Bioorganic & Medicinal Chemistry 19 (2011) 1802–1815

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and biological evaluation of 1,4-benzodiazepin-2-ones with antitrypanosomal activity

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ARTICLE INFO

Article history:
Received 3 November 2010
Revised 23 December 2010
Accepted 5 January 2011
Available online 14 January 2011

Keywords:
Parallel synthesis
Microwaves
Benzodiazepines
Trypanosoma

ABSTRACT

A library of 1,4-benzodiazepines has been synthesized and evaluated against *Trypanosoma brucei*, a causative parasite of Human African trypanosomiasis. Benzodiazepines possessing a P2- transporter motif were found to have MIC values as low as 0.78 μM.

1. Introduction

Human African Trypanosomiasis (HAT or sleeping sickness) is caused by two subspecies of the parasite *T. brucei*, namely *Trypanosoma brucei rhodesiense* (causing East African sleeping sickness) or *Trypanosoma brucei gambiense* (causing West African sleeping sickness).[1–5] Approximately 50,000 people are reported with this disease annually although over 300,000 are infected but have not been diagnosed or treated.

Current therapies for HAT have been in use for several decades (Fig. 1)[1–5] and, not surprisingly, have many shortcomings viz. high toxicity, prohibitive costs, undesirable routes of administration as well as poor efficacy. Melaminophenyl arsenicals, for example, melarsoprol, and diamidines, including pentamidine, possess amino-purine transporter (termed P2-) recognition motifs to facilitate access to the parasite; loss of this transporter accounts for added complications of drug resistance.[5]

There is an urgent need for more effective treatments for HAT. We recently reported a series of 1,4-benzodiazepin-2,5-diones (Fig. 2) structurally related to the paullone nucleus,[6] which inter alia were tractable for parallel synthesis, drug discovery and lead generation with antileishmanial and antitrypanosomal activities.[7] Given the structural similarity of this series with 1,4-benzodiazepine-2-ones[8] (BZDs), we will describe the synthesis and biological evaluation of a library of the latter and their evaluation as antitrypanosomal agents.

2. Results and discussion

C3 and C5 substituted BZDs **5**–**12** were synthesised from readily available 2-amino phenylketones **1**–**4** in a three step process, often without the need for purification.[8,9] The nitro analogue **14** was synthesised by treatment of **13** with KNO_3 in concentrated H_2SO_4 (Scheme 1, Table 1). N1-Substituted BZD analogues **17**–**40** were synthesised by treating BZDs **5**–**16** with sodium hydride and alkylation with the appropriate alkyl or benzyl halide.

All of the synthesised compounds were screened for antitrypanosomal activity against the bloodstream form of *T. b. brucei* strain S247 using the alamar blue assay technique. Minimum inhibitory concentrations (MIC) were determined by calculation of the percentage of control values and confirmed by microscopic examination. Preliminary biological evaluation of the parent N1 unsubstituted BZDs was performed and the following SAR was noted (Table 1):

Substitution at the *C*3 position is important for biological activity with the (*S*)-benzyl substituted compound **8** displaying an MIC value of 6.25 μM as opposed to 400 μM for the Valium-like **5** (entries 4 and 1, respectively).

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doi:10.1016/j.bmc.2011.01.010

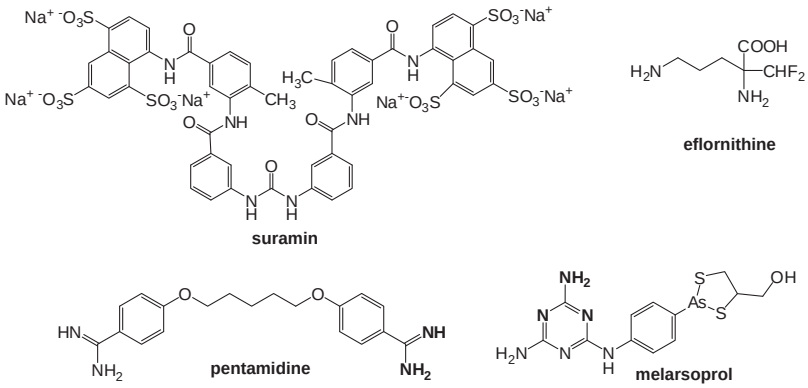
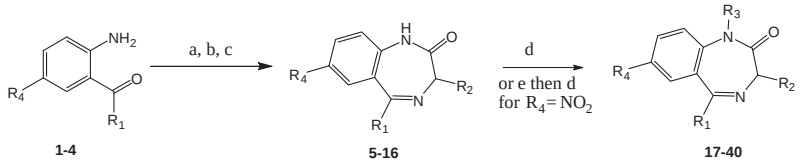


Figure 1. The structures of HAT acting agents.



Scheme 1. Reagents and conditions: (a) Boc-NHCH(R_2)CO_2H, EEDQ, DCM, rt; (b) TFA, DCM, rt; (c) CH_3COONH_4, AcOH, rt; (d) NaH, R_3Br or MeI, DMF; (e) KNO_3, H_2SO_4, 0 °C.

Table 1
Synthesised BZDs

Entry	BZD	R_1	R_2	R_3	R_4	MIC (μM)[e]
1	**5**	Ph	H	H	H	400
2	**6**	Ph	(*S*)-*i*Pr	H	H	100
3	**7**	Ph	(*R*)-*i*Pr	H	H	200
4	**8**	Ph	(*S*)-Bn	H	H	6.25
5	**9**	Ph	(*R*)-Bn	H	H	50
6	**10**	Ph	(*S*)-CH_2OBn	H	H	Nt[a]
7	**11**	*i*Pr	H	H	H	>400
8	**12**	*i*Pr	(*S*)-Bn	H	H	50
9	**13**	*c*-C_6H_{11}	(*S*)-*i*Pr	H	H	25
10	**14**	*c*-C_6H_{11}	(*S*)-*i*Pr	H	NO_2	12.5
11	**15**	*c*-C_6H_{11}	(*S*)-Bn	H	H	12.5
12	**16**	2-Py[b]	(*S*)-Bn	H	H	100
13	**17**	Ph	H	Me	H	nt
14	**18**	Ph	(*S*)-*i*Pr	Me	H	100
15	**19**	Ph	(*S*)-*i*Pr	Bn	H	200
16	**20**	Ph	(*S*)-*i*Pr	2-CH_2BiPh[c]	H	12.5
17	**21**	Ph	(*S*)-*i*Pr	8-CH_2Quin[d]	H	25
18	**22**	Ph	(*R*)-*i*Pr	Me	H	200
19	**23**	Ph	(*R*)-*i*Pr	Bn	H	12.5
20	**24**	Ph	(*S*)-Bn	Me	H	50
21	**25**	Ph	(*S*)-Bn	Bn	H	6.25
22	**26**	Ph	(*S*)-Bn	4-CH_2BiPh	H	6.25
23	**27**	Ph	(*S*)-Bn	3-CH_2BiPh	H	25
24	**28**	Ph	(*S*)-Bn	2-CH_2BiPh	H	6.25
25	**29**	Ph	(*S*)-Bn	8-CH_2Quin	H	25
26	**30**	Ph	(*S*)-Bn	CH_2CN	H	3.10
27	**31**	Ph	(*S*)-Bn	CH_2COOBn	H	nt
28	**32**	Ph	(*S*)-Bn	CH_2COOH	H	50
29	**33**	Ph	(*R*)-Bn	Me	H	400
30	**34**	Ph	(*R*)-Bn	Bn	H	12.5
31	**35**	Ph	(*S*)-CH_2OBn	4-CH_2BiPh	H	25
32	**36**	Ph	(*S*)-CH_2OBn	3-CH_2BiPh	H	25
33	**37**	Ph	(*S*)-CH_2OBn	8-CH_2Quin	H	12.5
34	**38**	*i*Pr	H	Me	H	400
35	**39**	*i*Pr	(*S*)-Bn	Me	H	25
36	**40**	*c*-C_6H_{11}	(*S*)-Bn	Bn	H	6.25

