 40–60 °C, THF = tetrahydrofuran. All the compounds are intended as racemic mixtures unless otherwise indicated. Analyses, performed on Carlo Erba elemental analyzer, indicated by the symbols of the elements or functions were within *0.4% of theoretical values.

6.1. General procedure for the synthesis of 1-alkylamino-2-nitrobenzene (4–7)

6.1.1. 1-[2-(*N*-Morpholino)ethyl]amino-2-nitrobenzene (4)

A mixture of 4-(2-aminoethyl) morpholine (9.24 g; 0.071 mol) and 2-fluoronitrobenzene **2** (5.0 g; 0.0355 mol) in tetrahydrofuran (50 mL) was stirred at 23 °C for 24 h. The mixture was filtered off and concentrated in vacuo to a residue that was purified by flash chromatography (eluting with cyclohexane–AcOEt 1:1) to give the title compound as an orange oil (8.6 g). Y = 98% TLC cyclohexane–AcOEt 1:1, R_f = 0.32. IR: 1620 and 1570 (C═C); 1514 and 1352 (NO_2) cm^{-1}. ^1H NMR: 8.50 (s, 1H); 8.18 (dd, 1H); 7.43 (t, 1H); 6.82–6.64 (d, 2H); 3.76 (m, 4H); 3.36 (m, 2H); 2.73 (t, 2H); 2.53 (m, 4H). MS: *m*/*z* = 252 $[M+H]^+$.

Compounds **5**–**7** were prepared as reported for **4**.

6.1.2. 5-Fluoro-*N*-[2-(*N*-morpholino)ethyl]-2-nitroaniline (5)

Y = 78.8% (yellow solid) Mp 103–4 °C. TLC cyclohexane–AcOEt 1:1, R_f = 0.39. IR (nujol): 3400 (NH); 1632 (C═C); 1570 and 1312 (NO_2) cm^{-1}. ^1H NMR: 8.64 (br s, 1H); 8.21 (m, 1H); 6.44 (m, 1H); 6.36 (m, 1H); 3.75 (t, 4H); 3.29 (m, 2H); 2.72 (t, 2H); 2.52 (t, 4H). MS: *m*/*z* = 270 $[M+H]^+$.

6.1.3. 1-(3-Methyl-1-butyl)amino-2-nitrobenzene (6)

Y = 59.7% (yellow oil) TLC cyclohexane–AcOEt 8:2, R_f = 0.79. IR (film): 3383 (NH), 1620 (C═C) cm^{-1}. ^1H NMR: 8.16 (dd, 1H), 8.02 (br s, 1H); 7.43 (m, 1H); 6.85 (d, 1H); 6.62 (m, 1H); 3.31 (m, 2H); 1.77 (m, 1H); 1.63 (m, 2H); 0.97 (d, 6H). MS: *m*/*z* = 208 $[M]^+$, 209 $[M+H]^+$.

6.1.4. *N*-(4-Methoxyphenylmethyl)-2-nitroaniline (7)

Y = 66.2% (orange solid). Mp 94–5 °C. TLC cyclohexane–AcOEt 8:2, R_f = 0.55. IR: 3400 (NH), 1610 (C═C), 1530 and 1370 (NO_2) cm^{-1}. ^1H NMR: 8.35 (s, 1H); 8.19 (dd, 1H); 7.39 (m, 1H); 6.89 (d, 2H); 6.83 (d, 1H); 6.65 (d, 1H); 4.47 (d, 2H); 3.80 (s, 3H). MS: *m*/*z* = 259 $[M+H]^+$.

6.2. Synthesis of 1-alkylamino-2-nitrobenzene (9, 10)

6.2.1. 1-(1-Adamantyl)carbonylamino-2-nitrobenzene (9)

A solution of 1-adamantanecarbonyl chloride (17.95 g; 0.0903 mol) in acetone (60 mL) was dropped into a solution of 2-nitroaniline **8** (10.4 g; 0.0753 mol) and triethylamine (12.6 mL; 0.0903 mol) in acetone (50 mL), at 23 °C under a nitrogen atmosphere. The mixture was stirred at 23 °C for 22 h, then further acetone (50 mL) was added. The mixture was heated at 70 °C for 3 h. The mixture was allowed to cool to rt, then filtered; the brown solid obtained was crystallized from acetone to give the title compound as a yellow solid (17.3 g). Y = 77% Mp 111–114 °C. TLC cyclohexane–AcOEt 10:2, R_f = 0.67. IR (nujol): 3342 (NH), 1697 (C═O), 1585 and 1337 (NO_2) cm^{-1}. ^1H NMR: 10.68 (s, 1H); 8.85 (d, 1H); 8.22 (d, 1H); 7.64 (t, 1H); 7.15 (t, 1H); 2.13 (m, 2H); 2.6–1.5 (m, 15H). MS: *m*/*z* = 301 $[M+H]^+$.

6.2.2. 2-(1-Adamantyl)methylamino-2-nitrobenzene (10)

Borane dimethylsulfide complex (10 M solution; 6.0 mL) was added, dropwise, under a nitrogen atmosphere, to a solution of 1-(1-adamantyl)carbonylamino-2-nitrobenzene **9** (13.5 g; 0.045 mol) in dry toluene (160 mL) previously cooled to 10 °C. The solution was stirred at 10 °C for 15 min, and then heated at 110 °C for 1 h. The solution was allowed to cool to rt, then a 10% potassium carbonate solution (50 mL) was added and the mixture was stirred at 23 °C for 40 min. The layers were separated; the organic extracts were washed with brine (50 mL), dried and concentrated in vacuo to a slurry solid, which was purified by flash chromatography (eluting with cyclohexane–AcOEt 10:1) to give the title compound as an orange solid (7.0 g). Y = 54% Mp 106–109 °C. TLC cyclohexane–AcOEt 10:1, R_f = 0.68. IR (nujol): 3371 (NH), 1620 (C═C), 1574–1377 (NO_2) cm^{-1}.^1H NMR: 8.29 (br s, 11H); 8.16 (d, 1H); 7.40 (m, 1H); 6.88 (d, 1H); 6.59 (m, 1H); 2.98 (d, 2H); 2.03 (m, 3H); 1.8–1.6 (m, 12H). MS: *m*/*z* = 286 $[M]^+$, 287 $[M+H]^+$

6.3. Synthesis of 1-alkylamino-1,2-phenylenediamine via alkylation of 11

6.3.1. *N*-Cyclohexylmethyl-1,2-phenylenediamine (12)

A solution of 1,2-phenylenediamine **11** (5.0 g; 0.046 mol), cyclohexylmethyl bromide (8.1 g; 0.046 mol) and sodium iodide (7.0 g; 0.046 mol) in dry dimethylformamide (250 mL) was stirred at 23 °C for 24 h under a nitrogen atmosphere. The solution was diluted with water (200 mL) and extracted with ethyl acetate (4 × 200 mL); the combined organic extracts were washed with brine (500 mL), dried and concentrated in vacuo to an oil, which was purified by flash chromatography (eluting with cyclohexane–AcOEt 8:2) to give the title compound as a white solid (1.8 g). Y = 19% Mp 72–75 °C. TLC cyclohexane–AcOEt 8:2, R_f = 0.55. IR (nujol): 3400, 3371 and 3271 (NH_2 and NH) cm^{-1}. ^1H NMR: 6.83 (m, 1H); 6.75–6.62 (m, 3H); 3.32 (br s, 3H); 2.95 (d, 2H); 1.86 (bd, 2H); 1.8–1.55 (m, 4H); 1.35–0.95 (m, 5H). MS: *m*/*z* = 204 $[M]^+$, 205 $[M+H]^+$

6.4. General procedure for the synthesis of 1-alkylamino-1,2-phenylenediamine (13–17)

6.4.1. 1-[2-(*N*-Morpholino)ethyl]-1,2-phenylenediamine (13)[42]

Potassium carbonate (36.3 g; 0.261 mol) and sodium hydrosulphite (30.3 g; 0.174 mol) were added to a suspension of 1-[2-(*N*-Morpholino)ethyl]amino-2-nitrobenzene **4** (8.6 g; 0.0343 mol) in 95% ethanol (200 mL) and water (200 mL). The mixture was stirred at 23 °C for 30 min, then acidified to pH 3 with concd hydrochloric acid. The mixture was concentrated in vacuo to half volume and treated with 10% sodium hydroxide solution (150 mL) until pH 10. The mixture was extracted with ethyl acetate (2 × 200 mL); the combined organic extracts were washed with brine (300 mL), dried and concentrated in vacuo to give the title compound as a brown oil (6.4 g). Y = 84.4%. TLC AcOEt–MeOH 95:5, R_f = 0.22. ^1H NMR: 6.82 (m, 1H); 6.74–6.64 (m, 3H); 4.1–3.8

(br s, 1H); 3.72 (t, 4H); 3.40 (m, 2H); 3.177 (t, 2H); 2.682 (t, 2H); 2.487 (t, 4H). MS: m/z = 222 $[M+H]^+$.

Compounds **14**–**17** were prepared as reported for **13**.

6.4.2. 5-Fluoro-1-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine (14)

Y = 75.5% (brown oil) TLC AcOEt–MeOH 9:1, R_f = 0.28. IR (film): 3337 (NH and NH_2); 1614 (C=C) cm^{-1}. ^1H NMR: 6.61 (m, 1H); 6.38–6.28 (m, 1H); 4.4–4.2 (br s, 1H); 3.72 (t, 4H); 3.40–2.80 (m, 2H); 3.13 (t, 2H); 2.58 (t, 2H); 2.48 (m, 4H). MS: m/z = 240 $[M+H]^+$

6.4.3. 1-(3-Methyl-1-butyl)-1,2-phenylenediamine (15)

Yield quantitative (brown oil) TLC cyclohexane–AcOEt 8:2, R_f = 0.36. IR: 3410–3342 (NH and NH_2), 1620 (C=C) cm^{-1}. ^1H NMR: 6.83–6.80 (m, 1H); 6.72–6.62 (m, 3H); 3.4–3.2 (br s, 2H); 3.11 (t, 2H); 1.76 (m, 1H); 0.56 (m, 2H), 0.96 (d, 6H). MS: m/z = 178 $[M]^+$.

6.4.4. 1-(1-Adamantyl)methyl-1,2-phenylenediamine (16)

Y = 81% (grey solid) Mp 101–104 °C. TLC cyclohexane–AcOEt 10:2, R_f = 0.36. IR (nujol): 3395 and 3342 (NH), 1616 (C=C) cm^{-1}. ^1H NMR: 6.82 (m, 1H); 6.72 (m, 1H); 6.67 (m, 1H); 6.64 (m, 1H); 3.33 (s, 3H); 2.77 (s, 2H); 2.02 and 1.8–1.52 (m, m, 15H). MS: m/z = 256 $[M]^+$, 287 $[M+H]^+$.

6.4.5. 1-(4-Methoxyphenyl)methyl-1,2-phenylenediamine (17)

Y = 83.5% (beige solid). Mp 91–92 °C TLC cyclohexane–AcOEt 8:2, R_f = 0.22. IR (nujol): 3398, 3310 and 3263 (NH and NH_2) cm^{-1}. ^1H NMR: 7.32 (d, 2H); 6.88 (d, 2H); 6.80–6.65 (m, 3H); 4.24 (s, 2H); 3.8 (s, 3H); 3.5–3.3 (br s, 2H). MS: m/z = 228 $[M]^+$, 229 $[M+H]^+$.

6.5. Synthesis of N,*N*′-disubstituted phenylenediamine 1a–1j

6.5.1. *N*-(3-methyl-1-butyl)-*N*′-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine (1a)

Glacial acetic acid (1.5 mL; 0.0258 mol) was added to a solution of 1-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine **13** (5.7 g; 0.0258 mol) and 3-methylbutyraldehyde (2.7 mL; 0.0258 mol) in methanol (100 mL). The mixture was stirred at 23 °C for 10 min, then sodiumcyanoborohydride (3.5 g; 0.0516 mol) was added portion wise. Stirring was continued for 3 h, then the mixture was concentrated in vacuo; the residue was diluted with ethyl acetate (500 mL), washed with a 5% sodium bicarbonate solution (2 × 100 mL) and brine (150 mL), dried and the solvents were evaporated in vacuo. Purification of the crude material by flash chromatography (eluting with cyclohexane–AcOEt 55:45) afforded the title compound as colorless oil (3.3 g). Y = 44% TLC cyclohexane–AcOEt 1:1, R_f = 0.27. IR: 1601 (C=O) cm^{-1}. ^1H NMR: 6.79–6.66 (m, 4H); 3.71 (m, 4H); 3.13 (m, 4H); 2.69 (m, 2H); 2.49 (m, 4H); 1.79–1.59 (m, 3H); 0.97 (d, 6H). MS: m/z = 291 $[M]^+$, 292 $[M+H]^+$.

