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Achiral, selective CCK_2 receptor antagonists based on a 1,3,5-benzotriazepine-2,4-dione template

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Abstract—Novel, achiral 1*H*-1,3,5-benzotriazepine-2,4(3*H*,5*H*)-diones have been prepared and structurally characterized. These compounds are potent CCK_2 receptor antagonists that display a high degree of selectivity over CCK_1 receptors.

1. Introduction

Cholecystokinin (CCK) and gastrin are related polypeptides that have the same C-terminal sequence (Gly-Trp-Met-Asp-Phe-NH_2).[1] CCK acts on CCK_1 receptors in the periphery where their activation mediates gall bladder contraction, bile release, gastric emptying and pancreatic enzyme secretion.[2] CCK can also stimulate CCK_1 receptors located in the CNS, an action that is associated with regulation of satiety.[3] CCK_2 receptors are present in the CNS also, where their stimulation by CCK is associated with the mediation of anxiety, panic and pain.[4,5] On the other hand, peripherally located CCK_2 receptors are primarily activated by gastrin. In addition to gastrin's role as a stimulant of gastric acid secretion, it is a key growth factor for the histamine-storing enterochromaffin-like cells of the stomach. Thus peripherally acting CCK_2 receptor antagonists have long attracted interest as one means of treating such gastrin-related conditions as gastro oesophageal reflux disorder, rebound hypersecretion (following cessation of anti-secretory therapy)[6] and proliferation of certain GI tract tumours.[7,8]

Amongst the many non-peptide ligands that have been devised for CCK receptors, those based on benzodiazepine (BDZ) ring systems are by far the most prominent.[9–11] In particular, the prototype non-peptide CCK_1 antagonist devazepide is a 1,4-BDZ-based ligand wherein the compound stereochemistry was pivotal to achieving high CCK_1 affinity and selectivity over CCK_2 receptors.[12] This same ring system formed the core of many potent and selective CCK_2 selective antagonists that followed. Equally, ligands for both CCK receptor subtypes based on a 1,5-BDZ ring system have been produced. In the case of CCK_2 receptor antagonists such as **1** (GR 199114X) their selectivity was influenced by similar structural and stereochemical influences as for the 1,4-BDZ-based compounds (Fig. 1).[13] However, a relatively minor structural modification to the side chain functionality in an analogous compound, specifically substitution of the methyl group of the *N*-1-anilidoacetamide substituent of **2** by isopropyl (**3**), produced a marked change in biological profile, causing switching from antagonist to agonist behaviour at CCK_1 receptors (Table 1).[14] More highly optimized derivatives of this type, such as **4** (GW7854), inhibited food intake in rats following oral administration and were explored as anorectic agents.[15] Subsequently 1,4-BDZ-based analogues, containing the same side chain motif, were also shown to display CCK_1 receptor-mediated agonism.[16]

Keywords: CCK; CCK_2; Gastrin; Antagonist; Benzotriazepine.
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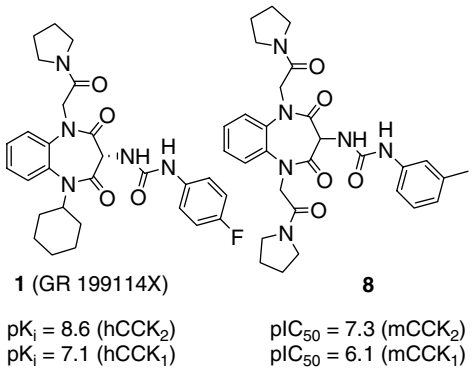


Figure 1. Literature activity values and structures of 1,5-BDZ-based CCK_2 antagonists.

As part of our effort to obtain orally active, non-peptide CCK_2 receptor antagonists, consideration of the influence of ligand stereochemistry on receptor selectivity of the BDZ-based antagonists led us to prepare 3-aza-analogues of 1,4-BDZ-based CCK_2 receptor antagonists such as **6** (YF476) (Fig. 2).[17] Significantly, these 1,3,4-benzotriazepine-based compounds retained selectivity for CCK_2 receptors over CCK_1 receptors even though they were achiral. The most highly optimized derivatives proved to be potent, orally active inhibitors of gastrin-mediated gastric acid secretion in vivo.[18] One obvious advantage of an achiral CCK_2 receptor antagonist is that the resolution step, required to obtain all of the 1,4-BDZ-based CCK_2 receptor antagonists as single entities hitherto, is avoided.

In this paper we extend the tactic that was used to obtain **7**, to 1,5-BDZ-based CCK_2 antagonists, thereby affording 1*H*-1,3,5-benzotriazepine-2,4(3*H*,5*H*)-diones. This change resulted in the pendant *N*-3 aryl substituent being attached to the 1,3,5-benzotriazepine ring by an acetamide rather than urea linkage, a modification for which there is precedent in the 1,5-BZD-based CCK ligands (e.g., **5**).[19] Although achiral 1,5-BDZ CCK_2 antagonists have been obtained previously,[20,21] these compounds were generally less effective than their structurally closest chiral counterparts, because they were limited to derivatives containing identical substituents on the *N*-1 and *N*-5 positions (**8**). However, the absence of a chiral centre in the case of 1,3,5-benzotriazepine-2,4(3*H*,5*H*)-diones enables non-identical substituents to be incorporated at *N*-1 and *N*-5 positions. The SAR of these novel compounds (**9**–**21**) with respect to their CCK receptor activity and subtype selectivity is explored.

2. Chemistry

There are few reports on the preparation and utility of 1,3,5-benzotriazepine-2,4-diones.[22–24] The synthesis of the 1,3,5-benzotriazepine-2,4-diones as potential CCK receptor ligands is described in Scheme 1. The differentially alkylated diamines (**22**–**25**) were prepared by alkylation of previously reported or commercially available *N*-alkyl-1,2-diamines.[13] Treatment of compounds **22**–**25** with phenyl isocyanatoformate[25] led to the *N*-1, *N*-5-disubstituted 1,3,5-benzotriazepine-2,4-diones **26**–**29**. Introduction of the *N*-3 carboxy alkyl substituent was achieved by base-mediated alkylation with a suitably protected haloalkyl carboxylic acid followed by deprotection to afford **30**–**34**. 1,3,5-Benzotriazepine-2,4-diones

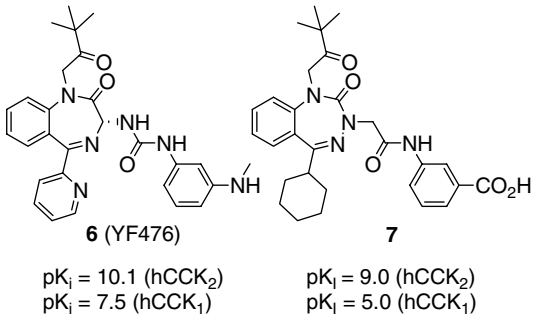
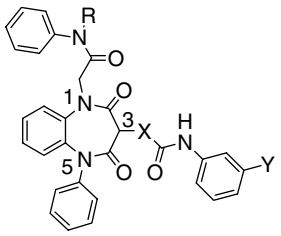