[a] nt = not tested.
[b] Py = pyridine.
[c] BiPh = biphenyl.
[d] Quin = quinoline.
[e] Mean value from duplicate assays.

Chirality at *C*3 appears to be important for biological activity with **9** displaying an eightfold decrease in activity compared with its enantiomer **8** (entries 5 and 4, respectively).

Replacing the R_1 = phenyl group at the *C*5 position with a more bulky lipophilic group cyclohexyl, as in **15**, leads to a slight drop in biological activity (entry 11). However, analogues with R_1 = 2-pyridyl **16** and R_1 = isopropyl **12** displayed poor antitrypanosomal activity (entries 12 and 8, respectively).

From these preliminary findings we considered **8** as a lead compound and made analogues based on R_1 = Ph and R_2 = (*S*)-Bn (Table 1). N1-Methylation of **8** as in **24** (Entry 20) leads to a drop in biological activity although N1-benzylation, as in **25** (entry 21), maintains the original activity of **8** indicating that there is space at *N*1 for relatively large substituents, further corroborated by the low MIC values for the 4- and 2-biphenylmethyl analogues[10] **26** and **28** (entry 22 and 24). We are, at present, unable to explain the unusually high MIC value for the positional isomer **27** (entry 23).

Compounds **24**, **26**, **28**, **30** and **33** have been characterised in the solid state by single crystal X-ray diffraction studies. The crystal structures of the two BZDs with large R_3 = biphenylmethyl substituents, **26** and **28** are shown in Figure 3 while the details of all the crystallographic studies are given in the Supplementary data. The geometry of the BZD portions of the five structures is similar and falls within the normal ranges for these parameters (Table S2). Despite the presence of several aromatic rings in these structures no π–π stacking interactions are observed. As can be seen in Figure 3 the N1 position in **26** and **28** is indeed congested. **26** is the only

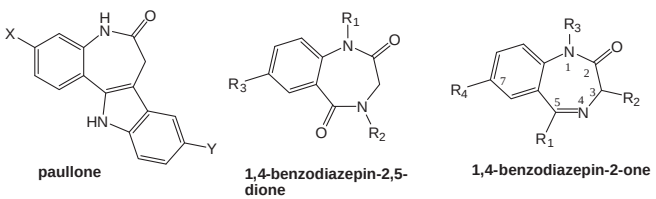


Figure 2. Structures of paullones and benzodiazepines.

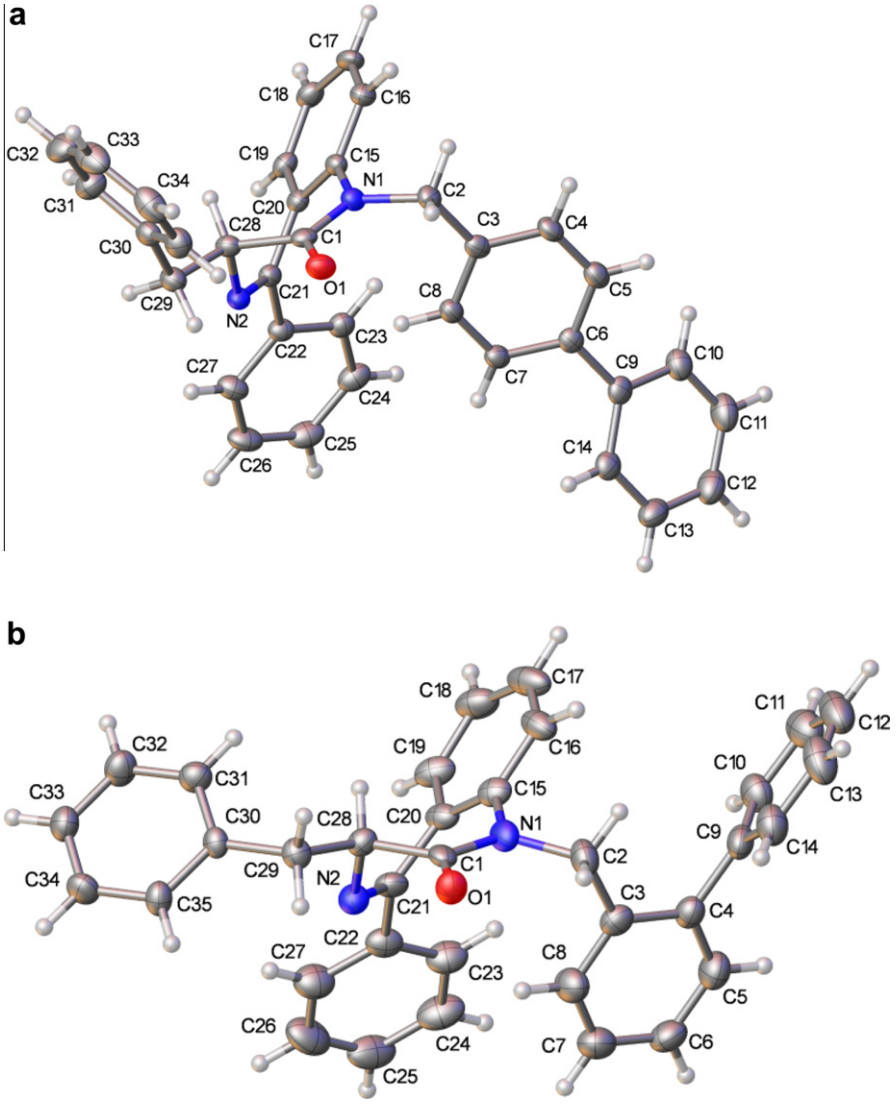


Figure 3. Solid state structures, with ellipsoids drawn at the 50% probability level, of: (a) **26** (the chloroform molecule has been omitted for clarity), (b) **28**.

one of the five structures that incorporates a solvent molecule, chloroform, in the crystal lattice. The chloroform molecules form a column about a 2_1 screw axis running through the centre of the unit cell in the (0 1 0) direction (Fig. S3).