6.5.2. *N*-Cyclohexylmethyl-*N*′-(2-diethylaminoethyl)-1,2-phenylenediamine (1b)

A solution of *N*-cyclohexylmethyl-1,2-phenylenediamine **12** (2.42 g; 0.0118 mol), sodium iodide (1.22 g; 0.0118 mol) and 2-diethylaminoethyl chloride hydrochloride (2.0 g; 0.0118 mol) in dry DMF (100 mL) was heated at reflux for 4 h under a nitrogen atmosphere. The solution was cooled to rt and concentrated in vacuo. The residue was diluted with a 10% sodium hydroxide solution (100 mL) and extracted with ethyl acetate (200 mL). The organic layer was washed with brine (150 mL), dried and concentrated in vacuo to an oil, which was purified by flash chromatography (eluting first with AcOEt then with AcOEt–MeOH 9:1) to give the title compound as a brown oil (1.7 g). Y = 47.5% TLC AcOEt, R_f = 0.18. IR: 1599 (C=C) cm^{-1}. ^1H NMR: 6.77 (m, 2H); 6.65 (m, 2H); 3.12 (m, 2H); 2.95 (d, 2H); 2.77 (m, 2H); 2.58 (m, 4H); 1.87 (m, 2H); 1.70–1.61 (m, 1H); 1.40–1.20 (m, 2H); 1.10–1.03 (m, 4H). MS: m/z = 304 $[M+H]^+$.

6.5.3. *N*-Cyclohexylmethyl-*N*′-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine (1c)

Compound synthesized as described for **1b**. Purification by flash chromatography (eluting with cyclohexane–AcOEt 65:35) to give the title compound as brown oil (1.31 g). Y = 29%, TLC cyclohexane–AcOEt 1:1, R_f = 0.46. IR (film): 3323 (NH), 1601 (C=C) cm^{-1}. ^1H NMR: 6.85–6.75 (m, 2H); 6.70–6.60 (m, 2H); 3.75 (m, 4H); 3.15 (t, 2H); 2.96 (d, 2H); 2.70 (t, 2H); 2.49 (m, 4H); 1.95–1.00 (m, 11H). MS: m/z = 317 $[M]^+$, 318 $[M+H]^+$.

6.5.4. *N*-(4-Methoxyphenyl)methyl-*N*′-(3-methyl-1-butyl)-1,2-phenylenediamine (1d)

Compound synthesized as described for **1b**. Reaction time: 4 h at 80 °C. Purification by flash chromatography (eluting with cyclohexane–AcOEt 9:1) to give the title compound as yellow oil (14.0 g). Y = 55%, TLC cyclohexane–AcOEt 9:1, R_f = 0.42. IR: 1610 and 1601 (C=C) cm^{-1}. ^1H NMR: 7.31 (d, 2H); 6.89 (d, 2H); 6.84–6.74 (m, 2H); 6.70 (d, 2H); 4.22 (s, 2H); 3.81 (s, 3H); 3.10 (t, 2H); 1.75 (m, 2H); 1.6–1.5 (m, 1H); 0.94 (d, 6H). MS: m/z = 298 $[M]^+$, 299 $[M+H]^+$.

6.5.5. *N*-(1-Adamantyl)methyl-*N*′-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine (1e)

A solution of 1-(1-adamantyl)methyl-1,2-phenylenediamine **16** (8.0 g; 0.0312 mol), sodium iodide (6.2 g; 0.037 mol), potassium carbonate (8.6 g; 0.0624 mol) and 2-(*N*-morpholino)ethyl chloride hydrochloride (7.0 g; 0.0375 mol) in dry xylene (150 mL) was heated at 160 °C for 4 h under a nitrogen atmosphere. The solution was cooled to rt and concentrated in vacuo. The residue was diluted with ethyl acetate (700 mL) and washed with a 10% sodium hydroxide solution (300 mL) and brine (300 mL). The organic layer was dried and concentrated in vacuo to oil, which was purified by flash chromatography (eluting with cyclohexane–AcOEt 6:4) to give the title compound as brown oil (6.7 g). Y = 58%, TLC cyclohexane–AcOEt 1:1, R_f = 0.39. IR: 1601 (C=C) cm^{-1}. ^1H NMR: 6.77 (m, 2H); 6.71–6.61 (m, 2H); 4.25 (m, 1H); 3.71 (m, 4H); 3.20–3.15 (t, 3H); 2.76 (s, 2H); 2.72 (t, 2H); 2.50 (m, 4H); 2.03 and 1.8–1.52 (m, m, 15H). MS: m/z = 269 $[M]^+$, 370 $[M+H]^+$.

6.5.6. *N*-(1-Adamantyl)methyl-*N*′-[2-(1-pyrrolidino)ethyl]-1,2-phenylenediamine (1f)

Compound synthesized as described for **1b**. Purification by flash chromatography (eluting with AcOEt–MeOH 95:5) to give the title compound as brown oil (0.54 g). Y = 19.6%, TLC AcOEt–MeOH 9:1, R_f = 0.33. IR: 3362–3302 (NH) cm^{-1}. ^1H NMR: 6.80 (m, 2H); 6.60 (m, 2H); 3.19 (t, 2H); 2.80 (t, 2H); 2.75 (s, 2H); 2.70 (m, 4H); 2.01 (m, 3H); 1.82–1.60 (m, 16H). MS: m/z = 354 $[M+H]^+$.

6.5.7. *N*-(1-Adamantanecarbonyl)-4-fluoro-*N*′-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine (1g)

A solution of 1-adamantanecarbonyl chloride (1.78 g; 0.00896 mol) in dry THF (30 mL) was added dropwise to a mixture of 5-Fluoro-1-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine **14** (1.9 g; 0.00815 mol) and triethylamine (1.36 mL; 0.00978 mol) in dry THF (70 mL). The mixture was heated at 60 °C for 1.5 h, and then the solvents were evaporated in vacuo. The residue was taken up with ethyl acetate (200 mL), washed with water (100 mL) and brine (50 mL), dried and concentrated in vacuo to give the title

compound as a white solid (3.23 g). Y = 98% Mp 172–174 °C. TLC cyclohexane–AcOEt 1:1, R_f = 0.31. IR (nujol): 3375, 3314 (NH and NH_2); 1647 (C=O); 1618 and 1600 (C=C) cm^{-1}. ^1H NMR: 7.12 (m, 1H); 6.90 (s, 1H); 6.63 (t, 1H); 6.44–6.36 (m, 2H); 3.71 (t, 4H); 3.09 (m, 2H); 2.65 (t, 2H); 2.46 (t, 4H); 2.11 (m, 3H); 2.00–1.50 (m, 12H). MS: m/z = 402 $[M+H]^+$.

6.5.8. *N*-(1-Adamantyl)methyl-4-fluoro-*N′*-[2-(*N*-morpholino) ethyl]-1,2-phenylenediamine (1h)

A solution of Vitride [sodium dihydro-bis (2-methoxyethoxy)aluminate] (5.7 mL; 0.02 mol) in toluene (10 mL) was added dropwise over 15 min to a cooled (0 °C) suspension of *N*-(1-adamantanecarbonyl)-4-fluoro-*N′*-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine **1g** (3.23 g; 0.008 mol) in toluene (40 mL). The mixture was stirred at 0 °C for further 10 min, then at 23 °C for 30 min. The reaction was quenched by adding ethyl acetate (20 mL) at 0 °C, over 15 min. After additional 15 min, the mixture was diluted with more ethyl acetate (100 mL) and washed with water (3 × 100 mL); the aqueous layer was extracted with ethyl acetate (200 mL), the combined organic extracts were washed with brine (150 mL), dried and concentrated in vacuo. The residue was purified by flash chromatography (eluting with cyclohexane–AcOEt 7:3) to give the title compound as a wax (2.2 g). Y = 70.5% TLC cyclohexane–AcOEt 1:1, R_f = 0.49. IR (nujol): 3300 (NH); 1612 (C=C) cm^{-1}. ^1H NMR: 6.58 (dd, 1H); 6.41–6.30 (m, 2H); 4.68 (br s, 1H); 3.71 (t, 4H); 3.10 (m, 2H); 2.70–2.67 (s, 4H); 2.49 (m, 4H); 2.03 (m, 3H); 1.80–1.30 (m, 12H). MS: m/z = 388 $[M+H]^+$.

6.5.9. *N*-(1-Adamantanecarbonyl)-*N′*-(4-methoxyphenyl) methyl-1,2-phenylenediamine (1i)

Compound synthesized as described for **1g**. Reaction time 3 h at 60 °C. Y = 73%, (white solid). Mp 171–3 °C, TLC cyclohexane–AcOEt 9:1, R_f = 0.70. IR (nujol) 3393 and 3304 (NH); 1639 (CO); 1612 (C=C) cm^{-1}. ^1H NMR: 7.29 (d, 2H); 7.34–7.24 (m, 1H); 6.87 (d, 2H); 6.82–6.74 (m, 2H); 4.26 (s, 2H); 4.17 (s, 1H); 3.80 (s, 3H); 2.08 (m, 3H); 1.97–1.74 (m, 12H). MS: m/z = 391 $[M+H]^+$.

6.5.10. *N*-(1-Adamantyl)methyl-*N′*-(4-Methoxyphenyl)methyl-1,2-phenylenediamine (1j)

Borane Dimethylsulfide complex (10 M solution in THF; 15.0 mL) was added, dropwise, under a nitrogen atmosphere, to a solution of *N*-(1-adamantanecarbonyl)-*N′*-(4-methoxyphenylmethyl)-1,2-phenylenediamine (2.3 g; 0.0059 mol) in dry THF (70 mL) previously heated to 60 °C, while dimethyl sulphide was distilled simoultaneously. The solution was stirred at 10 °C for 15 min, and then heated at 110 °C for 1 h. After cooling to +10 °C, a 10% potassium carbonate solution (30 mL) was added and the mixture was extracted with ethyl acetate (200 mL); the organic layer was washed with water (4 × 100 mL) and brine (2 × 100 mL), dried and concentrated in vacuo to a residue, which was purified by flash chromatography (eluting with cyclohexane–AcOEt 8:2) to give the boron complex as a white solid (1.91 g). Y = 83.8%, TLC cyclohexane–AcOEt 9:1, R_f = 0.76.

A suspension of this material (1.55 g; 0.004 mol) and sodium carbonate (4.27 g; 0.004 mol) in MeOH (100 mL) was refluxed for 3 h, then allowed to cool to rt and filtered over celite. The solution was concentrated in vacuo; the residue was taken up with DCM (200 mL), washed with water (2 × 150 mL) and brine (2 × 100 mL), dried and concentrated in vacuo to give the title compound as a white solid (1.28 g). Y = 85%, Mp 132–134 °C TLC cyclohexane–AcOEt 9:1, R_f = 0.76. IR (nujol): 3331 (NH); 1610 (C=C) cm^{-1}. ^1H NMR: 7.33 (m, 2H); 6.90 (m, 2H); 6.84–6.66 (m, 4H); 4.25 (s, 2H); 3.81 (s, 3H); 3.7–3.3 (bm, 2H); 2.76 (s, 2H); 2.00 (m, 3H); 1.80–1.50 (m, 12H). MS: m/z = 376 $[M]^+$, 377 $[M+H]^+$.

6.6. General procedure for the synthesis of 1,5-dialkyl-2,4-dioxo-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine

6.6.1. 2,4-Dioxo-1-(3-methylbutyl)-5-[2-(*N*-morpholino)ethyl]-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18a)

A solution of 2-phenylhydrazonomalonyl dichloride[25] (3.6 g; 0.01474 mol) in ethyl acetate (150 mL) was added dropwise to a solution of *N*-(3-methyl-1-butyl)-*N′*-[2-(*N*-morpholino)ethyl]-1,2-phenylenediamine **1a** (3.3 g; 0.01134 mol) in ethyl acetate (250 mL) and the mixture was stirred under reflux for 2 h. The mixture was concentrated in vacuo and the residue was purified by flash chromatography (eluting with AcOEt), to give the title compound as yellow solid (3.6 g). Y = 70%, Mp 76–8 °C. TLC cyclohexane–AcOEt 1:1, R_f = 0.13. IR (nujol): 1653 and 1626 (C=O) cm^{-1}. ^1H NMR: 11.18, 11.11 (2d, 1H); 7.45–7.42 (m, 1H); 7.40–7.15 (m, 7H); 6.99 (t, 1H); 4.60 (m, 1H); 4.5–4.4 (m, 1H); 3.73 (m, 1H); 3.66 (t, 4H); 3.65–3.50 (m, 1H); 2.65–2.45 (m, 2H); 2.48 (m, 4H); 1.65–1.55 (m, 1H); 1.55–1.40 (m, 2H); 0.91 (d, 3H); 0.88–0.87 (dd, 3H). MS: m/z = 464 $[M+H]^+$.

Compounds **18b**–**18f**, **18h** and **18j** were prepared as reported for **18a**.

6.6.2. 1-Cyclohexylmethyl-2,4-dioxo-5-(2-diethylaminoethyl)-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18b)

Y = 38% (pale yellow foam). TLC cyclohexane–AcOEt 1:1, R_f = 0.34. IR (nujol): 3441–3186 (NH); 1661 (C=O) cm^{-1}. ^1H NMR: 11.23, 11.15 (2d, 1H); 7.46–7.14 (m, 8H); 6.99 (m, 1H); 4.54–4.44 and 4.38 (m, dd, 2H); 3.68 (m, 1H); 3.37 (m, 1H); 2.84–2.66 (m, 2H); 2.66–2.50 (m, 2H); 2.56–2.50 (m, 2H); 1.72–1.50, 1.22–0.84 and 1.03 (m, m, t, 17H). MS: m/z = 476 $[M+H]^+$.