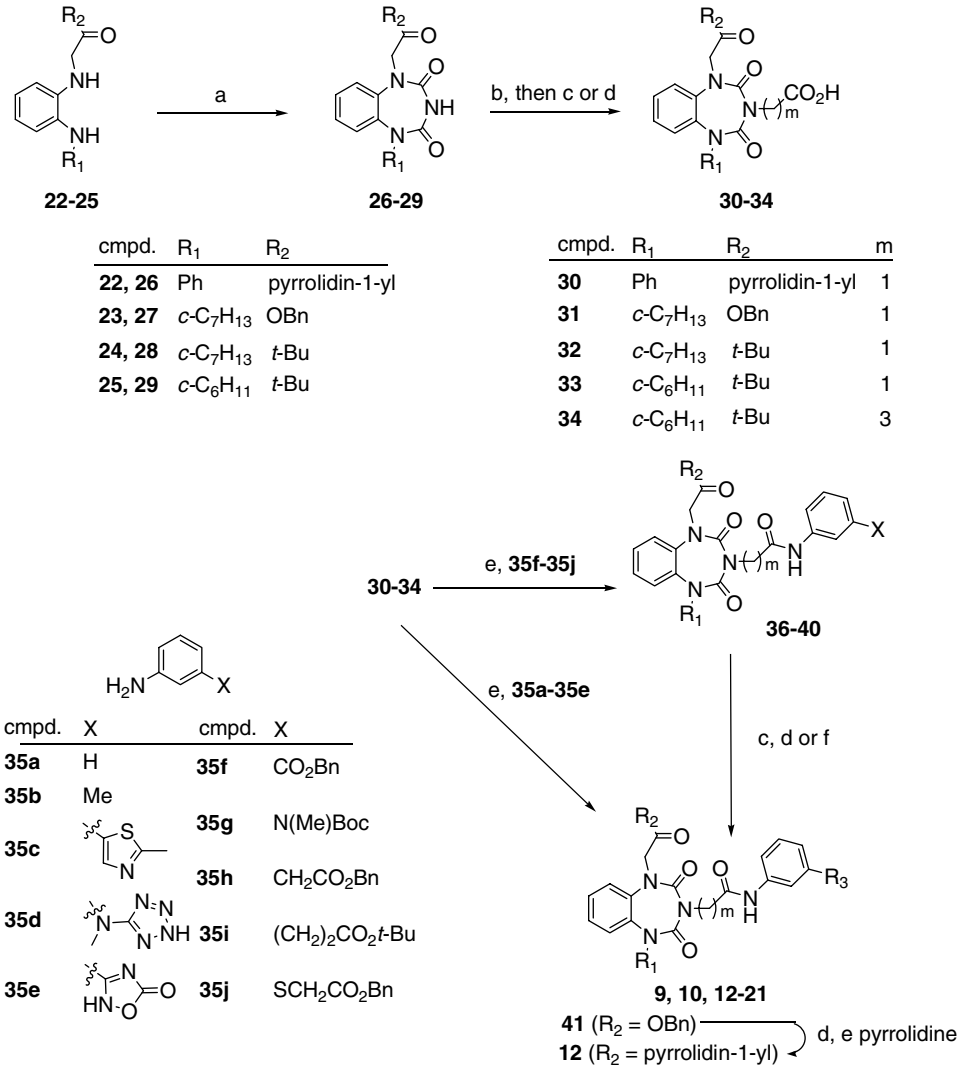
Figure 2. Literature activity values and structures of 1,4-BDZ and 1,3,4-benzotriazepine-based CCK_2 antagonists.

Table 1. Literature activity values and structures of 1,5-BDZ-based CCK_1 agonists



Compound[a]	R	X	Y	hCCK$_2$ (pK_i)	hCCK$_1$ (pK_i)	gpCCK$_1$		Ref.
						%CCK[b]	pEC$_{50}$	
2	Me	NH	H			Inactive	6.3[c]	14
3	*i*-Pr	NH	H	7.6	7.3	86	5.9	14
4 (GW7854)	*i*-Pr	NH	CO_2H	8.0	6.9	102	7.4	15
5	*i*-Pr	CH_2	H	6.1	6.8	50	6.4	19

[a] 3*R*/3*S* stereochemistry.
[b] Percentage contraction of isolated guinea-pig gallbladder produced by test compound (30 μM) relative to that produced by CCK-8 (1 μM).
[c] pK_B from shift of CCK-8 concentration–effect curve in presence of test compound (30 μM).



Scheme 1. Synthesis of 1,3,5-benzotriazepines. Reagents: (a) PhO_2CNCO/DMA; (b) NaH, $Br(CH_2)_mCO_2R$ ($m = 1, 3$; R = *t*-Bu, Bn)/DMF; (c) CF_3CO_2H; (d) H_2, Pd–C/THF–MeOH; (e) EDC, 1-HOBt, DMAP/DMF; (f) $NaOH/H_2O$–CH_3OH.

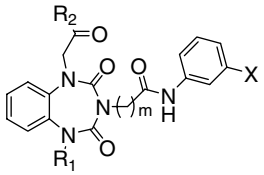
9, **12**, **14**, **18**–**21** were obtained directly on reaction of **30**, **32**–**34** with anilines (**35a**–**35e**) using standard amide bond forming conditions. Compounds **10**, **15**–**17** were obtained by initial reaction of **30**, **32** or **33** with anilines **35f**–**35j**, to afford intermediates **36**–**40**, followed by removal of the protecting group on the anilide substituent. Reaction of **31** with toluidine (**35b**) afforded intermediate **41**, which yielded compound **12** on manipulation of the *N*-1 side chain substituent.

3. Results and discussion

The activity of 1,3,5-benzotriazepines **9**–**21** as CCK_2 receptor antagonists was determined in a radioligand binding assay by displacement of [^{125}I]-BH-CCK-8S from human, recombinant CCK_2 receptors ($hCCK_2R$) expressed in NIH3T3 cell membranes.[26] To assess their selectivity for CCK_2 receptors over CCK_1 receptors, they were also tested in a similar manner in a human, recombinant CCK_1 receptor ($hCCK_1R$) radioligand binding assay.[17] Measurement of the inhibition, produced by selected examples, of the pentagastrin-stimulated acid secretion in a perfused, isolated rat stomach assay[27] was performed to demonstrate functional CCK_2 activity (Table 2). Compounds **10**, **15**–**17** and **19**–**21** were tested as *N*-methyl-D-glucamine salts. Compound **13** was tested as the hydrochloride salt.