Next, we synthesised a number of R_3 = nitrobenzyl analogues based around **25** with the intention of reducing the nitro group to an amine in order to synthesise guanidine derivatives to incorporate putative P2- binding motifs in our BZD library. The 4-nitro derivative **41** was used as a test substrate in order to establish the best reducing agent (Table 2). All selected agents gave excellent yields of the amine **49** (Scheme 2).

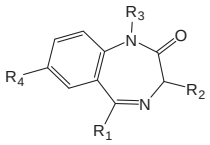
The nitro compounds **41**–**48** and **14** were reduced to their corresponding anilines **49**–**56** and **57**, respectively, mainly by molybdenum hexacarbonyl and DBU at 150 °C for 15 min in a microwave[13] (see Section 4) and other analogues were reduced by a simple flow cell reduction due to convenience of the work up (Table 2).

MIC values were obtained for the amines and echoed much of the SAR observed for **25**, that is, a benzyl group is tolerated although MIC values hit a plateau of 6.25 μM. We decided against testing nitro compounds due to expected toxicity and insolubility problems. *Boc*-protected guanidines were synthesised from their corresponding amine substituted BZDs **49–56** and **57** using $HgCl_2$ and 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea. Deprotection with 4 M HCl–dioxane, afforded the guanidine containing BZDs **58–66**. Compound **67** was synthesized from a Pbf-protected arginine analogue using a Fmoc coupling protocol followed by deprotection using a scavenger cocktail mixture of TFA/H_2O/TIS.

Gratifyingly, the introduction of a guanidine group (Table 3), a known P2- recognition motif, led to an approximate eightfold increase in trypanocidal activity comparing **58** to the parent benzodiazepine **8**. This may be due to the guanidine groups being recognised by P2- aminopurine transporters, which improves the uptake of the compounds into the trypanosomes thus exhibiting improved activity. However, the important observation from the series depicted in Table 3, is that analogues with a (*S*)-benzyl group at the *C*3 position are superior in activity to other analogues. The disubstituted **62** with an (*S*)-isopropyl substituent at the *C*3 position also shows good activity with an MIC of 0.78 μM similar to that of the (*S*)-benzyl substituted **58**, **59** and **63**. This could be due to the presence of two guanidine motifs that are recognised by P2- aminopurine transporters, which enhances the uptake. This explanation is corroborated by comparing **62** with derivative **61** (MIC = 6.25 μM) which has one guanidine motif.

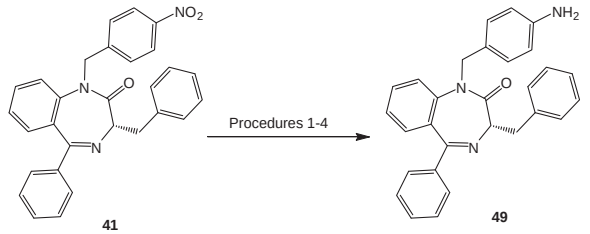
Finally, the N1-substituted carboxylic acid derivative **32** was synthesised via deprotection of the benzyl ester **31** (Scheme 3 and Table 1). Amide couplings were performed by treatment of

Table 2
Nitro and amine-substituted BZDs



BZD	R_1	R_2	R_3	R_4	MIC[a] (μM)
41	Ph	(*S*)-Bn	4-$NO_2C_6H_4CH_2$	H	nt
42	Ph	(*S*)-Bn	3-$NO_2C_6H_4CH_2$	H	nt
43	Ph	(*S*)-Bn	2-$NO_2C_6H_4CH_2$	H	nt
44	*c*-C_6H_{11}	(*S*)-*i*Pr	4-$NO_2C_6H_4CH_2$	H	nt
45	*c*-C_6H_{11}	(*S*)-*i*Pr	4-$NO_2C_6H_4CH_2$	NO_2	nt
46	*c*-C_6H_{11}	(*S*)-Bn	4-$NO_2C_6H_4CH_2$	H	nt
47	*c*-C_6H_{11}	(*S*)-Bn	3-$NO_2C_6H_4CH_2$	H	nt
48	2-Py	(*S*)-Bn	4-$NO_2C_6H_4CH_2$	H	nt
49	Ph	(*S*)-Bn	4-$NH_2C_6H_4CH_2$	H	12.5
50	Ph	(*S*)-Bn	3-$NH_2C_6H_4CH_2$	H	6.25
51	Ph	(*S*)-Bn	2-$NH_2C_6H_4CH_2$	H	6.25
52	*c*-C_6H_{11}	(*S*)-*i*Pr	4-$NH_2C_6H_4CH_2$	H	6.25
53	*c*-C_6H_{11}	(*S*)-*i*Pr	4-$NH_2C_6H_4$-CH_2	NH_2	6.25
54	*c*-C_6H_{11}	(*S*)-Bn	4-$NH_2C_6H_4CH_2$	H	6.25
55	*c*-C_6H_{11}	(*S*)-Bn	3-$NH_2C_6H_4CH_2$	H	6.25
56	2-Py	(*S*)-Bn	4-$NH_2C_6H_4CH_2$	H	25
57	*c*-C_6H_{11}	(*S*)-*i*Pr	H	NH_2	100

[a] Mean value from duplicate assays.



Procedure	Yield of 49 (%)[e)]
1[a)]	>90
2[b)]	>90
3[c)]	>90
4[d)]	>90

Scheme 2. Yields of amine **49** using different reducing conditions. Reagents and conditions: (a) MW/DBU (3 equiv), $Mo(CO)_6$, EtOH, 15 min, 150 °C; (b) H-Cube/Raney Ni, full H_2 mode, 60 °C, flow rate 1 mL/min;[11,12] (c) MW/$SnCl_2$. H_2O (5 equiv), 30 min, 130 °C, EtOH, followed by basic work-up;[13] (d) Overnight reflux/$SnCl_2{\cdot}2H_2O$ (5 equiv), 90 °C, EtOH followed by basic work-up; (e) by ^1H NMR analysis.