6.6.3. 1-Cyclohexylmethyl-2,4-dioxo-5-[2-(*N*-morpholino) ethyl]-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18c)

Y = 45.6% (yellow foam). TLC cyclohexane–AcOEt 1:1, R_f = 0.45. IR (nujol): 3161 (NH), 1659 (C=O) cm^{-1}. ^1H NMR: 11.23, 11.17 (2d, 1H); 7.44, 7.37–7.15 and 7.00 (m, m, m, 9H); 4.62–4.32 (m, 2H); 3.84–3.64 (m, 5H); 3.37 (m, 1H); 2.72–2.40 (m, 6H); 1.76–1.46, 1.12 and 0.92 (m, m, m, 11H). MS: m/z = 490 $[M+H]^+$.

6.6.4. 2,4-Dioxo-5-(4-methoxyphenyl)methyl-1-(3-methyl-1-butyl)-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18d)

Y = 69% (yellow solid). Mp 189–191 °C, TLC cyclohexane–AcOEt 8:2, R_f = 0.30. IR (nujol): 3279 (NH); 1651 (C=O), 1614 and 1601 (C=C) cm^{-1}. ^1H NMR:11.19, 11.18 (2d, 1H); 7.4–7.1 (m, 10H); 7.00 (t, 1H); 6.75–6.71 (dd, 2H); 5.63–5.57 (dd, 1H); 4.70–4.65 (dd, 1H); 4.5–4.3 (m, 1H); 3.75–3.74 (d, 3H); 3.54–3.40 (m, 1H); 1.51–1.4 (m, 1H); 1.2–1.1 (m, 2H); 0.85 (d, 3H); 0.83 (d, 3H). MS: m/z = 471 $[M+H]^+$.

6.6.5. 1-(1-Adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino) ethyl]-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18e)

Y = 62% (yellow solid). Mp 110–120 °C, TLC cyclohexane–AcOEt 1:5, R_f = 0.48. IR (nujol): 3179 (NH), 1661 (C=O) cm^{-1}. ^1H NMR: 11.23, 11.17 (2d, 1H); 7.44, 7.37–7.15 and 7.00 (m, m, m, 9H); 4.62–4.32 (m, 2H); 3.84–3.64 (m, 5H); 3.37 (m, 1H); 2.72–2.40 (m, 6H); 1.76–1.46, 1.12 and 0.92 (m, m, m, 11H). MS: m/z = 490 $[M+H]^+$.

6.6.6. 1-(1-Adamantyl)methyl-2,4-dioxo-5-[2-(1-pyrrolidino) ethyl]-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18f)

Y = 36.5% (yellow solid). Mp 105–110 °C, TLC AcOEt–MeOH 10:1, R_f = 0.44. IR: 3180 (NH), 1661 (C=O) cm^{-1}. ^1H NMR: 11.2 (br s, 1H); 7.46 (dd, 1H); 7.40–7.35, 7.35–7.15 and 7.00 (m, m, m, 8H); 4.63–4.53 (dd, 2H); 4.55–4.4 (m, 1H); 4.00–3.80 (m, 1H); 3.23 (d, 1H); 3.0–2.8 (m, 2H); 2.6 (m, 4H); 1.9, 1.82, 1.7–1.51 and 1.4 (m, m, m, m, 19H). MS: m/z = 526 $[M+H]^+$.

6.6.7. 1-(1-Adamantyl)methyl-2,4-dioxo-7-fluoro-5-[2-(*N*-morpholino)ethyl]-3-phenylhydrazono-2,3,4,5- tetrahydro-1*H*-1,5-benzodiazepine (18h)

Y = 73% (yellow foam). TLC cyclohexane–AcOEt 1:1, R_f = 0.42. IR (nujol) 1663 (C=O); 1603, 1590 (C=C) cm^{-1}. ^1H NMR: 11.23, 11.20 (2d, 1H); 7.55 (dd, 1H); 7.4–6.8 (m, 7H); 4.63–4.52 (dd, 1H); 4.4–4.1 (m, 1H); 3.96–3.75 (m, 5H); 3.14 (d, 1H); 3.04–2.60 (m, 2H); 2.57 (m, 4H); 1.90 (m, 3H); 1.70–1.30 (m, 12H). MS: m/z = 560 $[M+H]^+$.

6.6.8. 1-(1-Adamantyl)methyl-2,4-dioxo-5-(4-methoxyphenyl) methyl-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (18j)

Y = 92.3% (yellow solid). Mp 170–188 °C, TLC cyclohexane–AcOEt 8:2, R_f = 0.40. IR (nujol): 3441 (NH); 1661–1653 (C=O) cm^{-1}. ^1H NMR:11.25, 11.17 (2d, 1H); 7.39 (m, 2H); 7.32–7.10 (m, 8H); 7.00 (m, 1H); 6.87 (m, 2H); 5.31–5.15 (dd, 1H); 5.01–4.88 (dd, 1H); 4.62–4.50 (dd, 1H); 3.80 (d, 3H); 3.22 (dd, 1H); 1.85 (m, 3H); 1.70–1.42 and 1.34 (m, m, 12H). MS: m/z = 549 $[M+H]^+$.

6.7. General procedure for the synthesis of 3-amino-1,5-dialkyl-2,4-dioxo-2,3,4,5-tetrahydro-1H-1,5-benzodiazepine

6.7.1. 3-Amino-2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19a)

Zinc metal (3.8 g; 0.058 mol) was added portionwise to a solution of 2,4-dioxo-1-(3-methylbutyl)-5-(2-morpholinoethyl)-3-phenylhydrazono-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine **18a** (3.6 g; 0.00775 mol) in glacial acetic acid (60 mL); the mixture was stirred at 23 °C for 15 min, then it was diluted with ethyl acetate (150 mL) and filtered, washing the solid with ethyl acetate (100 mL) and a 10% sodium hydroxide solution (20 mL). More 10% sodium hydroxide solution (150 mL) was added to the filtrate, until pH 10, then the solution was extracted with ethyl acetate (150 mL). The organic layer was washed with brine (100 mL), dried and concentrated in vacuo. The residue was purified by flash chromatography (eluting with AcOEt–MeOH 8:2) to give the title compound as a light yellow solid (1.5 g). Y = 51%, Mp 117–119 °C, TLC AcOEt–MeOH 7:3, R_f = 0.24. IR: 1691 (C=O) cm^{-1}; ^1H NMR: 7.55–7.50 (m, 1H); 7.40–7.30 (m, 3H); 4.35 (m, 1H); 4.246 (m, 1H); 4.02 (s, 1H); 3.782 (m, 1H); 3.70 (m, 1H); 3.64 (m, 4H); 2.60–2.40 (m, 6H); 1.56–1.32 (m, 3H); 0.88 (d, 3H); 0.87 (d, 3H). MS: m/z = 375 $[M+H]^+$.

Compounds **19b–19f**, **19h** and **19j** were prepared as reported for **19a**.

6.7.2. 3-Amino-1-cyclohexylmethyl-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19b)

Y = 70% (yellow solid). Mp 94–5 °C, TLC DCM-MeOH 85:15, R_f = 0.78. IR: 1695 and 1664 (C=O) cm^{-1}. ^1H NMR: 7.63 (m, 1H); 7.40–7.30 (m, 3H); 4.39 (dd, 1H); 4.08 (m, 1H); 4.01 (s, 1H); 3.87 (m, 1H); 3.40 (dd, 1H); 2.84–2.50 (m, 6H); 2.1–1.3 (m, 11H); 1.032 (t, 6H). MS: m/z = 387 $[M+H]^+$.

6.7.3. 3-Amino-1-cyclohexylmethyl-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19c)

Y = 70.5% (white foam). Mp 180–182 °C, TLC AcOEt–MeOH 7:3, R_f = 0.37. IR (nujol): 1678 and 1651 (C=O), 1597 (C=C) cm^{-1}. ^1H NMR: 7.6–7.5 (m, 1H); 7.4–7.3 (m, 3H); 3.35 (dd, 1H); 4.16 (m, 1H); 4.02 (s, 1H); 3.9 (m, 1H); 3.7 (m, 4H); 3.42 (dd, 1H); 2.7 (m, 2H); 2.4(m, 4H); 1.8–1.3 (m, 11H). MS: m/z = 401 $[M+H]^+$.

6.7.4. 3-Amino-2,4-dioxo-5-(4-methoxyphenyl)methyl-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19d)

Y = 66.7% (white solid). Mp 115–116 °C, TLC AcOEt–MeOH 95:5, R_f = 0.25. IR: 1695 and 1663 (C=O) cm^{-1}. ^1H NMR: 7.43 (m, 1H); 7.28 (m, 3H); 7.06 (m, 2H); 6.73 (m, 2H); 5.45 (d, 1H); 4.74 (d, 1H); 4.21 (m, 1H); 4.08 (s, 1H); 3.73 (s, 3H); 3.43 (m, 1H); 2.10 (br s, 2H); 1.36 (m, 1H); 1.00–0.82 (m, 2H); 0.77 (d, 3H); 0.76 (d, 3H). MS: m/z = 382 $[M+H]^+$, 763 $[2M+H]^+$.

6.7.5. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19e)

Y = 67% (white foam). Mp 75–80 °C, TLC AcOEt–MeOH 10:1, R_f = 0.11. IR (nujol): 3371 and 3179 (NH); 1693 and 1666 (C=O), 1597 (C=C) cm^{-1}.^1H NMR: 7.75 (m, 1H); 7.4–7.28 (m, 3H); 4.42 (d, 1H); 4.13 (m, 1H); 4.05 (br s, 2H); 3.82 (t, 1H); 3.76 (m, 4H); 3.22 (d, 1H); 2.93 (m, 1H); 2.74 (m, 1H); 2.70–2.54 (m, 5H); 1.84 (m, 3H); 1.7–1.2 (m, 12H). MS: m/z = 453 $[M+H]^+$, 454 [M+2H]+, 475 $[M+Na]^+$

6.7.6. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-5-[2-(1-pyrrolidino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19f)

Y = 70% (white foam). TLC AcOEt–MeOH 7:3, R_f = 0.3. IR (nujol): 1680 and 1650 (C=O), 1591 (C=C) cm^{-1}. ^1H NMR: 7.64 (m, 1H); 7.4–7.24 (m, 3H); 4.2–4.1 (m, 1H); 4.04 (s, 1H); 3.95–3.84 (m, 1H); 3.23 (d, 1H); 3.04–2.85 (m, 2H); 2.62 (m, 4H); 1.9–1.2 (m, 19H). MS: m/z = 437 $[M+H]^+$

6.7.7. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-7-fluoro-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19h)

Y = 60.5% (white foam). TLC AcOEt–MeOH 8:2, R_f = 0.5. IR (nujol): 3400 (NH), 1693–1663 (CO); 1605 (C=C) cm^{-1}. ^1H NMR: 7.92 (dd, 1H); 7.33 (dd, 1H); 7.03 (m, 1H); 4.42 (d, 1H); 4.15–4.07 (m, 2H); 3.79 (m, 4H); 3.61 (m, 1H); 3.12–3.02 (m, 2H); 2.65–2.51 (m, 5H); 2.3–1.84 (m, 5H); 1.70–1.40 and 1.30–1.14 (m, m, 12H). MS: m/z = 471 $[M+H]^+$.

6.7.8. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-5-(4-methoxyphenyl)methyl-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (19j)

Y = 79% (yellow solid). Mp 223–225 °C, TLC AcOEt–MeOH 95:5, R_f = 0.34. IR: 1700 and 1670 (C=O) cm^{-1}. ^1H NMR: 7.40–7.12 (m, 6H); 6.91 (m, 2H); 5.66 (d, 1H); 4.45 (d, 1H); 4.29 (m, 1H); 4.21 (s, 1H); 3.82 (s, 3H); 3.22 (d, 1H); 1.85 and 1.80–1.18 (m, m, 17H). MS: m/z = 460 $[M+H]^+$, 920 $[2M+H]^+$

6.8. General procedure for the synthesis of *N*-[1,5-dialkyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepi*N*-3-yl]-*N*′-arylurea

6.8.1. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepi*N*-3-yl}-*N*′-phenylurea (20)

Phenyl isocyanate (0.041 ml: 0.38 mmol) was added to a solution of 3-amino-2,4-dioxo-1-(3-methylbutyl)-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine **19a** (0.16 g;

0.35 mmol) in acetonitrile (7 mL). The reaction mixture was stirred at 23 °C for 30 min, and then filtered to give the title compound as white solid (0.100 g). Y = 50%, Mp 129–130 °C, TLC AcOEt–MeOH 95:5, R_f = 0.46. IR (nujol): 3400 (NH); 1695 and 1637 (C=O) cm^{-1}. ^1H NMR: 7.60–7.54 (m, 1H); 7.46–7.24 (m, 7H); 7.05 (t, 1H); 6.75 (s, 1H); 6.22 (d, 1H); 5.09 (d, 1H); 4.40–4.20(m, 2H); 3.80–3.60 (m, 6H); 2.60–2.35 (m, 6H); 1.60–1.35 (m; 3H); 0.88–0.86 (d, 6H). MS: m/z = 494 $[M+H]^+$. Anal. ($C_{27}H_{32}N_{5O4}$) C, H, N.