Initial examples, **9** and **10**, displayed micromolar affinity at $hCCK_2$ receptors with selectivity over $hCCK_1$ receptors. Strict comparison with the structurally closest 1,5-BZD analogues[13] is made difficult as the latter compounds are racemic and were characterised in functional bioassays. Nonetheless, based on its activity in the rat stomach assay, where it did not produce acid output on its own but inhibited pentagastrin-stimulated acid secretion with comparable potency to that determined in the $hCCK_2$ radioligand binding assay, compound **10** can be considered to be around 250-fold less potent than its closest 1,5-BZD analogue. Previously published SAR on the 1,5-BDZ CCK_2 antagonists had established that enhanced CCK_2 affinity could be achieved by replacing the *N*-5 phenyl ring by alicylic groups such as cyclohexyl

Table 2. Biological data for 1,3,5-benzotriazepine-2,4-dione-based CCK_2 antagonists[a]



Compound	R_1	R_2	X	*m*	CCK_2[b]	CCK_1[c]	CCK_2[d]
9	Ph	Pyrrolidin-1-yl	H	1	6.00 ± 0.07	<5.0	NT[e]
10	Ph	Pyrrolidin-1-yl	CO_2H	1	6.42 ± 0.10	<5.0	6.15 ± 0.21
11	*c*-C_7H_{13}	Pyrrolidin-1-yl	CH_3	1	7.63 ± 0.09	6.21	IA[f]
12	*c*-C_7H_{13}	*t*-Bu	CH_3	1	8.12 ± 0.04	6.35	NT[e]
13	*c*-C_6H_{11}	*t*-Bu	$NHCH_3$	1	8.02	5.63 ± 0.13	7.71 ± 0.33
14	*c*-C_6H_{11}	*t*-Bu	2-Methyl-thiazol-4-yl	1	8.02	5.81	7.70 ± 0.35
15	*c*-C_7H_{13}	*t*-Bu	CH_2CO_2H	1	8.50 ± 0.08	5.41 ± 0.05	7.36 ± 0.21
16	*c*-C_6H_{11}	*t*-Bu	$CH_2CH_2CO_2H$	1	8.20	5.63 ± 0.04	7.58 ± 0.42
17	*c*-C_7H_{13}	*t*-Bu	SCH_2CO_2H	1	9.05	6.55 ± 0.09	8.35 ± 0.33
18	*c*-C_6H_{11}	*t*-Bu	*N*-Methyl-((2*H*)-tetrazol-5-yl)-amino	1	8.37	5.68 ± 0.06	7.90 ± 0.31
19	*c*-C_7H_{13}	*t*-Bu	1,2,4-Oxadiazol-3-yl-5(2*H*)-one	1	9.37	6.93 ± 0.12	8.49 ± 0.33
20	*c*-C_6H_{11}	*t*-Bu	1,2,4-Oxadiazol-3-yl-5(2*H*)-one	1	8.92 ± 0.41	6.50	7.35 ± 0.28
21	*c*-C_6H_{11}	*t*-Bu	1,2,4-Oxadiazol-3-yl-5(2*H*)-one	3	7.68	7.14 ± 0.02	7.06 ± 0.35

[a] Data were generally obtained from at least three separate experiments. Where no SEM was recorded, the data were from two experiments.
[b] pK_I ± SEM values obtained from competition with 20 pM [^{125}I]-BH-CCK-8S for recombinant, human CCK_2 receptors expressed in NIH3T3 cell membranes.
[c] pK_I ± SEM values obtained from competition with 20 pM [^{3}H]-L-364,718 for recombinant, human CCK_1 receptors expressed in CHO-K1 cells from at least two separate experiments.
[d] pA_2 ± SEM values, estimated from single shifts of pentagastrin concentration–effect curves in isolated, lumen-perfused immature rat stomachs.
[e] Not tested.
[f] Inactive at concentration tested (10^{-6} M).

and cycloheptyl.[13] In the 1,3,5-benzotriazepine series the potency increased similarly with the preparation of **11** as judged by comparison with that of **9**. Although **11** differed structurally from **9** still further in the nature of the *N*-3 substituent, in that it contained a tolyl acetamide, rather than a phenyl acetamide substituent at the *N*-3 position, it was at least 40-fold more potent at CCK_2 receptors than **9**, maintaining a high margin of selectivity over CCK_1 receptors. At a concentration of 10 μM, **11** failed to produce a shift of the pentagastrin concentration–effect curve in the rat functional bioassay, that might have been expected based on its affinity in the CCK_2 binding assay. This difference in activity between the CCK_2 assays for **11** can be ascribed to differing affinity for human and rat CCK_2 receptors. However, it can also be attributed to low aqueous solubility of the compound, as this property is more apparent in the functional tissue bioassay which is less tolerant of organic co-solvents that can otherwise be used to aid dissolution of poorly water soluble compounds in the radioligand binding assay.

Compound **12** displayed a further increase in affinity relative to **11** at $hCCK_2$ receptors. This increase is similar to that produced in the case of 1,4-BDZ containing CCK_2 antagonists, arising from substitution of *N*-1 acetamide substituents by alkylketomethylene groups such as pinacoloyl.[28] This apparent congruence in SAR between 1,3,5-benzotriazepine containing compounds and the 1,5-BDZ-based CCK_2 antagonists is consistent with the apparent similarity in core ring conformation. Single crystals of intermediate **26** were selected for X-ray diffraction determination and the ORTEP diagram is presented in Figure 3. Although **26** lacks a substituent on the *N*-3 position, the seven-membered ring adopts a pseudo-boat conformation that is not significantly different to that observed for 1,5-BDZ-based CCK_2 antagonists.[13,29]

As the presence of a polar substituent on the 3-aryl ring of **10** appeared to confer functional activity (since this was not observed for compound **11**) further derivatives that incorporated polar groups were prepared. The relationship between functional activity and the presence of polar substituents was further demonstrated in the case of the weakly basic compounds **13** and **14**, which, although they contained a cyclohexyl rather than a cycloheptyl group at the *N*-5 position, showed only marginally lower potency in the rat functional bioassay than they produced in the $hCCK_2$ binding assay. Moreover, the incorporation of these substituents to obtain **13** and **14** did not significantly

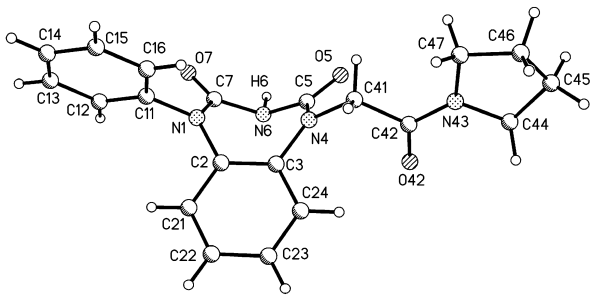


Figure 3. ORTEP perspective view of **26** showing 30% probability displacement ellipsoids.