31 with the requisite piperazine analogues using HATU, EDCI and DMAP in DMF. BZDs bearing an *N*1 piperazinyl ethanone group capped with a pyridine or pyrimidine groups were synthesised as potential guanidine bioisosteric P2- transporter motifs. Unfortunately, neither the 2-pyrimidyl or pyridyl analogues **68** and **69**, respectively, displayed any improvement in activity on **8** and had similar MIC values to the unsubstituted terminal piperazine **70**.

We have also investigated the transport of these BZDs in different *T. brucei* strains and the SAR echoes what we previously found (vide infra). Hence, the parent compound **8** exhibited relatively weak trypanocidal activity against the *T. brucei* wild-type whereas the addition of a guanidine moiety appears to improve the efficacies of subsequent derivatives **58**, **59** and **64** by approximately 100-fold (Fig. 4a). The increase in efficacy also appears to be influenced by the positioning of the guanidine group on its benzyl linker where the *meta*- substituted analogues **59** and **64** showed slightly better trypanocidal activity compared to their *para*- counterpart, **58**, most probably due to better binding to transporter(s) during uptake into cells.

The compounds were also tested against two other cell lines, *TbAT1*$^{-/-}$ and B48 of which both have lost the putative P2- transporter whilst B48 has lost an additional high affinity pentamidine transporter (HAPT1) under drug pressure selection.[13] The absence of one or both transporters renders the mutant parasites resistant toward compounds with an amidine moiety such as melarsoprol and the diamidines. Surprisingly, **8** (parent compound) appeared to be more toxic towards *TbAT1*$^{-/-}$ and B48 compared to the 427 wild-type (IC_{50} values of 7.84 and 9.23 μM, respectively, Fig. 4b and c). It is highly possible that the benzodiazepine has other route(s) of entry, for example, diffusion and/or membrane-lipid interaction which allows its uptake in the absence of the P2- and HAPT1 transporters. Upon addition of the guanidine moiety, the subsequent benzodiazepine derivatives **58**, **59** and **64** were thought to gain cell entry via the P2- and/or HAPT1 transporter(s) as shown with the diminution of their trypanocidal activities against the mutant parasites, albeit all IC_{50} values were still within the low micromolar range, that is, more efficacious than parent compound without the guanidine moiety (Table 4).

3. Conclusion

A library of BZDs has been synthesised and shown to have moderate to good trypanocidal activity. The presence of a guanidine moiety often confers substantial trypanocidal activity to the benzodiazepine although the introduction of such a potential P2- transporter motif is not a guaranteed prerequisite for trypanocidal activity since **67** exhibited poor HAT activity. Although the uptake of guanidine-benzodiazepine derivatives (**8**, **58**, **64**) into trypanosomes appeared to occur via the P2- and/or HAPT1 transporters, of which one or both are absent in mutant parasite strains *TbAT1*$^{-/-}$ and B48, the diminution of trypanocidal activity is outweighed by the better efficacy compared to the parent compound **8** without guanidine.[14,15] It is also possible that the BZDs have other route(s) for cell entry as shown by **8** which was more efficacious against *TbAT1*$^{-/-}$ and B48 compared to the 427 wild-type. At present, the mode of action of our BZDs is unknown and investigations are ongoing.

4. Experimental

NMR, MS and general experimental details are as outlined in previous publications.[8a] DCM = dichloromethane.

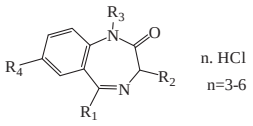
4.1. Assays

The MIC is the lowest drug concentration that inhibits growth or viability of the parasite. Suramin was assayed as a positive control alongside the compounds against *T. b. brucei* to ascertain the reliability of the assay conducted and an MIC of 0.13 μM was obtained. This activity is comparable to that reported by Raz *et al.* against *T. b. rhodesiense* and *T. b. gambiense*. It is worth noting that there are variations in bioactivity of compounds from strain to strain.[14]

4.2. In vitro

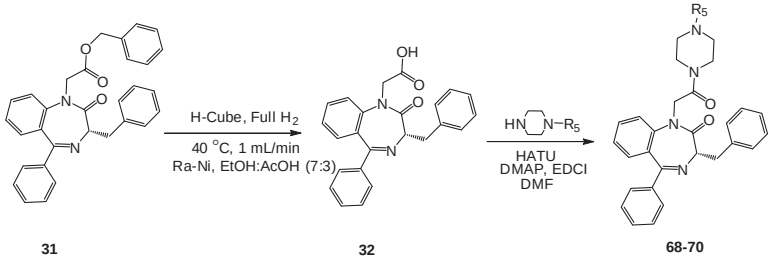
Toxicity assay of **8** and derivatives against *T. b. brucei* 427 wild-type, *TbAT1*$^{-/-}$ and B48.

Table 3
Guanidine-substituted BZDs



BZD	R_1	R_2	R_3	R_4	MIC[a] (μM)
58	Ph	(*S*)-Bn	4-$CH_2C_6H_4$–NHC(=NH)NH_2	H	0.78
59	Ph	(*S*)-Bn	3-$CH_2C_6H_4$–NHC(=NH)NH_2	H	0.78
60	Ph	(*S*)-Bn	2-$CH_2C_6H_4$–NHC(=NH)NH_2	H	1.56
61	*c*-C_6H_{11}	(*S*)-*i*Pr	4-$CH_2C_6H_4$–NHC(=NH)NH_2	H	6.25
62	*c*-C_6H_{11}	(*S*)-*i*Pr	4-$CH_2C_6H_4$–NHC(=NH)NH_2	NHC (=NH)NH_2	0.78
63	*c*-C_6H_{11}	(*S*)-Bn	4-$CH_2C_6H_4$–NHC(=NH)NH_2	H	0.78
64	*c*-C_6H_{11}	(*S*)-Bn	3-$CH_2C_6H_4$–NHC(=NH)NH_2	H	1.56
65	2-Py	(*S*)-Bn	4-$CH_2C_6H_4$–NHC(=NH)NH_2	H	3.13
66	*c*-C_6H_{11}	(*S*)-*i*-Pr	H	NHC (=NH)NH_2	1.56
67	Ph	$CH_2CH_2CH_2NHC(=NH)NH_2$	H	H	400

[a] Mean value from duplicate assays.



BZD	R_5	MIC (μM)[a]
68		12.5
69		6.25
70	H	12.5

Scheme 3. Synthesis of alternative potential P2- transporter motifs.