Compounds **20–36** were prepared as reported for **20**.

6.8.2. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(4-chloro) phenylurea (21)

Y = 65% (white solid). Mp 210–212 °C, TLC AcOEt–MeOH 95:5, R_f = 0.53. IR (nujol): 1693–1641 (C=O) cm^{-1}. ^1H NMR: 7.56 (m, 1H); 7.48 (br s, 1H); 7.42 (m, 1H); 7.40–7.32 (m, 2H); 7.21 (d, 2H); 7.13 (d, 2H); 6.59 (d, 1H); 5.07 (d, 1H); 4.36 (m, 1H); 4.2 (m, 1H); 3.82–3.68 (m, 2H); 3.62 (m, 4H); 2.58–2.48 (m, 1H); 2.5–2.3 (m, 5H); 1.6–1.4 (m, 3H); 0.88 (d, 3H); 0.87 (d, 3H). MS: m/z = 528 $[M+H]^+$. Anal. ($C_{27}H_{34}ClN_{5O4}$) C, H, N.

6.8.3. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(3-methyl)phenylurea (22)

Y = 42% (white solid). Mp 154–156 °C, TLC AcOEt–MeOH 95:5, R_f = 0.6. IR (nujol): 3306 (NH); 1693, 1663 and 1637 (C=O) cm^{-1}. ^1H NMR: 7.55 (m, 1H); 7.48 (m, 1H); 7.38–7.32 (m, 2H); 7.18 (br s, 1H); 7.14 (t, 1H); 7.08 (d, 1H); 6.85 (d, 1H); 6.87 (s, 1H); 6.30 (d, 1H); 5.09 (d, 1H); 4.37 (m, 1H); 4.28 (m, 1H); 3.80–3.60 (m, 2H); 3.62 (m, 4H); 2.52 (m, 1H); 2.40–2.32 (m, 5H); 2.28 (s, 3H); 1.55–1.35 (m, 3H). MS: m/z = 508 $[M+H]^+$., 530 $[M+Na]^+$. Anal. ($C_{28}H_{37}N_5O_4$) C, H, N.

6.8.4. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(4-trifluoromethyl)phenylurea (23)

Y = 81% (white solid). Mp 208–210 °C, TLC AcOEt–MeOH 95:5, R_f = 0.65. IR (nujol): 3300 (NH); 1701 and 1651 (C=O) cm^{-1}. ^1H NMR: 8.00 (br s, 1H); 7.58 (m, 1H); 7.45 (m, 1H); 7.40–7.35 (m, 2H); 7.29 (m, 4H); 6.91 (d, 1H); 5.08 (d, 1H); 4.40 (m, 1H); 4.16 (m, 1H); 3.85–3.75 (m, 2H); 3.63 (m, 4H); 2.52 (m, 1H); 2.50–2.30 (m, 5H); 1.64–1.44 (m, 3H); 0.904 (d, 3H); 0.88 (d, 3H). MS: m/z = 562 $[M+H]^+$. Anal. ($C_{28}H_{34}F_3N_{5O4}$) C, H, N.

6.8.5. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(4-bromo)phenylurea (24)

Y = 80% (white solid). Mp 202–205 °C, TLC AcOEt–MeOH 95:5, R_f = 0.63. IR (nujol): 3306 (NH); 1691, 1664 and 1641 (C=O) cm^{-1}. ^1H NMR: 7.57 (br s, 1H); 7.55 (m, 1H); 7.42 (m, 1H); 7.40–7.35 (m, 2H); 7.27 (m, 2H); 7.14 (m, 2H); 6.66 (d, 1H); 5.07 (d, 1H); 4.40 (m, 2H); 4.14 (m, 1H); 3.80–3.50 (m, 2H); 3.63 (m, 4H); 2.60 (m, 2H); 2.30 (m, 4H); 1.60–1.40 (m, 3H); 0.89 (d, 3H); 0.87 (d, 3H). MS: m/z = 572 $[M+H]^+$. Anal. ($C_{27}H_{34}BrN_{5O4}$) C, H, N.

6.8.6. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(4-fluoro)phenylurea (25)

Y = 41% (white solid). Mp 185–187 °C, TLC AcOEt–MeOH 95:5, R_f = 0.61. IR (nujol): 3304 (NH); 1701 and 1643 (C=O) cm^{-1}. ^1H NMR: 7.55 (m, 1H); 7.44–7.40 (m, 1H); 7.38–7.24 (m, 1H); 7.30 (br s, 1H); 7.27 (m, 2H); 6.91 (t, 2H); 6.48 (d, 1H); 5.08 (d, 1H); 4.35 (m, 1H); 4.19 (m, 1H); 3.80–3.60 (m, 2H); 3.62 (m, 4H); 2.58–2.48 (m, 1H); 2.45–2.30 (m, 5H); 1.55–1.35 (m, 3H); 0.87 (d, 3H); 0.86 (d, 3H). MS: m/z = 512 $[M+H]^+$, 534 $[M+Na]^+$. Anal. ($C_{27}H_{34}FN_{5O4}$) C, H, N.

6.8.7. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(4-ethoxy)phenylurea (26)

Y = 63% (white solid). Mp 184–186 °C, TLC AcOEt–MeOH 95:5, R_f = 0.56. IR (nujol): 1697, 1664 and 1634 (C=O) cm^{-1}. ^1H NMR: 7.53 (m, 1H); 7.42–7.30 (m, 3H); 7.23 (d, 1H); 6.82 (d, 2H); 6.81 (br s, 1H); 6.27 (d, 1H); 5.07 (d, 1H); 4.35 (m, 1H); 4.22 (m, 1H); 3.99 (q, 2H); 3.80–3.68 (m, 2H); 3.62 (m, 4H); 2.60–2.30 (m, 6H); 1.58–1.30 (m, 6H); 0.86 (dd, 6H). MS: m/z = 538 $[M+H]^+$. Anal. ($C_{29}H_{39}N_{5O4}$) C, H, N.

6.8.8. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-[(4-bromo-2-methyl)phenyl]urea (27)

Y = 51% (white solid). Mp 195–197 °C, TLC AcOEt–MeOH 95:5, R_f = 0.61. IR (nujol): 3314 (NH), 1697, 1668 and 1634 (C=O) cm^{-1}. ^1H NMR: 7.60–7.25 (m, 7H); 6.45 (br s, 2H); 6.20 (d, 1H); 5.05 (d, 1H); 4.40–4.16 (m, 2H); 3.80–3.68 (m, 2H); 2.56–2.30 (m, 6H); 2.25 (s, 3H); 1.58–1.30 (m, 3H); 0.88 (d, 3H); 0.85 (d, 3H). MS: m/z = 586 $[M+H]^+$. Anal. ($C_{28}H_{36}BrN_{5O4}$) C, H, N.

6.8.9. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-[(4-methoxy-2-methyl)phenyl]urea (28)

Y = 56%, Mp 244–246 °C, TLC AcOEt–MeOH 95:5, R_f = 0.47. IR (nujol): 1680 and 1663 (C=O) cm^{-1}. ^1H NMR: 7.53 (m, 1H); 7.40–7.30 (m, 4H); 6.80–6.34 (m, 2H); 6.01 (br s, 1H); 5.86 (d, 1H); 5.03 (d, 1H); 4.40–4.20 (m, 2H); 3.80–3.56 and 3.79 (m and s, respectively, 9H); 2.54–2.34 and 2.31 (m and s, respectively, 9H); 1.50–1.30 (m, 3H); 0.86 (d, 3H); 0.83 (d, 3H). MS: m/z = 538 $[M+H]^+$. Anal. ($C_{29}H_{39}N_{5O4}$) C, H, N.

6.8.10. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(2-isopropyl)phenylurea (29)

Y = 70% (white solid). Mp 218–220 °C, TLC AcOEt–MeOH 95:5, R_f = 0.48. IR (nujol): 3323 (NH); 1701, 1663 and 1630 (C=O) cm^{-1}. ^1H NMR: 7.53 (m, 2H); 7.40–7.35 (m, 1H); 7.35–7.30 (m, 3H); 7.25–7.20 (m, 2H); 6.33 (br s, 1H); 6.07 (d, 1H); 5.05 (d, 1H); 4.40 (m, 1H); 4.23 (m, 1H); 3.73 (m, 1H); 3.68–3.58 (m, 5H); 3.21 (m, 1H); 2.46 (m, 1H); 2.44–2.34 (m, 5H); 1.47 (m, 1H); 1.45–1.30 (m, 2H); 1.22 (d, 6H); 0.86 (d, 3H); 0.83 (d, 3H). MS: m/z = 536 $[M+H]^+$. Anal. ($C_{30}H_{41}N_{5O4}$) C, H, N.

6.8.11. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)]-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-[(2-chloro-6-methyl)phenyl]urea (30)

Y = 51% (white solid). Mp 195–197 °C, TLC AcOEt–MeOH 95:5, R_f = 0.54. IR (nujol): 1669 and 1637 (C=O) cm^{-1}. ^1H NMR: 7.55–7.50 (m, 1H); 7.40–7.35 (m, 1H); 7.35–7.32 (m, 2H); 7.29 (dd, 1H); 7.18 (dd, 1H); 7.14 (t, 1H); 6.09 (br s, 1H); 5.82 (d, 1H); 5.01 (d, 1H); 4.36 (m, 1H); 4.25 (m, 1H); 3.73 (m, 1H); 3.68–3.58 (m, 5H); 2.50–2.30 (m, 6H); 2.47 (s, 3H); 1.45–1.30 (m, 1H); 1.47 (m, 2H); 0.85 (d, 3H); 0.84 (d, 3H). MS: m/z = 542 $[M+H]^+$. Anal. ($C_{28}H_{36}ClN_{5O4}$) C, H, N.

6.8.12. *N*-[1-cyclohexylmethyl-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl]-*N*′-phenylurea (31)

Y = 66% (white solid). Mp 186–188 °C, TLC DCM-MeOH 9:1, R_f = 0.8. IR (nujol): 3400 (NH), 1699, 1666 and 1641 (C=O) cm^{-1}. ^1H NMR: 7.66 (m, 1H); 7.42–7.22 (m, 7H); 7.04 (m, 1H); 6.83 (s, 1H); 6.27 (d, 1H); 5.09 (d, 1H); 4.35 (dd, 1H); 4.07 (m, 1H); 3.84 (m, 1H); 3.40 (dd, 1H); 2.74 (m, 1H); 2.64–2.48 (m, 5H); 1.70–1.30, 1.16–0.78 and 1.01 (m, m and t, 17H) MS: m/z = 506 $[M+H]^+$. Anal. ($C_{29}H_{39}N_{5O3}$ + 0.75 H_2O) C, H, N.

6.8.13. *N*-{1-cyclohexylmethyl-2,4-dioxo-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-phenylurea (32)

Y = 69% (white solid). Mp 198–200 °C, TLC AcOEt–MeOH 1:1, R_f = 0.75. IR (nujol): 3300 (NH), 1697, 1664 and 1637 (C=O) cm^{-1}. ^1H NMR: 7.65 (m, 1H); 7.41 (m, 1H); 7.38–7.25 (m, 6H); 7.05 (tt, 1H); 6.78 (br s, 1H); 6.23 (bd, 1H); 5.09 (d, 1H); 4.32 (dd, 1H); 4.16 (m, 1H); 3.88 (m, 1H); 3.70 (t, 4H); 3.43 (dd, 1H); 2.7–2.60 (m, 1H); 2.48 (t, 4H); 2.54–2.44 (m, 1H); 1.70–1.50 (m, 4H); 1.50–1.40 (m, 4H); 1.10–0.8 (m, 5H). MS: *m*/*z* = 520 $[M+H]^+$. Anal. ($C_{29}H_{37}N_{5O4}$) C, H, N.

6.8.14. *N*-{1-cyclohexylmethyl-2,4-dioxo-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-(3-methyl)phenylurea (33)

Y = 82.4% (white solid). Mp185–187 °C, TLC AcOEt-cyclohexane 8:2, R_f = 0.17. IR: 1697 and 1666 (C=O), 1537 (C=C) cm^{-1}. ^1H NMR: 7.65 (m, 1H); 7.43–7.08 (m, 6H); 6.97 (br s, 1H); 6.86 (d, 1H); 6.21 (bd, 1H); 5.09 (d, 1H); 4.32 (dd, 1H); 4.14 (m, 1H); 3.88 (m, 1H); 3.70 (m, 4H); 3.43 (dd, 1H); 2.65 (m, 1H); 2.48 (m, 5H); 2.30 (s, 3H); 1.7–0.8 (m, 11H). MS: *m*/*z* = 534 $[M+H]^+$. Anal. ($C_{30}H_{39}N_{5O4}$) C, H, N.