alter the affinity, relative to **12**, for $hCCK_2$ receptors. In considering incorporation of acid substituents their choice was influenced by those that had not only conferred in vitro functional activity in the case of 1,3,4-benzotriazepines, but which had also proved effective in achieving inhibition of pentagastrin-stimulated acid secretion in in vivo assays.[18] To this end the acetic acid (**15**), propionic acid (**16**) and thioacetic acid (**17**) containing compounds were prepared, each displaying high affinity at $hCCK_2$ receptors, and potency in the functional bioassay within 10-fold of that determined in the radioligand binding assay. Selectivity over CCK_1 receptors of at least 300-fold was maintained for these compounds. Acid surrogates had proved to be particularly effective previously and in line with this trend the *N*-methyl amino-tetrazole (**18**) and oxadiazolone (**19** and **20**) derivatives showed a similar profile to the carboxylic acid containing compounds across the in vitro bioassays, achieving around 1 nM affinity at $hCCK_2$ receptors. In terms of its overall profile, **20** displayed a greater difference in cross species activity than the equivalent 1,3,4-benzotriazepine-based compound.[18]

Selectivity over CCK_1 receptors was maintained throughout for these 1,3,5-benzotriazepine-based CCK_2 antagonists. Only by lengthening the *N*-3 chain, as in **21**, was receptor selectivity significantly diminished. This arose due to the combination of reduced CCK_2 activity and higher CCK_1 affinity being produced by this change. In the absence of functional assay data it remains unclear whether **21** has intrinsic activity at CCK_1 receptors. Further manipulation of **21**, through changing the nature of the *N*-1 substituent, may ultimately provide CCK_1 selective ligands, particularly since these would bear a strong structural relationship to 1,5-BZD-based CCK_1 agonists such as **5**.

4. Conclusion

In summary, the achiral 1,3,5-benzotriazepine ring system has been employed as a template for the synthesis of a series of potent CCK_2 receptor antagonists. Except for compound **21** these display a high degree of selectivity over CCK_1 receptors. Influenced by some of the trends in SAR evident in the earlier BDZ-based CCK ligands we have prepared derivatives that indicate a high degree of congruence in CCK_2 receptor activity between the 1,3,5-benzotriazepine-based compounds and the BDZs. The parallels in SAR, that are consistent with the structural similarities of the respective ring system, suggest these 1,3,5-benzotriazepine-containing compounds interact with the CCK_2 receptor most likely in a similar manner. Where precise comparisons can be made, the 1,3,5-benzotriazepine containing compounds display a higher degree of selectivity over CCK_1 receptors than the BDZ-based compounds, which when coupled with their ease of synthesis, since a resolution step is avoided, offer a distinct advantage over the prior art.

5. Experimental

Flash column chromatography was performed on Merck silica gel 60 (40–63 μm) using the reported solvent systems or on a Biotage Quad purification unit. ^1H NMR spectra were recorded on a Bruker DRX-300 instrument and the chemical shifts (δ_H) were recorded relative to an internal standard Abbreviations: DCM, dichloromethane; DMA, *N*,*N*-dimethylacetamide; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DMAP, 4-(dimethylamino)pyridine; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid. Crystallographic data (excluding structure factors) for compounds **26** and **29** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CDC 659555 and CDC 659556, respectively. Copies of these data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, (fax: +44 (0)1223 336033). Tables of bond lengths and angles for compounds **26** and **29** can be found in Supplementary data.

Compounds **10**, **15**–**17** and **19**–**21** were tested as *N*-methyl-D-glucamine salts. These salts were prepared by stirring an aqueous mixture of the compound with one equivalent of *N*-methyl-D-glucamine until a solution was obtained (a minimum amount of 1,4-dioxan was added if necessary to complete dissolution) and the solutions were freeze-dried. Compound **13** was tested as the hydrochloride salt which was obtained by suspending the free-base in saturated methanolic-HCl. The solution was evaporated to dryness and the resultant hydrochloride salt freeze-dried from an aqueous solution as above.

5.1. 2-((2-Phenylamino)-phenylamino)-pyrrolidin-1-yl acetamide (22)

A mixture of *N*-phenyl-1,2-phenylenediamine (1 g, 5.4 mmol), 2-bromo-1-pyrrolidin-1-yl ethanone[30] (1.05 g, 5.5 mmol) and K_2CO_3 (0.84 g, 6.1 mmol) in DMF (20 mL) was heated at 60 °C for 16 h. On cooling, the mixture was suspended in H_2O/EtOAc (1:1/80 mL). The organic layer was separated, washed successively with H_2O (2× 40 mL), brine (2× 40 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent followed by chromatography (EtOAc/DCM (1:5)) of the residue afforded **22** as a red oil (1.01 g, 63%). ^1H NMR ($CDCl_3$) 7.32 (3H, m), 7.07 (1H, m), 6.83–6.64 (5H, m), 5.42 (1H, br s), 5.11 (1H, br s), 3.81 (2H, s), 3.49 (2H, t), 3.38 (2H, t), 1.94 (2H, m), 1.86 (2H, m).

5.2. (2-Cycloheptylamino-phenylamino)-acetic acid benzyl ester (23)

A mixture of *N*-cycloheptyl-benzene-1,2-diamine[13] (1.0 g, 5.0 mmol), K_2CO_3 (0.69 g, 5.0 mmol) and 2-benzyl bromoacetate (1.15 g, 5.0 mmol) in DMF (20 mL) was stirred at ambient temperature for 16 h. The mixture was suspended in H_2O/EtOAc (1:1/40 mL). The organic layer was separated, washed successively with H_2O (2× 20 mL), brine (2× 20 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent afforded **23** as a red oil (1.73 g, 98%). ^1H NMR ($CDCl_3$) 7.38 (5H, m), 6.82 (1H, dd), 6.75 (1H, dt), 6.62 (1H, dd), 6.58 (1H, dd), 5.22 (2H, s), 3.94 (3H, m), 3.42 (1H, m), 3.40 (1H, m), 1.97 (2H, m), 1.71–1.56 (10H, m).

5.3. 1-(2-Cycloheptylamino-phenylamino)-3,3-dimethyl-butan-2-one (24)

Compound **24** was obtained by the method used in the preparation of **23** except that 1-bromo-3,3-dimethyl-butan-2-one was used instead of 2-benzyl bromoacetate (91%). ^{1}H NMR ($CDCl_3$) 6.77 (2H, m), 6.61 (2H, m), 4.32 (1H, br s), 4.11 (2H, s), 3.43 (1H, m), 3.27 (1H, m), 1.72 (1H, m), 1.62-1.41 (11H, m), 1.27 (9H, s).

5.4. 1-(2-Cyclohexylamino-phenylamino)-3,3-dimethyl-butan-2-one (25)

Compound **25** was obtained by the method used in the preparation of **23** except that *N*-cyclohexyl-benzene-1,2-diamine[13] and 1-bromo-3,3-dimethyl-butan-2-one were used instead of *N*-cycloheptyl-benzene-1,2-diamine and 2-benzyl bromoacetate, respectively (100%). ^{1}H NMR ($CDCl_3$) 6.78–6.71 (3H, m), 6.57 (1H, m), 4.20 (1H, br s), 4.10 (2H, s), 3.23 (2H, m), 2.09 (2H, m), 1.82–1.76 (3H, m), 1.41–1.25 (14H, m).