4.3. Method

T. b. brucei wild-type (s427), *TbAT1*$^{-/-}$ and B48 cell lines were cultured to the optimum density of 1–2 × 10^6 cells mL^{-1} in HMI-9 supplemented with 10% foetal calf serum under environmental conditions of 37 °C and 5% CO_2. The same strain was used for MIC determinations. Solutions of test compounds were prepared in culture media at stock concentration of 200 μM and diluted serially (1:2) across the 96-well, flat-bottom solid white plates to give a total of 11 decreasing concentrations (100 μL $well^{-1}$). The last well of each series was left blank, that is, 'drug free' (negative control). Each cell line was prepared at the concentration of 4 × 10^4 cells mL^{-1} and added to the respective compound series (100 μL $well^{-1}$) separately. Plates were incubated at 37 °C/5% CO_2 for 48 h prior to addition of Alamar Blue solution (20 μL $well^{-1}$, 0.49 mM in 1X PBS, pH 7.4) followed by another 24-h incubation. Assay end points were measured fluorometrically with the fluorescence spectrometer (FluoStar, BMG LabTech, Germany) and Optima programme set at $\lambda_{excitation}$ 544 nm and $\lambda_{emission}$ 590 nm. Data was analysed using Prism 5.0 software to obtain IC_{50} values. The experiment was performed in duplicate and repeated three times.

4.4. 1-(2-Aminophenyl)-2-methylpropan-1-one (2)

Under an N_2 atmosphere, isopropyl magnesium chloride (2 M in Et_2O; 100 mL, 200.00 mmol) was added dropwise to a solution of 2-aminobenzonitrile (11.80 g, 100.00 mmol) in Et_2O (100 mL) at 0 °C. After stirring for 16 h at rt, the reaction mixture was cooled to −60 °C by carefully adding liquid nitrogen to the external bath. 5 N HCl (100 mL) was added dropwise to the reaction mixture while stirring. After allowing the reaction mixture to reach rt the aqueous layer was separated and the pH altered to 10, by carefully adding NaOH. The resulting mixture was extracted with ethyl acetate (100 mL), washed with brine (100 mL × 2) and dried ($MgSO_4$). Filtration and concentration under reduced pressure afforded a yellow solid (5.15 g, 45%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.75 (1H, d); 7.24 (1H, t); 6.63 (2H, m); 6.27 (2H, br s); 3.57 (1H, m); 1.19 (6H, d).

4.5. (2-Aminophenyl)(cyclohexyl)methanone (3)

This was synthesised on a 50.00 mmol scale by the same method used to obtain **2** but cyclohexane magnesium chloride (2 M in Et_2O; 50 mL) was used instead of isopropyl magnesium chloride

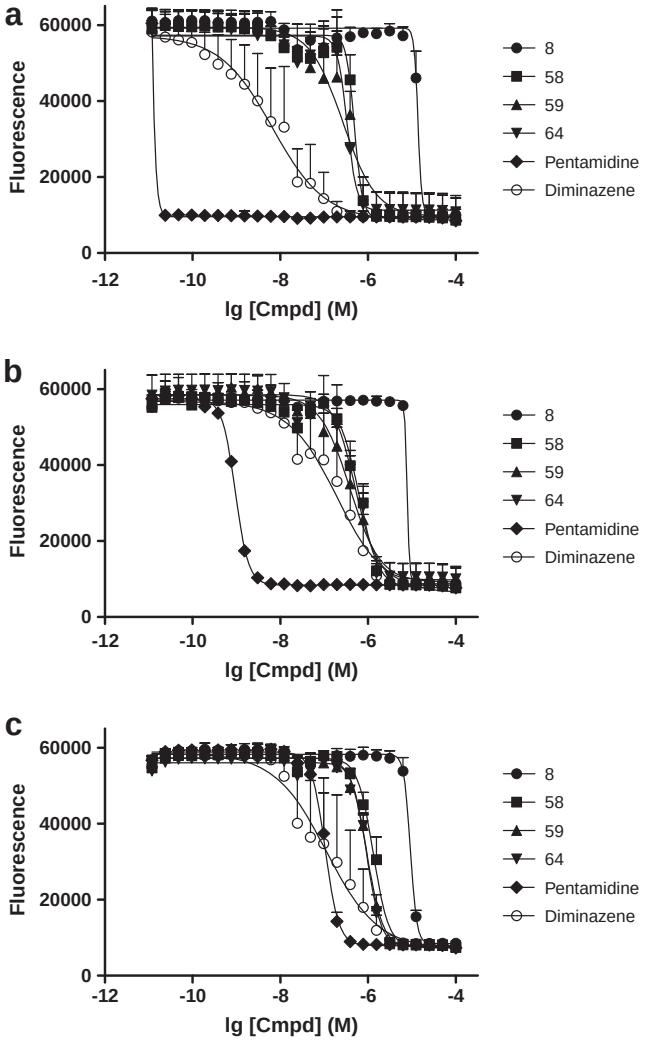


Figure 4. In vitro toxicity profiles of compounds against (a) 427 wild-type, (b) TbAT1$^{-/-}$ and (c) B48. Bars represent SEMs.

(Brown solid; 34%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.73 (1H, d); 7.22 (1H, t); 6.62 (2H, m); 6.27 (2H, br s); 3.25 (1H, m); 1.86–1.30 (10H, m).

4.6. (2-Aminophenyl)(pyridin-2-yl)methanone (4)

n-Butyl lithium (46.50 mL, 74.50 mmol) was added to mixture of 2-aminobenzonitrile (4.00 g, 33.85 mmol) and bromopyridine (9.10 g, 57.55 mmol) in toluene (~100 mL) under nitrogen at −50 °C. The reaction mixture was stirred for 1 h at ~−50 °C and allowed to warm to 0 °C over 1 h. 3 N HCl (100 mL) was added with cooling such that the reaction temperature remained <10 °C. The organic layer was separated and re-extracted with 3 N HCl (100 mL). The combined acid extracts were washed with toluene (50 mL), then carefully rebasified with NaOH (~50 mL) to pH 10 and kept in the fridge overnight, which led to the formation of a brown precipitate, which was collected by filtration and washed with water (100 mL × 3) and air-dried (3.23 g, 45%). Used as crude.