6.8.15. *N*-{1-(1-adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-phenylurea (34)

Y = 50% (white solid). Mp 188–190 °C, TLC cyclohexane–AcOEt 1:1, R_f = 0.18. IR (nujol): 3317 (NH); 1699 and 1666 (C=O) cm^{-1}. ^1H NMR: 7.80 (m, 1H); 7.50–7.20 (m, 7H); 7.05 (tt, 1H); 6.81 (br s, 1H); 6.24 (d, 1H); 5.12 (d, 1H); 4.39 (d, 1H); 4.14 (m, 1H); 3.90–3.60 (m, 5H); 3.23 (d, 1H); 2.94–2.76 (m, 2H); 2.56 (m, 4H); 1.83 (bs; 3H); 1.70–1.1 (m, 12H). MS: *m*/*z* = 571 $[M+H]^+$; 572 [M+2H]+. Anal. ($C_{34}H_{41}N_{5O4}$) C, H, N.

6.8.16. *N*-{1-(1-adamantyl)methyl-2,4-dioxo-5-[2-(1-pyrrolidino)ethyl]-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl}-*N*′-phenylurea (35)

Y = 65% (white solid). Mp 155–160 °C, TLC AcOEt–MeOH 10:1, R_f = 0.43. IR (nujol): 3200 (NH), 1695 and 1664 (C=O) cm^{-1}. ^1H NMR: 7.65 (m, 1H); 7.4–7.28 (m, 7H); 7.03 (t, 1H); 6.91 (s, 1H); 6.31 (d, 1H); 5.11 (d, 1H); 4.38 (d, 1H); 4.15 (m, 1H); 3.88 (m, 1H); 3.22 (d, 1H); 2.94 (m, 2H); 2.66–2.54 (m, 4H); 1.86–1.76 (m, 7H); 1.58 (d, 3H); 1.46 (d, 3H); 1.25 (d, 3H); 1.20 (d, 3H). MS: *m*/*z* = 556 $[M+H]^+$. Anal. ($C_{33}H_{41}N_{5O3}$) C, H, N.

6.8.17. *N*-{1-(1-adamantyl)methyl-2,4-dioxo-7-fluoro-5-[2-(*N*-morpholino)-ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (36)

Y = 65% (white foam). TLC cyclohexane–AcOEt 1:1, R_f = 0.37. IR (nujol): 3327 (NH), 1695–1660 (CO) cm^{-1}. ^1H NMR: 7.98 (dd, 1H); 7.35–7.24 (m); 7.04 (m, 2H); 6.90 (br s, 1H); 6.29 (d, 1H); 5.14 (d, 1H); 4.38 (d, 1H); 4.14 (m, 1H); 3.78 (t, 4H); 3.62 (m, 1H); 3.12 (d, 1H); 3.00 (m, 1H); 2.70–2.60 (m, 3H); 2.58–2.48 (m, 2H); 1.83 (m, 3H); 1.60–1.20 (m, 12H). MS: *m*/*z* = 590 $[M+H]^+$. Anal. ($C_{33}H_{40}FN_{5O3}$ + 0.25 H_2O) C, H, N.

6.9. General procedure for the synthesis of 1-alkyl-3-amino-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine

6.9.1. 3-Amino-2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (37)

Ammonium cerium (IV) nitrate (17.45 g; 0.032 mol) was added to a solution of 3-amino-2,4-dioxo-5-(4-methoxyphenyl)methyl-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine **19d** (3.0 g; 0.008 mol) in acetonitrile (90 mL) and water (10 mL). The solution was stirred at 23 °C for 36 h, and then concentrated in vacuo to a slurry solid. This material was diluted with a 10% sodium hydroxide solution (150 mL), stirred at 23 °C for 30 min, then inorganic salts were filtered off. The aqueous solution was extracted with ethyl acetate (4 × 100 mL). The combined organic extracts were washed with brine (300 mL), dried and concentrated in vacuo to give an oil, that was purified by flash chromatography (eluting with DCM-MeOH 95:5) to give the title compound as a white solid (0.6 g). Y = 29%, Mp 148–150 °C, TLC DCM-MeOH 95:5, R_f = 0.20. IR (nujol): 3360–3100 (NH and NH_2), 1701,1674 and 1660 (C=O) cm^{-1}. ^1H NMR: 8.25 (br s, 1H); 7.39 (dd, 1H); 7.31 (dt, 1H); 7.26 (dt, 1H); 7.14 (dd, 1H); 4.32 (m, 1H); 4.03 (s, 1H); 3.68 (m, 1H); 2.2–1.6 (m, 2H); 1.48 (m, 1H); 1.39 (m, 2H); 0.87 (d, 3H); 0.82 (d, 3H). MS: *m*/*z* = 262 $[M+H]^+$, 523 $[2M+H]^+$.

6.9.2. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (38)

Compound synthesized as described for **37**. Y = 84% (yellow foam) TLC DCM-MeOH 95:5, R_f = 0.24. IR (nujol): 3213–3126 (NH and NH_2), 1705 and 1668 (C=O); 1600 (C=C) cm^{-1}. ^1H NMR: 7.86 (s, 1H); 7.42 (m, 1H); 7.30–7.2 (m, 2H); 7.13 (m, 1H); 4.45 (d, 1H); 4.06 (s, 1H); 3.25 (d, 1H); 1.80 (m, 3H); 1.70–1.20 (m, 12H). MS: *m*/*z* = 340 $[M+H]^+$, 679 $[2M+H]^+$

6.10. General procedure for the synthesis of *N*-[1-alkyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-arylurea

6.10.1. *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (39)

Phenyl isocyanate (0.183 mL; 1.68 mmol) was added to a solution of 3-amino-2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine **37** (0.4 g; 1.53 mmol) in dry dichloromethane (5 mL) under a nitrogen atmosphere. The mixture was stirred at 23 °C for 30 min, and then concentrated in vacuo. The residue was triturated with diethyl ether to give the title compound as a white solid (0.478 g). Y = 82%, Mp 249–250 °C, TLC DCM-MeOH 95:5, R_f = 0.38. IR (nujol): 3396, 3350 and 3223 (NH), 1699 and 1672 (C=O); 1601 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6): 10.76 (s, 1H); 9.14 (s, 1H); 7.65 (m, 1H); 7.40–7.21 (m, 7H); 6.90–6.82 (d, 2H); 4.71 (d, 1H); 4.29 (m, 1H); 3.71 (m, 1H); 1.34–1.20 (m, 3H); 0.80 (d, 3H); 0.74 (d, 3H). MS: *m*/*z* = 381 $[M+H]^+$.

Compounds **40–42** were synthesized as described for **39**.

6.10.2. *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-methoxyphenyl)urea (40)

Y = 66% (white solid). Mp 150–152 °C, TLC AcOEt–MeOH 95:5, R_f = 0.78. IR (nujol): 1717, 1705 and 1663 (C=O); 1601 and 1558 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6): 10.73 (s, 1H); 8.93 (s, 1H); 7.64 (m, 1H); 7.34 (m, 2H); 7.25–7.20 (d, 3H); 6.79 (d, 2H); 6.69 (d, 1H); 4.69 (d, 1H); 4.27 (m, 1H); 3.7 (m, 1H); 3.66 (s, 3H); 1.34 (m, 1H); 1.20 (m, 2H); 0.79 (d, 3H); 0.73 (d, 3H). MS: *m*/*z* = 410 $[M]^+$, 411 $[M+H]^+$.

6.10.3. *N*-[1-(1-adamantyl)methyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (41)

Y = 77.4% (white solid). Mp >280 °C, TLC DCM-MeOH 95:5, R_f = 0.46. IR (nujol): 3383–3215 (NH), 1697, 1676 and 1665 (C=O); 1597 (C=C) cm^{-1}. ^1H NMR (DMSO-d_6): 10.83 (s, 1H); 9.13 (s, 1H); 7.69 (m, 1H); 7.32 (m, 4H); 7.24 (m, 1H); 7.21 (m, 2H); 6.89 (d, 1H); 6.80 (d, 1H); 4.76 (d, 1H); 4.22 (d, 1H); 3.40 (d, 1H); 1.76 (m, 3H); 1.60–1.38 (m, 6H); 1.18 (m, 6H). MS: *m*/*z* = 459 $[M+H]^+$.

6.10.4. *N*-[1-(1-adamantyl)methyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-fluoro)phenylurea (42)

Y = 60.7% (beige solid). Mp >250 °C, TLC cyclohexane–AcOEt 1:1, R_f = 0.57. IR (nujol): 3395–3358 (NH), 1700–1650 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): 10.80 (s, 1H); 9.15 (s, 1H); 7.67 (dd, 1H); 7.35–7.25 (m, 4H); 7.24 (dd, 1H); 7.03 (t, 2H); 6.74 (d, 1H); 4.73 (d, 1H); 4.20 (d, 1H); 3.38 (d, 1H); 1.74 (br s, 3H); 1.58–1.38 (m, 6H); 1.16 (m, 6H). MS: m/z = 477 $[M+H]^+$.

6.11. General procedure for the synthesis of *N*-[1,5-dialkyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-arylurea

6.11.1. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (20)

Method A: Sodium hydride (19.8 mg; 0.66 mmol) was added to a solution of *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea **39** (110 mg; 0.29 mmol) in dry DMF (5 mL) under a nitrogen atmosphere. The mixture was stirred at 23 °C for 30 min, then 4-(2-chloroethyl)morpholine hydrochloride (67.6 mg; 0.36 mol) was added. The mixture was heated at 70 °C for 5 h. The mixture was cooled to 23 °C, then diluted with a 5% sodium hydrogen carbonate solution (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (60 mL), dried and concentrated in vacuo to oil. The latter was purified by flash chromatography (eluting with EA) and the solid obtained was further purified by trituration with diethyl ether to give the title compound as white solid (78 mg). Y = 54.5%.

Method B: A mixture of *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea **39** (50 mg; 0.13 mmol), potassium carbonate (54 mg; 0.39 mmol), 4-(2-chloroethyl)morpholine hydrochloride (26.6 mg; 0.143 mmol), acetone (10 mL) and water (1 mL) was stirred at 75 °C for 17 h. The suspension was cooled to 23 °C; inorganic compounds were filtered off and the filtrate was concentrated in vacuo. The residue was triturated with acetonitrile to give the title compound as white solid (45 mg). Y = 70% Mp 129–130 °C, TLC AcOEt–MeOH 95:5, R_f = 0.46. IR (nujol): 3400 (NH); 1695 and 1637 (C=O) cm^{-1}. ^1H NMR: 7.60–7.54 (m, 1H); 7.46–7.24 (m, 7H); 7.05 (t, 1H); 6.75 (s, 1H); 6.22 (d, 1H); 5.09 (d, 1H); 4.40–4.20 (m, 2H); 3.80–3.60 (m, 6H); 2.60–2.35 (m, 6H); 1.60–1.35 (m; 3H); 0.88–0.86 (d, 6H). MS: m/z = 494 $[M+H]^+$. Anal. ($C_{27}H_{32}N_{5O3}$) C, H, N.

Compounds **43–53** were synthesized as described for **20** following method B, except otherwise specified.

6.11.2. *N*-{2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(1-piperidino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (43)

Y = 64% (white solid). Mp 107–9 °C, TLC AcOEt–MeOH 95:5, R_f = 0.2. IR (nujol): 3429 and 3192 (NH), 1699 and 1647 (C=O), 1601 (C=C) cm^{-1}. ^1H NMR:7.6 (m, 1H); 7.44–7.30 (m, 7H); 7.05 (t, 1H); 6.75 (s, 1H); 6.22 (d, 1H); 5.08 (d, 1H); 4.4–4.2 (m, 2H); 3.8–3.65 (m, 2H); 2.5–2.3 (m, 6H); 1.56–1.3 (m, 9H); 0.87 (d, 3H); 0.84 (d, 3H). MS: m/z = 492 $[M+H]^+$. Anal. ($C_{28}H_{37}N_{5O3}$ + 1 H_2O) C, H, N.

6.11.3. *N*-[5-(2-dimethylaminoethyl)-2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (44)

Y = 76.6% (white solid). Mp 159–161 °C, TLC AcOEt–MeOH 95:5, R_f = 0.39. IR (nujol): 3350 (NH), 1695 and 1641 (C=O), 1601 (C=C) cm^{-1}. ^1H NMR: 7.54–7.20 (m, 8H); 7.05 (t, 1H); 6.71 (s, 1H); 6.20 (d, 1H); 5.08 (d, 1H); 4.4–4.25 (m, 2H); 3.8–3.6 (m, 2H); 2.5–2.3 (m, 2H); 2.17 (s, 6H); 1.50 (m, 1H); 1.45–1.35 (m, 2H); 0.87 (d, 3H); 0.85 (d, 3H). MS: m/z = 452 $[M+H]^+$. Anal. ($C_{25}H_{32}N_{5O3}$ + 0.5 H_2O) C, H, N.