5.5. 1-(2-Oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1*H*-1,3,5-benzotriazepine-2,4(3*H*,5*H*)-dione (26)

A solution of phenyl isocyanatoformate[25] (0.6 g, 3.7 mmol) in DMA (3 mL) was added dropwise to a solution of compound **22** (0.7 g, 2.4 mmol) in DMA (40 mL) maintained at 90 °C. Heating was continued at 90 °C for 16 h. After cooling to ambient temperature, the reaction mixture was suspended in H_2O/EtOAc (2:1/75 mL). The organic layer was separated, washed with brine (2× 50 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent followed by chromatography (EtOAc/DCM (1:5) to neat EtOAc) of the oil obtained afforded **26** as a white solid (0.43 g, 49%). ^{1}H NMR (DMSO-d_6) 9.08 (1H, s), 7.52-7.23 (6H, m), 7.18 (1H, m), 7.11 (1H, m), 7.74 (1H, d), 4.72 (2H, m), 3.51 (2H, m), 3.32 (2H, m), 1.92 (2H, m), 1.77 (2H, m).

5.6. (1-Benzyloxycarbonylmethyl-5-cycloheptyl-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid (27)

Compound **27** was obtained by the method used in the preparation of **26** except that **23** was used instead of **22** (20%). ^{1}H NMR ($CDCl_3$) 7.61–7.23 (9H, m), 6.31 (1H, s), 5.20 (2H, s), 4.60 (1H, d), 4.47 (1H, d), 3.93 (1H, m), 2.18–1.21 (12H, m).

5.7. 1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-1*H*-1,3,5-benzotriazepine-2,4(3*H*,5*H*)-dione (28)

Compound **28** was obtained by the method used in the preparation of **26** except that **24** was used instead of **22** (47%). ^{1}H NMR ($CDCl_3$) 7.32–7.19 (3H, m), 7.00 (1H, m), 6.23 (1H, br s), 4.78 (1H, d), 4.67 (1H, d), 3.96 (1H, m), 2.25 (2H, m), 1.94–1.79 (8H, m), 1.29 (11H, s).

5.8. 1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-1*H*-1,3,5-benzotriazepine-2,4(3*H*,5*H*)-dione (29)

Compound **29** was obtained by the method used in the preparation of **26** except that **25** was used instead of **22** (42%). ^{1}H NMR ($CDCl_3$) 7.37 (1H, m), 7.27 (2H, m), 6.99 (1H, m), 6.20 (1H, br s), 4.73 (2H, m), 3.80 (1H, m), 2.20–1.35 (7H, m), 1.34–1.26 (12H, m).

5.9. (2,4-Dioxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid (30)

Step A. Compound **26** (0.14 g, 0.38 mmol) and NaH (60% suspension in mineral oil/0.02 g, 0.6 mmol) were stirred in anhydrous DMF (10 mL) at ambient temperature for 0.5 h. *tert*-Butyl bromoacetate (0.11 g, 0.6 mmol) was added and stirring was continued for 16 h. The reaction mixture was suspended in H_2O/EtOAc (1:1/25 mL). The organic layer was separated, washed with H_2O (2× 20 mL), brine (2× 20 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent followed by chromatography (EtOAc/DCM (1:1)) of the oil obtained afforded (2,4-dioxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid *tert*-butyl ester as a beige solid (0.16 g, 89%). ^{1}H NMR ($CDCl_3$) 7.54 (2H, d), 7.42 (3H, m), 7.37 (1H, t), 7.17 (1H, dt), 7.07 (1H, dt), 6.84 (1H, dd), 4.67 (1H, d), 4.59 (1H, d), 4.25 (1H, d), 4.13 (1H, d), 3.54 (4H, m), 2.04–1.85 (4H, m), 1.40 (9H, s).

Step B. The product of the previous step was suspended in TFA (4 mL) and stirred at ambient temperature for 1 h. The solution was concentrated in vacuo and re-evaporated from DCM (10 mL). The oil obtained was dissolved in DCM (20 mL), washed with H_2O (20 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent afforded **30** as an orange amorphous solid (0.14 g, 96%). ^{1}H NMR ($CDCl_3$) 10.29 (1H, br s), 7.47–7.34 (6H, m), 7.27 (1H, t), 7.10 (1H, t), 6.86 (1H, m), 4.67 (2H, m), 4.25 (2H, m), 3.59 (4H, m), 2.06 (2H, m), 1.94 (2H, m).

5.10. (1-Benzyloxycarbonylmethyl-5-cycloheptyl-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid (31)

Compound **31** was obtained by steps A and B of the method used in the preparation of **30** except that **27** was used instead of **26** in step A (41%). ^{1}H NMR ($CDCl_3$) 10.15 (1H, br s), 7.37–7.24 (9H, m), 5.19 (2H, m), 4.62 (1H, d), 4.44 (1H, d), 4.17 (2H, s), 3.93 (1H, m), 2.19–1.44 (12H, m).

5.11. (1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid (32)

Compound **32** was obtained by steps A and B of the method used in the preparation of **30** except that **28** was used instead of **26** in step A (82%) ^{1}H NMR ($CDCl_3$) 7.30–7.17 (3H, m), 7.04 (1H, m), 4.74 (2H, m), 3.99 (3H, m), 2.28–1.52 (12H, m), 1.27 (9H, s).

5.12. (1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid (33)

Step A. (1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-ace-

tic acid benzyl ester was obtained by step A of the method used in the preparation of **30** except that **29** and benzyl 2-bromoacetate were used instead of **26** and *tert*-butyl bromoacetate, respectively (88%). ^{1}H NMR ($CDCl_3$) 7.33–7.18 (8H, m), 6.99 (1H, m), 5.09 (2H, m), 4.76 (1H, d), 4.68 (1H, d), 4.33 (1H, d), 4.23 (1H, d), 3.85 (1H, m), 2.18 (1H, m), 1.91–1.34 (6H, m), 1.33–1.25 (11H, m).

Step B. The product of the previous step (0.88 g, 1.7 mmol) in THF/MeOH (1:1/30 mL) was stirred with 10% Pd/C (20 mg) under a hydrogen atmosphere at ambient temperature for 16 h. The reaction mixture was filtered through a pad of Celite. Concentration of the filtrate in vacuo to afford an oil from which compound **33** was obtained as a white solid on crystallization from DCM/hexanes (0.72 g, 100%). ^{1}H NMR ($CDCl_3$) 10.40 (1H, br s), 7.36 (1H, m), 7.25 (2H, m), 7.03 (1H, m), 4.75 (2H, m), 4.05 (2H, s), 3.85 (1H, m), 2.27 (1H, m), 1.99–1.65 (6H, m), 1.43–1.20 (12H, s).