4.7. 5-Phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (5)

2-Aminobenzophenone **1** (4.94 g, 25.00 mmol), EEDQ (6.18 g, 25.00 mmol) and *Boc*-Gly-OH (4.38 g, 25.00 mmol) were combined in DCM (20 mL) and stirred overnight at rt. The organic layer was washed successively with HCl (40 mL, 10% solution) and saturated Na_2CO_3 (100 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was dissolved in TFA (30 mL) and DCM (20 mL) and stirred for 2.5 h at rt. After concentration, the resulting oil was dissolved in DCM (20 mL) and reconcentrated under reduced pressure then redissolved in DCM (20 mL). The organic layer was washed with saturated Na_2CO_3 (100 mL), dried ($MgSO_4$ as above) and concentrated. Thereafter, to the resulting oil, ammonium acetate (9.64 g, 125.00 mmol) and AcOH (30 mL) were added and the reaction mixture was stirred at rt overnight and then concentrated under reduced pressure to afford an oil. The latter was dissolved in ethyl acetate (30 mL) and washed with saturated Na_2CO_3 (3 × 40 mL). The organic layer was dried over $MgSO_4$ and filtered. After concentration of the solvent, Et_2O (10 mL) was added and the product precipitated as a white powder (2.32 g, 39%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 9.00 (1H, br s); 7.53–7.10 (9H, m); 4.31 (2H, br s). HRMS: *m*/*z* calcd for $C_{15}H_{13}N_2O$ $[M+H]^+$: 237.1022, found: 237.1020.

4.8. (*S*)-3-Isopropyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (6)

This was synthesised on a 10.00 mmol scale by the same method used to obtain **5** but instead of *Boc*-Gly-OH, L-*Boc*-Val-OH (2.47 g, 10.00 mmol) was used. Purification with flash chromatography (10:1:1 DCM/ethyl acetate/hexane) gave an orange solid (71%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 10.26 (1H, br s); 7.50–7.02 (9H, m); 3.10 (1H, d); 2.78 (1H, m); 1.21 (3H, d); 1.11 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.7 (C2); 167.7 (C5); 144.0 (ArC); 139.0 (ArC); 131.2 (ArC); 130.3 (ArC); 130.2 (ArC); 129.7 (2 × ArC); 129.3 (ArC); 128.2 (2 × ArC), 123.7 (ArC); 121.4 (ArC); 69.8 (C3); 35.1 (C16); 20.4 (C17); 19.3 (C18). HRMS: *m*/*z* calcd for $C_{18}H_{19}N_2O$ $[M+H]^+$: 279.1492, found: 279.1489. IR (ATR, cm^{-1}): 3204 (NH); 1682 (C=O); 1607 (C=N). Anal. Calcd for $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.66; H, 6.59; N, 10.50.

4.9. (*R*)-3-Isopropyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (7)

This was synthesised on a 10.00 mmol scale by the same method used to obtain **5** but instead of *Boc*-Gly-OH, D-*Boc*-Val-OH (2.47 g, 10.00 mmol) was used. Purification with flash chromatography (10:1:1 DCM/ethyl acetate/hexane) gave an orange solid (70%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 10.26 (1H, br s); 7.50–7.02 (9H, m); 3.10 (1H, d); 2.78 (1H, m); 1.21 (3H, d); 1.11 (3H, d).

Table 4
IC_{50} values of compounds against respective cell types and the calculated resistant factors in relative to 427 wild-type

BZD	IC_{50} (μM)			Resistant factor	
	427 wild-type (w.t.)	*TbAT1*$^{-/-}$	B48	*TbAT1*$^{-/-}$ versus w.t.	B48 versus w.t.
8	13.84 ± 0.08	7.84 ± 0.17	9.23 ± 0.01	0.57	0.67
58	0.49 ± 0.02	0.64 ± 0.02	1.31 ± 0.01	1.29	2.68
59	0.27 ± 0.04	0.39 ± 0.03	0.94 ± 0.01	1.45	3.52
64	0.33 ± 0.03	0.51 ± 0.03	0.93 ± 0.01	1.53	2.80

HRMS: *m*/*z* calcd for $C_{18}H_{19}N_2O$ $[M+H]^+$: 279.1492, found: 279.1489.

4.10. (*S*)-3-Benzyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (8)

This was prepared on 18.86 mmol scale by the same method used to obtain **5** but instead of *Boc*-Gly-OH, L-*Boc*-Phe-OH (3.72 g, 18.86 mmol) was used. White powder precipitated from Et_2O (47%). ^1H NMR (270 MHz) $CDCl_3$: δ 8.87 (1H, br s); 7.51–7.08 (14H, m); 3.81 (1H, t); 3.58 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 172.0 (C2); 169.4 (C5); 139.5 (ArC); 139.4 (ArC); 138.5 (ArC); 131.7 (ArC); 131.3 (ArC); 130.3 (ArC); 130.0 (2 × ArC); 129.9 (2 × ArC), 128.3 (2 × ArC), 128.2 (2 × ArC); 127.6 (ArC); 126.3 (ArC); 123.3 (ArC); 121.3 (ArC); 64.9 (C3); 37.8 (C16). HRMS: *m*/*z* calcd for $C_{22}H_{19}N_2O$ $[M+H]^+$: 327.1492, found: 327.1490. IR (ATR, cm^{-1}): 3209 (NH); 1678 (C=O); 1601 (C=N). Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58. Found: C, 80.69; H, 5.58; N, 9.03.

4.11. (*R*)-3-Benzyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (9)

This was synthesised on a 18.86 mmol scale by the same method used to obtain **5** but instead of *Boc*-Gly-OH, D-*Boc*-Phe-OH (3.72 g, 18.86 mmol) was used. White powder precipitated from Et_2O (59%). ^1H NMR (270 MHz) $CDCl_3$: δ 9.04 (1H, br s); 7.51–7.07 (14H, m); 3.81 (1H, t); 3.58 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 172.0 (C2); 169.4 (C5); 139.5 (ArC); 139.4 (ArC); 138.5 (ArC); 131.7 (ArC); 131.3 (ArC); 130.3 (ArC); 130.0 (2 × ArC); 129.9 (2 × ArC), 128.3 (2 × ArC), 128.2 (2 × ArC); 127.6 (ArC); 126.2 (ArC); 123.3 (ArC); 121.3 (ArC); 64.9 (C3); 37.8 (C16). HRMS: *m*/*z* calcd for $C_{22}H_{19}N_2O$ $[M+H]^+$: 327.1492. Found: 327.1489. IR (ATR, cm^{-1}): 3209 (NH); 1678 (C=O); 1601 (C=N). Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58. Found: C, 80.60; H, 5.59; N, 9.12.

4.12. 5-Isopropyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (11)

This was synthesised on a 5.00 mmol scale by the same method used to obtain **5**. Purification with flash chromatography (10:1 DCM/acetone) gave a yellow solid (22%). ^1H NMR (270 MHz) $CDCl_3$: δ 10.06 (1H, br s); 7.43 (1H, d); 7.27 (1H, q); 7.04 (2H, t); 3.95 (2H, br s); 3.01 (1H, m); 0.97 (6H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 176.7 (C2); 172.7 (C5); 137.5 (ArC), 130.6 (ArC); 128.0 (ArC); 127.4 (ArC); 123.52 (ArC); 120.9 (ArC); 55.5 (C3); 35.5 (C10); 20.4 (2 × C, C11, C12). HRMS: *m*/*z* calcd for $C_{12}H_{15}N_2O$ $[M+H]^+$: 203.1179, found: 203.1178. IR (ATR, cm^{-1}): 3069 (N–H); 1676 (C=O); 1625 (C=N). Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.08; H, 7.18; N, 14.08.