6.11.4. *N*-[5-(2-diethylaminoethyl)-2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (45)

Y = 55% (white solid). Mp 146–148 °C, TLC AcOEt–MeOH 8:2, R_f = 0.64. IR (nujol): 3315 (NH), 1703, 1666 and 1639 (C=O) cm^{-1}. ^1H NMR: 7.57 (m, 1H); 7.39 (m, 1H); 7.37–7.25 (m, 6H); 7.04 (t, 1H); 6.84 (br s, 1H); 6.26 (bd, 1H); 5.08 (d, 1H); 4.36–4.20 (m, 2H); 3.74 (m, 1H); 3.62 (m, 1H); 2.63 (m, 1H); 2.49 (q, 5H); 1.48 (m, 1H); 1.38 (m, 2H); 0.94 (t, 6H); 0.86 (d, 3H); 0.83 (d, 3H). MS: m/z = 480 $[M+H]^+$. Anal. ($C_{27}H_{37}N_{5O3}$) C, H, N.

6.11.5. *N*-[5-(2-dimethylaminoethyl)-2,4-dioxo-1-(3-methyl-1-butyl)-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-methoxyphenyl)urea (46)

Y = 55.6% (white solid). Mp 216–218 °C, TLC AcOEt–MeOH 8:2, R_f = 0.32. IR (nujol): 3400 (NH), 1699 and 1664 (C=O) cm^{-1}. ^1H NMR: 7.54–7.20 (m, 8H); 6.86 (d, 2H); 6.41 (br s, 1H); 6.06 (d, 1H); 5.05 (d, 1H); 4.32 (m, 2H); 3.78 (s, 3H); 3.73–3.60 (m, 2H); 2.45 (m, 2H); 2.19 (s, 6H); 1.55–1.30 (m, 3H); 0.86 (d, 3H); 0.84 (d, 3H). MS: m/z = 482 $[M+H]^+$. Anal. ($C_{26}H_{35}N_{5O3}$) C, H, N.

6.11.6. *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-methoxyphenyl)urea (47)

Y = 79.8% (white solid). Mp161–3 °C, TLC AcOEt–MeOH 9:1, R_f = 0.53. IR (nujol): 3346 and 3179 (NH), 1728, 1700 and 1653 (C=O) cm^{-1}. ^1H NMR: 7.54 (m, 1H); 7.42–7.32 (m, 3H); 7.26 (d, 2H); 6.86 (d, 2H); 6.43 (br s, 1H); 6.06 (d, 1H); 5.06 (d, 1H); 4.4–4.1 (m, 2H); 3.78 (s, 3H); 3.8–3.6 (m, 2H); 3.62 (t, 4H); 2.6–2.2 (m, 6H); 1.6–1.3 (m, 3H); 0.87 (d, 3H); 0.84 (d, 3H). MS: m/z = 524 $[M+H]^+$. Anal. ($C_{28}H_{37}N_{5O5}$) C, H, N.

6.11.7. *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-hydroxyphenyl)urea (48)

Aluminium iodide (0.194 g; 0.477 mmol) was added to a solution of *N*-[2,4-dioxo-1-(3-methyl-1-butyl)-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-methoxyphenyl)urea 47 (50 mg; 0.0954 mmol) in dry acetonitrile (20 mL) under a nitrogen atmosphere. The solution was heated at 90 °C for 24 h. Further aluminium iodide (0.194 g; 0.477 mmol) was added and the mixture heated at 90 °C for further 24 h. The mixture was cooled to rt, diluted with water (5 mL) and a 5% sodium thiosulfate solution (25 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with brine (50 ml), dried and concentrated in vacuo to a residue that was triturated with diethyl ether to give the title compound as a white solid (30 mg). Y = 61.6% Mp 104–105 °C (dec). TLC AcOEt–MeOH 95:5, R_f = 0.2. IR: 3450 and 3340 (NH and OH), 1697 and 1663 (C=O) cm^{-1}. ^1H NMR:7.55 (dd, 1H); 7.46–7.32 (m, 3H); 7.07 (m, 2H); 6.62 (m, 2H); 6.46–6.10 (m, 1H); 5.07 (d, 1H); 4.34–4.20 (m, 2H); 3.9–3.6 (m, 6H); 2.7–2.3 (m, 6H); 1.8–1.3 (m, 3H); 0.86 (d, 3H); 0.84 (d, 3H). MS: m/z = 510 $[M+H]^+$. Anal. ($C_{27}H_{35}N_{5O5}$) C, H, N.

6.11.8. *N*-[1-(1-adamantyl)methyl-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (49)

Y = 91% (white solid). Mp 143–145 °C, TLC AcOEt–MeOH 95:5, R_f = 0.55. IR: 3436 (NH), 1695 and 1666 (C=O); 1599 (C=C) cm^{-1}. ^1H NMR: 7.80 (d, 1H); 7.5–7.3 (m, 5H); 7.2–6.8 (m, 4H); 6.34 (d, 1H); 5.13 (d, 1H); 4.39 (d, 1H); 4.08 (m, 1H); 3.75 (m, 1H); 3.22 (d, 1H); 3.05–2.75 (m, 2H); 2.60 (m, 4H); 1.82 (m, 3H);

1.60–1.18 (m, 12H); 1.05 (t, 6H). MS: m/z = 558 $[M+H]^+$, 559 [M+2H]+. Anal. ($C_{33}H_{43}N_{5O3}$) C, H, N.

6.11.9. *N*-[1-(1-adamantyl)methyl-5-(2-dimethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (50)

Compound synthesized following method A. Y = 81.6% (white solid). Mp 150–152 °C, TLC AcOEt–MeOH 95:5, R_f = 0.31. IR: 3400 (NH), 1697 and 1666 (C=O) cm^{-1}. ^1H NMR: 7.66–7.41 (m, 1H); 7.40–7.20 (m, 7H); 7.07 (m, 1H); 6.50 (s, 1H); 6.08 (d, 1H); 5.10 (d, 1H); 4.39 (d, 1H); 4.11 (m, 1H); 3.79 (m, 1H); 3.23 (d, 1H); 2.90–2.66 (m, 2H); 2.33 (s, 6H); 1.83 (m, 3H); 1.66–1.40 (m, 6H); 1.24 (m, 6H). MS: m/z = 530 $[M+H]^+$. Anal. ($C_{31}H_{39}N_{5O3}$) C, H, N.

6.11.10. *N*-[1-(1-adamantyl)methyl-5-(3-dimethylaminopropyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea (51)

Compound synthesized following method A. Y = 73.3% (white solid). Mp 138–140 °C, TLC DCM-MeOH 9:1, R_f = 0.24. IR: 3315 (NH), 1699 and 1639 (C=O); 1610 (C=C) cm^{-1}. ^1H NMR: 7.52–7.24 (m, 8H); 7.04 (t, 1H); 6.87 (s, 1H); 6.27 (d, 1H); 5.09 (d, 1H); 4.39 (d, 1H); 4.08–3.80 (m, 2H); 3.24 (d, 1H); 2.45 (m, 2H); 2.28 (s, 6H); 2.20–2.00 (m, 2H); 1.98–1.70 (m, 3H); 1.60–1.20 (m, 12H). MS: m/z = 544 $[M+H]^+$. Anal. ($C_{32}H_{41}N_{5O3}$) C, H, N.

6.11.11. *N*-[1-(1-adamantyl)methyl-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-fluoro)phenylurea (52)

Compound synthesized following method B. Y = 52% (white solid). Mp 140–142 °C, TLC cyclohexane–AcOEt 2:8, R_f = 0.19. IR (nujol): 1693–1670 (C=O) cm^{-1}. ^1H NMR: 7.81 (dd, 1H); 7.4 (dd, 1H); 7.35–7.25 (m, 4H); 6.97 (t, 2H); 6.61 (br s, 1H); 6.10 (d, 1H); 5.08 (d, 1H); 4.38 (d, 1H); 4.09 (m, 1H); 3.74 (m, 1H); 3.22 (d, 1H); 3.00 (m, 1H); 2.82 (m, 1H); 2.62 (m, 4H);1.83 (s, 3H); 1.64–1.44 (m, 6H); 1.3–1.2 (m, 6H); 1.06 (t, 6H). MS: m/z = 576 $[M+H]^+$. Anal. ($C_{33}H_{42}FN_{5O3}$) C, H, N.

6.11.12. *N*-{1-(1-adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)-ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-(4-fluoro)phenylurea (53)

Y = 95% (white solid). Mp >270 °C, TLC cyclohexane–AcOEt 1:1, R_f = 0.05. IR: 1692 and 1682 (C=O) cm^{-1}. ^1H NMR: 7.80 (m, 1H); 7.41–7.22 (m, 5H); 6.95 (m, 2H); 6.87 (br s, 1H); 6.25 (d, 1H); 5.10 (d, 1H); 4.38 (d, 1H); 4.13 (m, 1H); 3.78–3.75 (m, 5H); 3.23 (d, 1H); 2.93 (m, 1H); 2.74 (m, 1H); 2.56 (m, 4H); 1.83 (m, 3H); 1.70–1.40 (m, 6H); 1.24 (m, 6H). MS: m/z = 590 $[M+H]^+$. Anal. ($C_{33}H_{40}FN_{5O4}$) C, H, N.

6.12. Optical resolution via HPLC

6.12.1. (−) and (+) *N*-{1-(1-adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (54) and (55)

Racemic *N*-[1-(1-adamantylmethyl)-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea 34 (1.58 g) was separated into its enantiomers via preparative chiral HPLC, using a Pirkle D-DNBPG C5 column (25 × 2 cm), flow rate 20 mL/min, λ = 254 nm (UV detector), and eluting with DCM-IPA 93:7 to give 0.434 g of isomer 1 and 0.491 g of isomer 2, both as white solids.

Compound **54** (isomer 1): HPLC: retention time 5.2 min, enantiomeric excess 100%. $[\alpha]_D$ = -40.1 (*c* 0.75 in $CHCl_3$). Mp 157–159 °C, TLC cyclohexane–AcOEt 1:1, R_f = 0.18. IR (nujol): 3400 (NH), 1699, 1668, 1641 (C=O) cm^{-1}. ^1H NMR: 7.80 (m, 1H); 7.44–7.24 (m, 7H); 7.05 (m, 1H); 6.75 (s, 1H); 6.21 (d, 1H); 5.12 (d, 1H); 4.39 (d, 1H); 4.14 (m, 1H); 3.78–3.75 (m, 5H); 3.22 (d, 1H); 2.93 (m, 1H); 2.75 (m, 1H); 2.57 (m, 4H); 1.83 (m, 3H); 1.64–1.18 (m, 12H). MS: m/z = 571 $[M+H]^+$, 572 [M+2H]+. Anal. ($C_{34}H_{41}N_{5O4}$) C, H, N.

Compound **55** (isomer 2): HPLC: retention time 6.9 min, enantiomeric excess 92%. $[\alpha]_D$ = +40.4 (*c* 0.6 in $CHCl_3$). Mp 156–160 °C, TLC cyclohexane–AcOEt 1:1, R_f = 0.18. IR (nujol): 3400 (NH); 1701, 1668, 1643 (C=O), 1601 (C=C) cm^{-1}. ^1H NMR: 7.80 (m, 1H); 7.44–7.24 (m, 7H); 7.05 (m, 1H); 6.79 (s, 1H); 6.22 (d, 1H); 5.12 (d, 1H); 4.39 (d, 1H); 4.13 (m, 1H); 3.79–3.75 (m, 5H); 3.22 (d, 1H); 2.93 (m, 1H); 2.75 (m, 1H); 2.57 (m, 4H); 1.83 (m, 3H); 1.70–1.16 (m, 12H). MS: m/z = 571 $[M+H]^+$, 572 [M+2H]+. Anal. ($C_{34}H_{41}N_{5O4}$) C, H, N.

6.12.2. (−) and (+) *N*-{1-(1-adamantyl)methyl-2,4-dioxo-7-fluoro-5-[2-(*N*-morpholino)-ethyl]-2,3,4,5-tetrahydro-1*H*–1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (56) and (57)

Racemic *N*-[1-(1-adamantylmethyl)-2,4-dioxo-7-fluoro-5-[2-(*N*-morpholino)-ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-phenylurea 36 (0.12 g) was separated into its enantiomers via preparative chiral HPLC, using a Pirkle DNBPG C5 column (25 × 2 cm), flow rate 1.0 mL/min, λ = 235 nm (UV detector), and eluting with DCM-IPA 93:7 to give 0.048 g of isomer 1 and 0.045 g of isomer 2, both as white foams.

Compound **56** (isomer 1): HPLC: retention time 4.4 min, enantiomeric excess 100% (column Pirkle DNBPG S5 25 × 0.46 cm, 5μ, flow rate=1 mL/min, mobile phase DCM-IPA 93:7 λ =235 nm). IR (nujol): 3321 (NH), 1705, 1670 and 1653 (C=O); 1601 (C=C) cm^{-1}. ^1H NMR: 7.98 (dd, 1H); 7.37 (dd, 1H); 7.36–7.24 (m, 4H); 7.06 (m, 2H); 6.70 (s, 1H); 6.17 (bd, 1H); 5.13 (d, 1H); 4.39 (d, 1H); 4.14 (m, 1H); 3.78 (m, 4H); 3.62 (m, 1H); 3.12 (d, 1H); 3.01 (m, 1H); 2.70–2.48 (m, 5H); 1.83 (m, 3H); 1.68–1.14 (m, 12H). MS: m/z = 590 $[M+H]^+$. Anal. ($C_{33}H_{40}FN_{5O4}$) C, H, N.