5.13. (1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-butyric acid (34)

Step A. Compound **29** (0.25 g, 0.7 mmol) and NaH (60% suspension in mineral oil/0.04 g, 1.0 mmol) were stirred in anhydrous DMF (10 mL) at ambient temperature for 0.5 h. Benzyl 4-bromobutanoate[31] (0.3 g, 1.2 mmol) was added and the mixture was heated at 60 °C for 72 h. On cooling, the reaction mixture was suspended in H_2O/EtOAc (1:1/25 mL). The organic layer was separated, washed with brine (2× 20 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent followed by chromatography (EtOAc/DCM (1:20–1:5)) of the residue afforded (1-cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-butyric acid benzyl ester as a colourless oil. (0.21 g, 57%). ^{1}H NMR ($CDCl_3$) 7.34–7.29 (5H, m), 7.27–7.16 (3H, m), 6.96 (1H, m), 5.01 (2H, s), 4.68 (2H, m), 3.83 (1H, m), 3.51 (2H, m), 2.15–1.75 (8H, m), 1.66–1.18 (11H, m).

Step B. Compound **34** was obtained by step B of the method used in the preparation of 20 except that the product of the previous step was used instead of (1-cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid benzyl ester (100%). ^{1}H NMR ($CDCl_3$) 7.32–7.18 (3H, m), 6.97 (1H,m), 4.68 (2H, m), 3.76–3.71 (3H, m), 3.50 (2H,m), 2.07–1.80 (8H,m), 1.70–1.23 (11H, m).

5.14. 2-(2,4-Dioxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-phenyl-acetamide (9)

A mixture of compound **30** (0.15 g, 0.4 mmol), EDC (0.1 g, 0.5 mmol), HOBt (0.07 g, 0.5 mmol), NEt_3 (0.12 mL, 0.9 mmol), DMAP (10 mg) and aniline (**35a**) (0.04 g, 0.4 mmol) in DMF (5 mL) was stirred at ambient temperature for 18 h. The reaction mixture was diluted with EtOAc (20 mL), washed successively with H_2O (2× 20 mL), brine (2× 20 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent afforded an oil from which **9** was obtained as an off-white solid from DCM/hexanes (0.08 g, 43%). ^{1}H NMR ($CDCl_3$) 8.49 (1H, br s), 7.54 (2H, d), 7.43–7.33 (9H, m), 7.18 (1H, m), 7.12 (1H, m), 6.88 (1H, d), 4.79 (1H, d), 4.60 (1H, d), 4.32 (2H, s), 3.53 (4H, m), 1.99–1.84 (4H, m). HRMS (ES, $[M+H]^+$) calcd for $C_{28}H_{27}N_5O_4$ 498.2136, found 498.2131.

5.15. 2-(1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-*m*-tolyl-acetamide (12)

Compound **12** was obtained by the method used in the preparation of **9** except that **32** and *m*-toluidine (**35b**) were used instead of **30** and **35a**, respectively (16%). ^{1}H NMR ($CDCl_3$) 8.20 (1H, br s), 7.39–7.10 (7H, m), 6.86 (1H, m), 4.77 (2H, m), 4.20 (2H, m), 4.05 (1H, m), 2.36 (3H, s), 2.30–1.74 (12H, m), 1.25 (9H, s). HRMS (ES, $[M+H]^+$) calcd for $C_{30}H_{38}N_4O_4$ 519.2966, found 519.2966.

5.16. 2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-(3-(2-methyl-thiazol-4-yl)-phenyl)-acetamide (14)

Compound **14** was obtained by the method used in the preparation of **9** except that **33** and 3-(2-methyl-thiazol-4-yl)-phenylamine (**35c**)[32] were used instead of **30** and **35a**, respectively (70%). ^{1}H NMR ($CDCl_3$) 8.27 (1H, br s), 7.80 (1H, s), 7.46 (1H, d), 7.30 (3H, m), 7.28 (3H, m), 7.08 (1H, m), 4.83 (1H, d), 4.70 (1H, d), 4.21 (2H, m), 3.91 (1H, m), 2.89 (3H, s), 2.31 (1H, m), 1.94–1.85 (5H, m), 1.41 (1H, m), 1.35 (12H, m). HRMS (ES, $[M+H]^+$) calcd for $C_{32}H_{37}N_5O_4$ 588.2639, found 588.2633.

5.17. 2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-(3-(methyl-(2*H*-tetrazol-5-yl)-amino)-phenyl)-acetamide (18)

Compound **18** was obtained by the method used in the preparation of **9** except that **33** and *N*-methyl-*N*-(2*H*-tetrazol-5-yl)-benzene-1,3-diamine (**35d**)[33] were used instead of **30** and **35a**, respectively (6%). ^{1}H NMR ($CDCl_3$) 10.50 (1H, br s), 8.50 (1H, br s), 7.36 (2H, m), 7.26 (4H, m), 7.02 (1H, m), 7.00 (1H, m), 4.79 (1H, d), 4.67 (1H, d), 3.89 (1H, m), 3.55 (3H, s), 2.20 (1H, m), 1.96–1.63 (6H, m), 1.36–1.23 (12H, m). HRMS (ES, $[M+H]^+$) calcd for $C_{30}H_{37}N_9O_4$ 588.3041, found 588.3046.

5.18. 2-(1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-(3-(5-oxo-2,5-dihydro-[1,2,4]oxadiazol-3-yl)-phenyl)-acetamide (19)

Compound **19** was obtained by the method used in the preparation of **9** except that **32** and 3-(3-amino-phenyl)-2*H*-[1,2,4]oxadiazol-5-one (**35e**)[34] were used instead of **30** and **35a**, respectively (39%). ^{1}H NMR ($CDCl_3$) 10.80 (1H, br s), 9.15 (1H, s), 8.02 (1H, s), 7.57–7.07 (8H, m), 4.85 (1H, d), 4.64 (1H, d), 4.31 (2H, s), 4.10

(1H, m), 1.72–1.25 (12H, m), 1.20 (9H, s). The compound was further characterized as the *N*-methyl-D-glucamine salt. Anal. Found: C, 55.98; H, 6.77; N, 10.91%; $C_{31}H_{36}N_6O_6{\cdot}C_7H_{17}NO_5{\cdot}1.5H_2O{\cdot}0.7$dioxan requires: C, 56.16; H, 7.12; N, 11.24%.

5.19. 2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-(3-(5-oxo-2,5-dihydro-[1,2,4]oxadiazol-3-yl)-phenyl)-acetamide (20)

Compound **20** was obtained by the method used in the preparation of **9** except that **33** and 3-(3-amino-phenyl)-2*H*-[1,2,4]oxadiazol-5-one (**35e**)[34] were used instead of **30** and **35a**, respectively (67%). ^{1}H NMR ($CDCl_3$) 9.15 (1H, s), 7.57–7.07 (9H, m), 4.82 (1H, d), 4.63 (1H, d), 4.31 (2H, m), 3.89 (1H, m), 2.17 (1H,m), 1.84–1.64 (6H, m), 1.27–1.15 (12H, m). The compound was further characterized as the *N*-methyl-D-glucamine salt. HRMS (ES, $[M+H]^+$) calcd for $C_{30}H_{34}N_6O_6{\cdot}C_7H_{17}NO_5$ 770.3719, found 770.3727.