4.13. (*S*)-3-Benzyl-5-isopropyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (12)

This was synthesised on a 5.00 mmol scale by the same method used to obtain **5**. Purification with flash chromatography (10:1 DCM/acetone) obtained white solid (22%). ^1H NMR (270 MHz) $CDCl_3$: δ 10.18 (1H, br s); 7.53–7.15 (9H, m); 3.67 (1H, t); 3.54 (2H, m); 3.12 (1H, m); 1.32 (3H, d). 0.95 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 174.4 (C2); 172.8 (C5); 139.3 (ArC), 137.2 (ArC), 130.6 (ArC), 129.8 (2 × ArC), 128.6 (ArC), 127.8 (2 × ArC), 127.4 (ArC), 125.8 (ArC), 123.6 (ArC), 121.1 (ArC), 63.9 (C3), 37.4 (C10), 35.6 (C17), 21.3 (C18), 19.9 (C19). HRMS: *m*/*z* calcd for $C_{19}H_{21}N_2O$ $[M+H]^+$: 293.1648, found: 293.1651. IR (KBr, cm^{-1}): 3208 (NH); 1682 (C=O); 1626 (C=N). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 77.59; H, 7.10; N, 9.93.

4.14. (*S*)-5-Cyclohexyl-3-isopropyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (13)

This was made on a 4.92 mmol by the same method used to obtain **5**. Purification with flash chromatography (20:1 DCM/acetone) gave a light yellow solid (53%). ^1H NMR (270 MHz) $CDCl_3$: δ 9.48 (1H, br s), 7.53 (1H, d); 7.36 (1H, t); 7.10 (2H, q); 2.87 (1H, d); 2.69 (1H, t); 2.52 (1H, m); 1.94–1.06 (10H, m); 0.98 (6H, q). ^{13}C NMR (75 MHz) $CDCl_3$: δ 173.5 (C2); 172.25 (C5); 137.7 (ArC); 130.7 (ArC); 128.9 (ArC); 127.6 (ArC); 123.7 (ArC); 121.3 (ArC); 68.6 (C3); 45.9 (C16); 32.0 (C10); 30.5 (C11); 28.9 (C15), 26.7 (C13); 26.2 (2 × C, C12, C14); 20.4 (C17); 18.9 (C18). HRMS: *m*/*z* calcd for $C_{18}H_{25}N_2O$ $[M+H]^+$: 285.1961, found: 285.1964 IR (cm^{-1}): 3209 (NH); 1684 (C=O); 1624 (C=N). Anal. Calcd for $C_{18}H_{24}N_2O$: C, 76.02; H, 8.51; N, 9.85. Found: C, 75.86; H, 8.67; N, 10.20.

4.15. (*S*)-5-Cyclohexyl-3-isopropyl-8-nitro-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (14)

To solution of **13** (0.12 g, 0.42 mmol) in concentrated H_2SO_4 (5 mL) was added KNO_3 (0.05 g, 0.5 mmol) in concentrated H_2SO_4 (5 mL) at 0 °C. The solution was stirred at rt for 16 h. The resulting mixture was extracted with DCM (20 mL × 2) and the combined extracts were washed with H_2O (20 mL), saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried ($MgSO_4$). After filtration, the solvent was concentrated under reduced pressure yielded yellow solid (83%). ^1H NMR (270 MHz) $CDCl_3$: δ 9.71 (1H, br s), 8.48 (1H, d); 8.28 (1H, dd); 7.23 (1H, d); 2.92 (1H, d); 2.77 (1H, t, CH, H16); 2.59 (1H, m, CH); 2.03–1.12 (10H, m, 5 × CH_2,); 1.08–1.00 (6H, dd). ^{13}C NMR (75 MHz) $CDCl_3$: δ 172.5 (C2); 172.1 (C5); 143.2 (ArC); 1426 (ArC); 128.8 (ArC); 125.6 (ArC); 123.9 (ArC); 121.9 (ArC); 68.9 (C3); 46.1 (C16); 32.1 (C10); 30.4 (C11); 28.9 (C15), 26.4 (C13); 26.0 (C12); 25.9 (C14); 20.2 (C17); 18.7 (C18). HRMS: *m*/*z* calcd for $C_{18}H_{24}N_3O_3$ $[M+H]^+$: 330.1812, found: 330.1812. IR (cm^{-1}): 1689 (C=O); 1614 (C=N); 1530 and 1341 (NO_2). Anal. Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.24; H, 7.17; N, 13.22.

4.16. (*S*)-3-Benzyl-5-cyclohexyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (15)

This was synthesised on a 4.92 mmol by the same method used to obtain **5**. Purification with flash chromatography (20:1 DCM/acetone) gave a white solid (93%). ^1H NMR (270 MHz) $CDCl_3$: δ 10.09 (1H, br s), 7.49–7.06 (9H, m); 3.61 (1H, t), 3.45 (2H, m); 2.17 (1H, t); 1.96–0.97 (10H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 174.1 (C2); 172.9 (C5); 139.6 (ArC); 137.6 (ArC); 130.8 (ArC); 130.0 (2 × ArC); 128.9 (ArC); 128.1 (2 × ArC), 127.7 (ArC), 126.1 (ArC); 123.8 (ArC); 121.4 (ArC); 64.2 (C3); 45.9 (C16); 37.7 (C10); 32.2 (C11); 30.3 (C15), 26.7 (C13); 26.2 (2 × C, C12, C14). HRMS: *m*/*z* calcd for $C_{22}H_{25}N_2O$ $[M+H]^+$: 333.1961, found: 333.1962. IR (cm^{-1}): 3206 (NH); 1681.7 (C=O); 1623.2 (C=N). Anal. Calcd for $C_{22}H_{24}N_2O{\cdot}0.1DCM$: C, 77.86; H, 7.15; N, 8.22. Found: C, 77.85; H, 7.34; N, 8.72.