Compound **57** (isomer 2): HPLC: retention time 6.0 min, enantiomeric excess 98% (column Pirkle DNBPG S5 25 × 0.46 cm, 5μ, flow rate = 1 mL/min, mobile phase DCM-IPA 93:7 λ = 235 nm). $[\alpha]_D$ = +24.6° (*c* 9.0 in $CHCl_3$). IR (nujol): 3339 (NH), 1701 and 1668 (C=O), 1599 (C=C) cm^{-1}. ^1H NMR: 7.97 (dd, 1H); 7.37 (dd, 1H); 7.36–7.24 (m, 4H); 7.06 (m, 2H); 6.63 (br s, 1H); 6.13 (bd, 1H); 5.12 (d, 1H); 4.39 (d, 1H); 4.14 (m, 1H); 3.79 (m, 4H); 3.63 (m, 1H); 3.13 (d, 1H); 3.01 (m, 1H); 2.70–2.48 (m, 5H); 1.84 (m, 3H); 1.70–1.12 (m, 12H). MS: m/z = 590 $[M+H]^+$. Anal. ($C_{33}H_{40}FN_{5O4}$) C, H, N.

6.12.3. (−) and (+) *N*-{1-(1-adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)-ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-(4-fluoro)phenylurea (58) and (59)

Racemic *N*-[1-(1-adamantylmethyl)-2,4-dioxo-5-[2-(*N*-morpholino)-ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-*N*′-(4-fluoro)phenylurea 53 (0.184 g) was separated into its enantiomers via preparative chiral HPLC, using a Pirkle DNBPG C5 column (25 × 0.46 cm), flow rate 1.0 mL/min, λ = 254 nm (UV detector), and eluting with DCM-IPA 93:7 to give the first isomer (0.022 g) as a white solid. The second isomer (0.057 g), was further purified by flash chromatography (eluting with AcOEt–MeOH 9:1) to give pure isomer 2 (0.021 g) as a white solid.

Compound **58** (isomer 1): HPLC: retention time 5.0 min, enantiomeric excess 100% (column Pirkle DNBPG C5 25 × 0.46 cm, 5μ, flow rate=1 mL/min, mobile phase DCM-IPA 97:3, λ = 254 nm). [α $]_D$=-38.0° (*c* 6.75 in $CHCl_3$). IR ($CHCl_3$): 1691 and 1680 (C=O) cm^{-1}. ^1H NMR: 7.79 (m, 1H); 7.41 (m, 1H); 7.33 (m, 2H); 7.25 (m, 2H); 7.03 (br s, 1H); 6.93 (t, 2H); 6.33 (d, 1H); 5.10 (d, 1H); 4.39 (d, 1H); 4.14 (m, 1H); 3.78 (m, 1H); 3.75 (m, 4H); 3.23 (d, 1H); 2.93 (m, 1H); 2.75 (m, 1H); 2.56 (m, 4H); 1.84 (m, 3H); 1.68–1.14 (m, 12H). MS: m/z = 590 $[M+H]^+$. Anal. ($C_{33}H_{40}FN_{5O4}$) C, H, N.

Compound **59** (isomer 2): HPLC: retention time 6.3 min, enantiomeric excess 95% (column Pirkle DNBPG C5 25 × 0.46 cm, 5 μ, flow rate=1 mL/min, mobile phase DCM-IPA 97:3, λ = 254 nm). $[\alpha]_D$ = +31.3° (*c* 6.75 $CHCl_3$). IR ($CHCl_3$): 1691 and 1680 (C=O) cm^{-1}. ^1H NMR: 7.79 (m, 1H); 7.41 (m, 1H); 7.33 (m, 2H); 7.25 (m, 2H); 7.03 (br s, 1H); 6.93 (t, 2H); 6.33 (d, 1H); 5.10 (d, 1H); 4.39 (d, 1H); 4.14 (m, 1H); 3.78 (m, 1H); 3.75 (m, 4H); 3.23 (d, 1H); 2.93 (m, 1H); 2.75 (m, 1H); 2.56 (m, 4H); 1.84 (m, 3H); 1.68–1.14 (m, 12H). MS: *m*/*z* = 590 $[M+H]^+$. Anal. ($C_{33}H_{40}FN_{5O4}$) C, H, N.

6.13. Optical resolution via Edman degradation

6.13.1. 2-tert-Butoxycarbonylamino-*N*-[(1-cyclohexylmethyl)-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenylpropionamide (60b)

1,3-Dicyclohexylcarbodiimide (0.168 g; 0.82 mmol) and 1-hydroxybenzotriazole hydrate (0.096 g; 0.71 mmol) were added to a solution of *N*-(tert-butoxycarbonyl)-D-phenylalanine34 (0.207 g; 0.78 mmol) in ethyl acetate (15 mL) and the mixture was stirred at 23 °C for 1 h. Then, a solution of intermediate 3-amino-1-cyclohexylmethyl-5-[2-(diethylaminoethyl)]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine 19b (0.273 g; 0.71 mmol) in ethyl acetate (20 mL) was added, and the mixture was stirred at 23 °C for additional 3 h. Inorganic materials were filtered off, washing the solid with ethyl acetate (50 mL); the filtrate was dried, concentrated in vacuo and purified by flash chromatography (eluting with DCM-MeOH 99:2 to 97:3) to give the title compound (0.395 g) as a white foam. Y = 87.7%, TLC DCM-MeOH 97:3, R_f = 0.22. IR (nujol): 3300 (NH), 1707 and 1668 (C=O) cm^{-1}. 1H NMR: 7.67 (m, 1H); 7.42–7.10 (m, 9H); 4.98 (d, 1H); 4.92 (d, 1H); 4.52 (m, 1H); 4.35 (m, 1H); 4.06 (m, 1H); 3.84 (m, 1H); 3.40 (m, 1H); 3.24 (m, 1H); 3.04 (m, 1H); 2.73 (m, 1H); 2.56 (m, 5H); 1.68–0.78 (m, 11H); 1.37 (s, 9H); 1.02 (t, 6H). MS: *m*/*z* = 634 $[M+H]^+$.

6.13.2. 2-tert-Butoxycarbonylamino-*N*-{[1-(1-adamantylmethyl)-5-[2-(*N*-morpholino)ethyl]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]}-3-phenylpropionamide (60e)

It was synthesized as described for 60b. Purification by flash chromatography (eluting with cycloexane-AcOEt 1:1 to AcOEt 100%). Y = 80% (white foam), TLC cyclohexane–AcOEt 2:1, R_f = 0.2. IR: 1695 (C=O) cm^{-1}. 1H NMR: 7.99 (m, 1H); 7.37 (m, 1H); 7.40–7.20 (m, 6H); 7.06 (m, 1H); 5.03 (m, 1H); 4.93 (m, 1H); 4.52 (m, 1H); 4.39 (m, 1H); 4.15 (m, 1H); 3.80 (m, 4H); 3.70–3.40 (m, 1H); 3.30–3.10 (m, 1H); 3.12 (m, 1H); 3.10–2.90 (m, 2H); 2.74–2.48 (m, 5H); 2.00–1.10 (m, 24H). MS: *m*/*z* = 718 $[M+H]^+$.

6.13.3. 2-D-Amino-*N*-[1-(cyclohexylmethyl)-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenylpropionamide (61b and 62b)

2-tert-Butoxycarbonylamino-*N*-[(1-cyclohexylmethyl)-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenylpropionamide 60b (0.375 g; 0.59 mmol) was dissolved in a mixture of trifluoroacetic acid (1.35 mL) and dicloromethane (1.35 mL) and stirred at 23 °C for 30 min. The reaction mixture was concentrated in vacuo and triturated with diethyl ether, filtered and dried, to give the trifluoroacetic salt of the title compound as a white foam (0.652 g) that was used without further purification. IR (nujol): 1670 (C=O) cm^{-1}. 1H NMR: 9.56 (m, 1H); 9.26 (dd, 1H); 8.02 (s, 3H); 7.8–7.65 (m, 2H); 7.55–7.45 (m, 2H); 7.4–7.2 (m, 5H); 4.93 (dd, 1H); 4.4–4.1 (m, 5H); 3.63 (dd, 1H); 3.5–3.2 (m, 6H); 2.8 (dd, 1H); 1.52–0.8 (m, 11H).

This material (0.640 g; 0.99 mmol) was suspended in ethyl acetate (10 mL) and extracted with a 5% ammonia solution (2 × 5 mL). The organic layer was washed with brine, dried and concentrated in vacuo to give the title compounds as diastereomeric mixture. Separation of the two diastereomers was achieved by flash chromatography on silica gel (eluting with DCM/MeOH in gradient from 98:2 to 95:5, then 90:10) to give intermediate Compound **61b** (0.058 g; Y = 18%) and intermediate **62b** (0.060 g; Y = 19%) both as yellow oils. Some starting material (0.040 g) was also recovered.

Compound **61b**: TLC DCM-MeOH 94:6, R_f = 0.32. IR (nujol): 3379 (NH), 1711, 1691 and 1649 (C=O) cm^{-1}. 1H NMR: 8.53 (d, 1H); 7.69 (m, 1H); 7.45–7.2 (m, 8H); 5.05 (d, 1H); 4.39 (dd, 1H); 4.08 (m, 1H); 3.87 (m, 1H); 3.67 (dd, 1H); 3.40 (dd, 1H); 3.31 (dd, 1H); 2.64 (dd, 1H); 2.77–2.57 (m, 6H); 1.7–1.4 (m, 5H); 1.1–0.8 (m, 12H).

62b: TLC DCM-MeOH 94:6, R_f = 0.26. IR (nujol): 3381 (NH), 1709, 1691 and 1651 (C=O) cm^{-1}. 1H NMR: 8.50 (d, 1H); 7.64 (m, 1H); 7.44–7.18 (m, 8H); 5.05 (d, 1H); 4.36 (dd, 1H); 4.16 (m, 1H); 3.89 (m, 1H); 3.66 (dd, 1H); 3.41 (dd, 1H); 3.3 (dd, 1H); 2.66 (dd, 1H); 2.79 (m, 1H); 2.7–2.60 (m, 5H); 1.7–1.4 (m, 22H); 1.2–0.88 (m, 12H).

6.13.4. *N*-{[1-(1-adamantyl)methyl-5-[2-(*N*-morpholino)ethyl]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]}-2-D-amino-3-phenylpropionamide (61e and 62e) They were synthesized as described for 61b and 62b. Racemate: Y = 79%

Compound **61e**: Y = 41% (white foam). TLC AcOEt–MeOH 98:2, R_f = 0.38. IR (nujol): 3373 (NH), 1703 and 1688 (C=O) cm^{-1}. 1H NMR: 8.52 (dd, 1H); 7.99 (m, 1H); 7.37 (m, 1H); 7.31 (m, 2H); 7.24 (m, 3H); 7.06 (m, 1H); 5.10 (d, 1H); 4.41 (2d, 1H); 4.13 (m, 1H); 3.79 (m, 4H); 3.67 (m, 1H); 3.68–3.56 (m, 1H); 3.30 (m, 1H); 3.13 (d, 1H); 3.01 (m, 1H); 2.79 (m, 1H); 2.70–2.48 (m, 6H); 1.84–1.14 (m, 17H). MS: *m*/*z* = 618 $[M+H]^+$.

Compound **62e**: Y = 28% (white foam). TLC AcOEt–MeOH 98:2, R_f = 0.22. IR (nujol): 3379 (NH), 1703 and 1688 (C=O) cm^{-1}. 1H NMR: 8.49 (d, 1H); 7.78 (m, 1H); 7.44 (m, 1H); 7.40–7.20 (m, 7H); 5.09 (d, 1H); 4.40 (d, 1H); 4.19 (m, 1H); 3.85 (m, 1H); 3.75 (t, 4H); 3.66 (dd, 1H); 3.30 (dd, 1H); 3.24 (d, 1H); 2.92 (m, 1H); 2.75 (m, 1H); 2.66 (dd, 1H); 2.64–2.52 (m, 4H); 1.84 (m, 3H); 1.70–1.40 (m, 6H); 1.24 (m, 6H). MS: *m*/*z* = 618 $[M+H]^+$.

6.13.5. *N*-[1-cyclohexylmethyl-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenyl-2-(3-phenylthioureido)-propionamide (63b)

Phenylisothiocyanate (0.033 g; 0.24 mmol) was added to a solution of 2-amino-*N*-[1-(cyclohexylmethyl)-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenylpropionamide (**61b**) (0,123 g; 0.23 mmol) in DCM (7 mL). The solution was stirred at 23 °C for 3 h and at 50 °C for 3 h. The solvent was evaporated and the residue was purified by flash chromatography (eluting with cyclohexane–AcOEt 1:1) to give the title compound as white foam (0.130 g). Y = 82.6%, TLC DCM-MeOH 96:4, R_f = 0.22. IR: 3308–3200 (NH); 1703 and 1664 (C=O) cm^{-1}. 1H NMR: 7.7–7.6 (m, 4H); 7.4–7.2 (m, 5H); 6.96–6.90 (m, 6H); 6.51 (d, 1H); 5.36 (m, 1H); 4.92 (dd, 1H); 4.4–4.3 (m, 1H); 4.2 (m, 1H); 3.8 (m, 1H); 3.4 (m, 2H); 3.22 (m, 1H); 2.81–2.50 (m, 6H); 1.70–1.50 (m, 5H); 1.50–1.30 (m, 8H); 1.10–0.80 (m, 6H). MS: *m*/*z* = 669 $[M+H]^+$.