5.20. 4-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-(3-(5-oxo-2,5-dihydro-[1,2,4]oxadiazol-3-yl)-phenyl)-butyramide (21)

Compound **21** was obtained by the method used in the preparation of **9** except that **34** and 3-(3-amino-phenyl)-2*H*-[1,2,4]oxadiazol-5-one (**35e**)[34] were used instead of **30** and **35a**, respectively (20%). ^{1}H NMR ($CDCl_3$) 8.65 (1H, s), 7.89 (1H, s), 7.68 (1H, m), 7.53 (1H, m), 7.37–7.20 (5H, m), 6.94 (1H, m), 4.90 (1H, d), 4.52 (1H, d), 3.84 (1H, m), 3.51 (2H, m), 2.18–1.47 (9H, m), 1.35–1.26 (16H, m). The compound was further characterized as the *N*-methyl-D-glucamine salt. Anal. Found: C, 56.52; H, 6.75; N, 11.40%. $C_{32}H_{38}N_6O_6{\cdot}C_7H_{17}NO_5{\cdot}1.5H_2O{\cdot}0.1$-dioxan requires: C, 56.76; H, 7.10; N, 11.76%.

5.21. 3-(2-(2,4-Dioxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-benzoic acid benzyl ester (36)

Compound **36** was obtained by the method used in the preparation of **9**, except that 3-amino-benzoic acid benzyl ester (**35f**) was used instead of **35a**. ^{1}H NMR ($CDCl_3$) 8.67 (1H, br s), 7.85–7.73 (3H, m), 7.53–7.21 (14H, m), 6.90 (1H, m), 5.34 (2H, s), 4.68 (2H, m), 4.30 (2H, m), 3.54 (4H, m), 2.04 (2H, m), 1.91 (2H, m).

5.22. 3-(2-(2,4-Dioxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-benzoic acid (10)

Compound **10** was obtained by step B of the method used in the preparation of **33** except that **36** was used instead of (1-cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid benzyl ester (34%). ^{1}H NMR (DMSO-d_6) 10.24 (1H, br s), 8.19 (1H, s), 7.69–7.17 (12H, m), 6.83 (1H, d), 4.91 (1H, d), 4.70 (1H, d), 4.26 (2H, m), 3.44 (4H, m), 1.90 (2H, m), 1.77 (2H, m). The compound was further characterized as the *N*-methyl-D-glucamine salt. Anal. Found C, 54.20; H, 6.03; N, 10.68%; $C_{29}H_{27}N_5O_6{\cdot}C_7H_{17}NO_5{\cdot}3.5H_2O$ requires: C, 54.06; H, 6.43; N, 10.51%.

5.23. (3-(2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenyl)-methyl-carbamic acid *tert*-butyl ester (37)

Compound **37** was obtained by the method used in the preparation of **9** except that **33** and (3-amino-phenyl)-methyl-carbamic acid *tert*-butyl ester (**35g**)[17] were used instead of **30** and **35a**, respectively (89%). ^{1}H NMR ($CDCl_3$) 8.30 (1H, br s), 7.43–7.27 (4H, m), 7.19–7.10 (3H, m), 6.95 (1H, d), 4.78 (1H, d), 4.73 (1H, d), 4.16 (2H, m), 3.90 (1H, m), 3.23 (3H, s), 2.28 (1H, m), 1.89–1.45 (6H, m), 1.45 (9H, s), 1.34–1.25 (12H, m).

5.24. 2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-(3-methylamino-phenyl)-acetamide (13)

Compound **13** was obtained by step B of the method used in the preparation of **35j** except that **37** was used instead of benzyl-3-(*N*-*tert*-butyloxycarbonylamino)phenylthioacetate (32%). ^{1}H NMR ($CDCl_3$) 8.10 (1H, br s), 7.42 (1H, m), 7.28 (2H, m), 7.11–7.01 (3H, m), 6.45 (1H, m), 6.36 (1H, d), 4.78 (1H, d), 4.67 (1H, d), 4.19 (2H, m), 3.90 (1H, m), 2.81 (3H, s), 2.28 (1H, m), 1.83–1.38 (6H, m), 1.35–1.21 (12H, m). The compound was further characterized as the hydrochloride salt. Anal. Found: C, 62.82; H, 6.76; N, 12.12%; $C_{39}H_{37}N_5O_4{\cdot}HCl{\cdot}0.1$dioxan requires: C, 62.51; H, 6.92; N, 12.40%.

5.25. (3-(2-(1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenyl)-acetic acid benzyl ester (38)

Compound **38** was obtained by the method used in the preparation of **9** except that **33** and **35h**[35] were used instead of **30** and **35a**, respectively (31%). ^{1}H NMR ($CDCl_3$) 8.20 (1H, br s), 7.37–7.27 (11H, m), 7.20 (1H, m), 7.07 (1H, m), 5.12 (2H, s), 4.77 (1H, d), 4.70 (1H, d), 4.18 (1H, d), 4.13 (1H, d), 4.09 (1H, m), 3.61 (2H, s), 2.04–1.32 (11H, m), 1.26 (9H, s).

5.26. (3-(2-(1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenyl)-acetic acid (15)

Compound **15** was obtained by step B of the method used in the preparation of **33** except that **38** was used instead of (1-cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid benzyl ester (96%). ^{1}H NMR ($CDCl_3$) 8.20 (1H, br s), 7.34-7.21 (5H, m), 7.20 (1H, m), 7.19 (1H, m), 6.97 (1H, m), 4.74 (1H, d), 4.63 (1H, d), 4.17 (2H, m), 4.00 (1H, m), 3.46 (2H, s), 1.89–1.54 (12H, m), 1.24 (9H, s). The compound was further characterized as the *N*-methyl-D-glucamine salt. Anal. Found: C, 57.91; H, 7.59; N, 7.97%; $C_{31}H_{38}N_4O_6{\cdot}C_7H_{17}NO_5{\cdot}1.5$-$H_2O{\cdot}$dioxan requires: C, 57.78; H, 7.62; N, 8.02%.

5.27. 3-(3-(2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenyl)-propionic acid *tert*-butyl ester (39)

Compound **39** was obtained by the method used in the preparation of **9** except that **33** and (3-amino-phenyl)-propionic acid *tert*-butyl ester (**35i**)[18] were used instead of **30** and **35a**, respectively (100%). ^{1}H NMR ($CDCl_3$) 8.79 (1H, br s), 7.41 (1H, m), 7.40–7.28 (5H, m), 7.10 (1H, m), 6.94 (1H, m), 4.85 (1H, d), 4.68 (1H, d), 4.15 (2H, s), 3.89 (1H, m), 2.90 (2H, m), 2.62 (2H, m), 2.20 (1H, m), 1.79–1.39 (6H, m), 1.34–1.27 (12H, m), 1.25 (9H, s).