6.13.6. *N*-[1-cyclohexylmethyl-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenyl-2-(3-phenylthioureido)-propionamide (64b)

It was synthesized as described for **63b**. Y = 76.2% (white foam), TLC DCM-MeOH 97:3, R_f = 0.47. IR (nujol): 3288 (NH); 1701 and 1661 (C=O) cm^{-1}. 1H NMR: 7.70–7.60 (m, 4H); 7.40–7.20 (m, 5H); 6.96–6.90 (m, 6H); 6.51 (d, 1H); 5.36 (m, 1H); 4.92 (dd, 1H); 4.40–4.30 (m, 1H); 4.2 (m, 1H); 3.8 (m, 1H); 3.4 (m, 2H); 3.22 (m, 1H); 2.8–2.5 (m, 6H); 1.70–1.50 (m, 5H); 1.50–1.30 (m, 8H); 1.10–0.80 (m, 6H). MS: *m*/*z* = 669 $[M+H]^+$.

6.13.7. *N*-{[1-(1-adamantyl)methyl-5-[2-(*N*-morpholino)ethyl]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]}-3-phenyl-2-(3-phenylthioureido)-propionamide (63e)

It was syntesized as described for **63b**. Y = 90% (white foam), TLC cyclohexane–AcOEt 1:1, R_f = 0.38. IR (nujol): 3317 (NH); 1705 and 1666 (C=O) cm^{-1}. 1H NMR: 7.71 (dd, 1H); 7.56 (br s, 1H); 7.4–7.15 (m, 12H); 6.92 (d, 2H); 6.50 (d, 1H); 5.34 (m, 1H); 4.95 (d, 1H); 4.38 (d, 1H); 4.11 (m, 1H); 3.82 (m, 1H); 3.75 (t, 4H); 3.42 (dd, 1H); 3.21 (d, 1H); 3.20 (d, 1H); 2.92 (m, 1H); 2.73 (m, 1H); 2.58 (m, 4H); 1.84 (br s, 3H); 1.65–1.45 (m, 6H); 1.23 (m, 6H). MS: m/z = 735 $[M+H]^+$.

6.13.8. *N*-{[1-(1-adamantyl)methyl-5-[2-(*N*-morpholino)ethyl]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]}-3-phenyl-2-(3-phenylthioureido)-propionamide (64e)

It was synthesized as described for 63b. Y = 93% (white foam), TLC cyclohexane–AcOEt 1:1, R_f = 0.35. IR (nujol): 3310 (NH); 1705 and 1666 (C=O) cm^{-1}. 1H NMR: 7.81 (br s, 1H); 7.77 (m, 1H); 7.39 (m, 1H); 7.35–7.15 (m, 11H); 6.94 (d, 2H); 6.55 (d, 1H); 5.34 (m, 1H); 4.97 (d, 1H); 4.39 (d, 1H); 4.09 (m, 1H); 3.79 (m, 1H); 3.74 (t, 4H); 3.48 (dd, 1H); 3.21 (d, 1H); 3.17 (dd, 1H); 2.89 (m, 1H); 2.69 (m, 1H); 2.55 (m, 4H); 1.83 (br s, 3H); 1.55–1.45 (m, 6H); 1.23 (m, 6H). MS: m/z = 735 $[M+H]^+$.

6.13.9. 3-Amino-1-cyclohexylmethyl-5-[2-(diethylaminoethyl)]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (65b)

N-[1-(Cyclohexylmethyl)-5-(2-diethylaminoethyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl]-3-phenyl-2-(3-phenylthioureido)propionamide **63b** (0.130 g; 0.19 mmol) was dissolved in trifluoroacetic acid (8.5 mL) and stirred at 40 °C for 18 h. The solution was concentrated in vacuo, and the residue was diluted with ethyl acetate (20 mL) and washed with a 5% sodium bicarbonate solution (20 mL) and brine (20 mL). The organic phase was dried and concentrated in vacuo; the residue was purified by flash chomatography (eluting with DCM–MeOH 99:1–96:4) to give the title compound (0.045 g) and recovered starting material (0.028 g). Y = 61%, TLC DCM-MeOH 96:4, R_f = 0.10. IR: 1695 and 1664 (C=O) cm^{-1}. ^1H NMR: 7.63 (m, 1H); 7.40–7.30 (m, 3H); 4.39 (dd, 1H); 4.08 (m, 1H); 4.01 (s, 1H); 3.87 (m, 1H); 3.40 (dd, 1H); 2.84–2.50 (m, 6H); 2.1–1.3 (m, 11H); 1.03 (t, 6H). MS: m/z = 387 $[M+H]^+$. The enantiomeric purity of the title compound (100%) was indirectly determined by chiral HPLC chromatography by conversion into the corresponding phenylurea derivative (column: Pirkle D-DNBPG, eluent DCM-IPA 1:1, retention time: 5.90 min).

6.13.10. 3-Amino-1-cyclohexylmethyl-5-[2-(diethylaminoethyl)]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (66b)

It was synthesized as described for **65b**. Y = 55% (white foam), TLC DCM-MeOH 97:3, R_f = 0.10. IR: 1695 and 1664 (C=O) cm^{-1}. ^1H NMR: 7.63 (m, 1H); 7.40–7.30 (m, 3H); 4.39 (dd, 1H); 4.08 (m, 1H); 4.01 (s, 1H); 3.87 (m, 1H); 3.40 (dd, 1H); 2.84–2.50 (m, 6H); 2.1–1.3 (m, 11H); 1.03 (t, 6H). MS: m/z = 387 $[M+H]^+$. The enantiomeric purity of the title compound (100%) was indirectly determined by chiral HPLC chromatography by conversion into the corresponding phenylurea derivative (column: Pirkle D-DNBPG, eluent DCM-IPA 1:1, retention time: 6.50 min).

6.13.11. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (65e)

It was synthesized as described for **65b**. Y = 48% (white foam), TLC AcOEt–MeOH 10:1, R_f = 0.11. IR (nujol): 3371 and 3179 (NH); 1693 and 1666 (C=O), 1597 (C=C) cm^{-1}.^1H NMR: 7.75 (m, 1H); 7.4–7.28 (m, 3H); 4.42 (d, 1H); 4.13 (m, 1H); 4.05 (br s, 2H); 3.82 (t, 1H); 3.76 (m, 4H); 3.22 (d, 1H); 2.93 (m, 1H); 2.74(m, 1H); 2.70–2.54 (m, 5H); 1.84 (m, 3H); 1.7–1.2 (m, 12H). MS: m/z = 453 $[M+H]^+$, 454 [M+2H]+, 475 $[M+Na]^+$. The enantiomeric purity of the title compound (97%) was indirectly determined by chiral HPLC chromatography by conversion into the corresponding phenylurea derivative (column: Pirkle D-DNBPG, eluent DCM-IPA 1:1, retention time: 5.20 min).

6.13.12. 1-(1-Adamantyl)methyl-3-amino-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine (66e)

It was synthesized as described for **65b**. Y = 55% (white foam) + 17% recovered SM. TLC AcOEt–MeOH 10:1, R_f = 0.11. IR (nujol): 3371 and 3179 (NH); 1693 and 1666 (C=O), 1597 (C=C) cm^{-1}.^1H NMR: 7.75 (m, 1H); 7.4–7.28 (m, 3H); 4.42 (d, 1H); 4.13 (m, 1H); 4.05 (br s, 2H); 3.82 (t, 1H); 3.76 (m, 4H); 3.22 (d, 1H); 2.93 (m, 1H); 2.74(m, 1H); 2.70–2.54 (m, 5H); 1.84 (m, 3H); 1.7–1.2 (m, 12H). MS: m/z = 453 $[M+H]^+$, 454 [M+2H]+, 475 $[M+Na]^+$. The enantiomeric purity of the title compound (92%) was indirectly determined by chiral HPLC chromatography by conversion into the corresponding phenylurea derivative (column: Pirkle D-DNBPG, eluent DCM-IPA 1:1, retention time: 6.90 min).

6.13.13. *N*-[1-(1-cyclohexylmethyl)-2,4-dioxo-5-(2-diethylaminoethyl)-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl]-*N*′-phenylurea (67)

Phenyl isocyanate (0.013 mL; 0.122 mmol) was added to a solution of the 3-amino-1-cyclohexylmethyl-5-[2-(diethylaminoethyl)]-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepine **65b** (0.045 g; 0.116 mmol) in dry acetonitrile (1.4 mL). The reaction mixture was stirred at 23 °C for 30 min, and then concentrated in vacuo. The residue was triturated with diethyl ether, filtered and dried to give the title compound as a white solid (0.050 g). Y = 85.2%, Mp 180–182 °C, IR: 3450 (NH), 1697 and 1666 (C=O) cm^{-1}. ^1H NMR: 7.65 (m, 1H); 7.42–7.22 (m, 7H); 7.04 (m, 1H); 6.88 (s, 1H); 6.30 (d, 1H); 5.08 (d, 1H); 4.35 (dd, 1H); 4.08 (m, 1H); 3.88 (m, 1H); 3.40 (dd, 1H); 2.78 (m, 1H); 2.60 (m, 5H); 1.8–1.3 (m, 9H); 1.14–0.80 (m, 8H). MS: m/z = 506 $[M+H]^+$. Chiral HPLC: (column: Pirkle D-DNBPG, eluent DCM-IPA 1:1) retention time 5.90 min; enantiomeric purity 100%. $[\alpha]_D$ = -30.6 (*c* 1.05 in $CHCl_3$, Hg light). Anal. ($C_{29}H_{39}N_{5O3}$) C, H, N.

6.13.14. *N*-[1-(1-cyclohexylmethyl)-2,4-dioxo-5-(2-diethylaminoethyl)-2,3,4,5-tetrahydro-1*H*-benzodiazepin-3-yl]-*N*′-phenylurea (68)

It was synthesized as described for **67**. Y = 90.6% (white solid), Mp 178–180 °C, IR (nujol): 3321 (NH), 1701, 1668 and 1650 (C=O) cm^{-1}. ^1H NMR: 7.65 (m, 1H); 7.42–7.24 (m, 7H); 7.06 (m, 1H); 6.60 (s, 1H); 6.14 (d, 1H); 5.07 (d, 1H); 4.35 (dd, 1H); 4.10 (m, 1H); 3.91 (m, 1H); 3.41 (dd, 1H); 2.81 (m, 1H); 2.63 (m, 5H); 1.70–1.30 (m, 9H); 1.15–0.80 (m, 8H). MS: m/z506=$[M+H]^+$. Chiral HPLC: (column: Pirkle D-DNBPG, eluent DCM-IPA 1:1) retention time 6.50 min; enantiomeric purity: 100%. $[\alpha]_D$ = +34.1 (*c* 0.245 in $CHCl_3$, Hg light). Anal. ($C_{29}H_{39}N_{5O3}$) C, H, N.

6.13.15. (−)-*N*-{1-(1-Adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (54)

It was synthesized as described for 67. Y = 95%, HPLC: retention time 5.2 min. For the analytical characterization, see the description following the HPLC separation of the racemic compound **34**.

6.13.16. (+)-*N*-{1-(1-Adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (55)

It was synthesized as described for 67. Y = 90%, HPLC: retention time 6.9 min. For the analytical characterization, see the description following the HPLC separation of the racemic compound 34.

6.14. Optical resolution via tosyl mandelate

(−)-*N*-{1-(1-Adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino) ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (**54**) and (+)-*N*-{1-(1-Adamantyl)methyl-2,4-dioxo-5-[2-(*N*-morpholino)ethyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-3-yl}-*N*′-phenylurea (**55**) were obtained starting from compound 19e as outlined in Scheme 5 and according to the experimental procedure reported in reference[38] (Curotto G. et al)

6.15. Biological section

For the in vitro assays, inhibition constants (K_i) were determined for all compounds in Guinea Pig brain (CCK2) and rat pancreas (CCK1) membranes using [^{3}H]-CCK8s at a concentration of 0.2 nM as the radiolabelled standard; the membranes were incubated at 25 and 37 °C, respectively. Receptor density (Bmax) and the KD for the radiolabelled standard were determined in separate saturation binding isotherms. Non specific binding was determined in the presence of L-365,260 for CCK2 and L-364,718 for CCK1 receptors. Bound was separated from free ligands by filtration over Whatman GF/C filters with a Brandel M-48 cell harvester. Filters were rinsed and analyzed by liquid scintillation counting on a Packard TriCarb 1900CA.

The black/white box model has been used in order to evaluate the potency as well as the duration of action of the more interesting compounds.[39,41,42] The box is divided in two parts: black and white, where the white side is illuminated (cause of anxiogenesis). Animals, treated with the benzodiazepines administered orally 1 hour before the experiments, are placed in the centre of the white compartment; then the time spent exploring both the white and the black sides are taken: the longer the animal stays in the white side the better is the anxiolytic effect of the compound. The anxiolytic effect was measured at 2, 4, 6 and 8 h.

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