5.28. 3-(3-(2-(1-Cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenyl)-propionic acid (16)

Compound **16** was obtained by step B of the method used in the preparation of **30** except that **39** was used instead of (2,4-dioxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-5-phenyl-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid *tert*-butyl ester (83%). ^{1}H NMR ($CDCl_3$) 8.79 (1H, br s), 7.41 (1H, m), 7.40–7.28 (5H, m), 7.10 (1H, m), 6.94 (1H, m), 4.85 (1H, d), 4.68 (1H, d), 4.15 (2H, s), 3.89 (1H, m), 2.90 (2H, m), 2.62 (2H, m), 2.20 (1H, m), 1.79–1.39 (6H, m), 1.34–1.27 (12H, m). The compound was further characterized as the *N*-methyl-D-glucamine salt. HRMS (ES, $[M+H]^+$) calcd for $C_{31}H_{38}N_4O_6{\cdot}C_7H_{17}NO_5$ 758.3971, found 758.3964.

5.29. (3-Amino-phenylsulfanyl)-acetic acid benzyl ester (35j)

Step A. A mixture of *N*-*tert*-butoxycarbonyl 3-aminothiophenol[36] (2.7 g, 12 mmol), benzyl bromoacetate (3.0 g, 13 mmol) and K_2CO_3 (2.1 g, 15 mmol) in acetone (40 mL) was stirred at ambient temperature for 72 h. Diethyl ether was added and the insoluble material removed by filtration. The filtrate was evaporated and chromatography (Et_2O/pentane (1:3)) of the residue afforded benzyl-3-(*N*-*tert*-butyloxycarbonylamino)phenylthioacetate as a white solid (2.67 g, 60%). ^{1}H NMR ($CDCl_3$) 7.34–7.18 (8H, m), 7.05 (1H, m), 6.34 (1H, br s), 5.16 (2H, s), 3.68 (2H, s), 1.53 (9H, s).

Step B. The product of the previous step (2.67 g, 7.2 mmol) in TFA (25 mL) was stirred at ambient temperature for 1 h. After removal of the volatiles in vacuo, the resulting oil was dissolved in DCM (60 mL), washed with a saturated $NaHCO_3$ solution (2× 60 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent afforded **35j** as an oil (1.76 g, 90%). ^{1}H NMR ($CDCl_3$) 7.34–7.18 (7H, m), 6.72–6.81 (2H, m), 6.54 (1H, br s), 5.18 (2H, s), 3.62 (2H, s).

5.30. (3-(2-(1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenylsulfanyl)-acetic acid benzyl ester (40)

Compound **40** was obtained by the method used in the preparation of **9** except that **32** and **35j** were used instead of **30** and **35a**, respectively (89%). ^{1}H NMR ($CDCl_3$) 8.33 (1H, br s), 7.40–7.30 (10H, m), 7.27–7.05 (3H, m), 5.14 (2H, s), 4.74 (2H, m), 4.16 (2H, m), 4.05 (1H, m), 4.00 (2H, s), 2.20–1.58 (12H, m), 1.29 (12H, m).

5.31. (3-(2-(1-Cycloheptyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetylamino)-phenylsulfanyl)-acetic acid (17)

Compound **40** (0.12 g, 0.17 mmol) was dissolved in MeOH (5 mL) and 1 N NaOH (0.25 mL, 0.25 mmol) and after stirring at ambient temperature for 16 h 10% $KHSO_4$ solution (2 mL) added. The resulting white precipitate was collected by filtration, then dissolved in DCM (30 mL) and dried ($MgSO_4$). Filtration and evaporation of the solvent and trituration of the residue with hexanes afforded **17** as a white solid, which was isolated by filtration and dried in vacuo (0.08 g, 77%). ^{1}H NMR ($CDCl_3$) 8.50 (1H, br s), 7.39–7.28 (6H, m), 7.15–7.05 (3H, m), 4.80 (1H, m), 4.70 (1H, m), 4.17 (2H, s), 4.03 (1H, m), 3.62 (2H, s), 2.29–1.56 (12H, m), 1.26 (12H, m). The compound was further characterized as the *N*-methyl-D-glucamine salt. Anal. Found: C, 53.52; H, 6.97; N, 7.89%; $C_{31}H_{38}N_4O_6S{\cdot}C_7H_{17}NO_5{\cdot}3.5H_2O$ requires: C, 53.51; H, 7.33; N, 8.21%.

5.32. (5-Cycloheptyl-2,4-dioxo-3-(*m*-tolylcarbamoyl-methyl)-2,3,4,5-tetrahydro-1,3,5-benzotriazepin-1-yl)-acetic acid benzyl ester (41)

Compound **41** was obtained by the method used in the preparation of **9**, except that **31** and *m*-toluidine (**35b**) were used instead of **30** and **35a**, respectively (88%). ^{1}H NMR ($CDCl_3$) 8.21 (1H, br s), 7.61–7.29 (7H, m), 7.22 (2H, m), 6.87 (1H, m), 5.19 (2H, m), 4.64 (1H, d), 4.46 (1H, d), 4.20 (2H, s), 3.98 (1H, m), 2.26 (3H, s), 2.25–1.70 (12H, m).

5.33. 2-(1-Cycloheptyl-2,4-dioxo-5-(2-oxo-2-pyrrolidin-1-yl-ethyl)-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-*N*-*m*-tolyl-acetamide (11)

Step A. (5-Cycloheptyl-2,4-dioxo-3-(*m*-tolylcarbamoyl-methyl)-2,3,4,5-tetrahydro-1,3,5-benzotriazepin-1-yl)-acetic acid was obtained by step B of the method used in the preparation of **33** except that **41** was used instead of (1-cyclohexyl-5-(3,3-dimethyl-2-oxo-butyl)-2,4-dioxo-1,2,4,5-tetrahydro-1,3,5-benzotriazepin-3-yl)-acetic acid benzyl ester (48%). ^{1}H NMR ($CDCl_3$) 8.31 (1H, br s), 7.38–7.29 (3H, m), 7.23–7.14 (4H, m), 6.87 (1H, m), 4.52 (2H, m), 4.20 (2H, m), 4.02 (1H, m), 2.25 (3H, m), 2.21–1.40 (12H, m).

Step B. Compound **11** was obtained by the method used in the preparation of **9**, except that the product of the previous step and pyrrolidine were used instead of **30** and **35a**, respectively (91%). ^{1}H NMR ($CDCl_3$) 8.24 (1H, br s), 7.41–7.14 (7H, m), 6.86 (1H, m), 4.48 (2H, s), 4.21 (2H, m), 4.04 (1H, m), 3.49 (4H, m), 2.35 (3H, s), 2.30–1.51 (16H, m). HRMS (ES, $[M+H]^+$) calcd for $C_{30}H_{37}N_5O_4$ 532.2918, found 532.2921.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.12.047.

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