4.17. (*S*)-3-Benzyl-5-(pyridin-2-yl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (16)

This was synthesised on a 5.67 mmol scale by the same method used to obtain **5**. Purification with flash chromatography (9:1 DCM/acetone) gave an off-white solid (50%). ^1H NMR (270 MHz) $CDCl_3$: δ 8.56 (1H, d); 8.16 (1H, br s); 7.96 (1H, d); 7.77 (1H, t); 7.51–7.07 (10H, m); 3.88 (1H, t); 3.58 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 170.8 (C2); 167.9 (C5); 156.7 (ArC); 148.7 (ArC); 139.2 (ArC); 138.0 (ArC); 136.7 (ArC); 131.7 (ArC); 131.7

(ArC); 129.9 (2 × ArC); 128.2 (2 × ArC); 126.6 (ArC); 126.2 (ArC); 124.4 (ArC); 124.2 (ArC); 123.3 (ArC); 121.0 (ArC); 65.1 (C3); 37.5 (C16). HRMS: *m*/*z* calcd for $C_{22}H_{18}N_3O$ $[M+H]^+$: 328.1444, found: 328.1448. IR (ATR, cm^{-1}): 3206 (NH); 1684 (C=O); 1610 and 1565 (2 × C=N). Anal. Calcd for $C_{21}H_{17}N_3O{\cdot}0.05DCM$: C, 76.24; H, 5.20; N, 12.67. Found: C, 76.03; H, 5.31; N, 12.95.

4.18. 1-Methyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (17)

Compound **5** (0.79 g, 3.30 mmol) with NaH (0.16 g, 3.95 mmol) were stirred in DMF for 1.5 h under nitrogen. Methyl iodide (308 μL, 4.90 mmol) was added to the reaction mixture, which was stirred for overnight at rt. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with brine (30 mL × 3), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Compound **17** was obtained as yellow solid (0.603 g, 73%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.61–7.14 (9H, m); 4.82 (1H, d); 3.77 (1H, d); 3.40 (3H, s). ^{13}C NMR (75 MHz) $CDCl_3$: δ 170.5 (C2); 170.3 (C5); 144.2 (ArC); 138.9 (ArC); 131.4 (ArC); 130.6 (ArC); 130.4 (ArC); 129.6 (2 × ArC); 128.9 (ArC); 128.3 (2 × ArC); 123.8 (ArC); 121.1 (ArC); 57.1 (C16); 3.9 (C3). HRMS: *m*/*z* calcd for $C_{16}H_{15}N_2O$ $[M+H]^+$: 251.1179, found: 251.1176. IR (ATR, cm^{-1}): 1664 (C=O); 1609 (C=N). Anal. Calcd for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found: C, 76.61; H, 5.96; N, 11.25.

4.19. (*S*)-3-Isopropyl-1-methyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (18)[11]

This was made on a 1.19 mmol scale by the same method used to obtain **17**. Yellow solid (74%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.61–7.13 (9H, m); 3.40 (3H, s); 3.06 (1H, d); 2.76 (1H, m); 1.13 (3H, d); 1.00 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.6 (C2); 167.6 (C5); 143.9 (ArC); 138.9 (ArC); 131.1 (ArC); 130.2 (ArC); 130.1 (ArC); 129.6 (2 × ArC); 129.1 (ArC); 128.1 (2 × ArC); 123.6 (ArC); 121.3 (ArC); 69.7 (C3); 34.9 (C19); 29.4 (C16); 20.2 (C17); 19.2 (C18). HRMS: *m*/*z* calcd for $C_{19}H_{19}N_2O$ $[M-H]^+$: 291.1492, found: 291.1490. IR (ATR, cm^{-1}): 1665 (C=O); 1604 (C=N). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 77.59; H, 6.99; N, 9.80.

4.20. (*S*)-1-Benzyl-3-isopropyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (19)

This was synthesized on a 1.00 mmol scale by the same method used to obtain **17**. White powder (24%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.48–6.98 (14H, m); 5.64 (1H, d); 4.82 (1H, d); 3.19 (1H, d); 2.84 (1H, m); 1.16 (3H, d); 1.07 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 168.9 (C2); 168.3 (C5); 142.2 (ArC); 138.9 (ArC); 137.1 (ArC); 131.1 (ArC); 130.9 (ArC); 130.2 (ArC); 130.1 (ArC); 129.7 (2 × ArC); 128.6 (2 × ArC); 128.1 (2 × ArC), 127.5 (2 × ArC); 127.2 (ArC); 124.3 (ArC); 122.5 (ArC); 70.1 (C3); 50.2 (C23); 29.5 (C16); 20.3 (C24); 19.2 (C25). HRMS: *m*/*z* calcd for $C_{25}H_{25}N_2O$ $[M+H]^+$: 369.1961, found: 369.1958. IR (cm^{-1}): 1665 (C=O); 1604 (C=N). Anal. Calcd for $C_{25}H_{24}N_2O$: C, 81.49; H, 6.57; N, 7.60. Found: C, 81.11; H, 6.59; N, 7.98.

4.21. (*S*)-1-(Biphenyl-2-ylmethyl)-3-isopropyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin2(3*H*)-one (20)

This was made on a 0.50 mmol scale by the same method used to obtain **17**. Yellow solid (88%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.49–6.90 (18H, m); 5.62 (1H, d); 4.89 (1H, d); 3.19 (1H, d); 2.93 (1H, m); 1.19 (3H, d); 1.07 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.0 (C2); 168.2 (C5); 141.8 (ArC); 141.3 (ArC); 140.5 (ArC); 138.8 (ArC); 134.2 (ArC); 130.9 (ArC); 130.2 (ArC); 129.9 (ArC); 129.7 (2 × ArC); 129.6 (2 × ArC); 129.4 (2 × ArC); 128.2 (2 × ArC); 128.0 (2 × ArC); 127.8 (ArC);127.3 (ArC); 127.0 (ArC);126.6 (ArC); 123.9 (ArC); 121.9 (ArC); 69.9 (C3); 46.6 (C16); 29.4 (C29); 20.2 (C30); 19.1 (C31). HRMS: *m*/*z* calcd for $C_{31}H_{29}N_2O$ $[M+H]^+$: 445.2274, found: 445.2270. IR (cm^{-1}): 1683 (C=O); 1603 (C=N). Anal. Calcd for $C_{31}H_{28}N_2O{\cdot}0.4DCM$: C, 78.81; H, 6.07; N, 5.85. Found: C, 78.72; H, 6.40; N, 5.82.

4.22. (*S*)-3-Isopropyl-5-phenyl-1-(quinolin-6-ylmethyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (21)

This was prepared on a 0.50 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (10:2 DCM/acetone) yielded a brown oil (82%). ^1H NMR (270 MHz) $CDCl_3$: δ 8.80 (1H, dd); 8.03 (1H, dd); 7.58–7.00 (13H, m); 5.96 (2H, t); 3.29 (1H, d); 2.89 (1H, m); 1.18 (3H, d); 1.09 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.2 (C2); 168.4 (C5); 149.4 (ArC); 146.1 (ArC); 142.8 (ArC); 139.1 (ArC); 136.7 (ArC); 135.1 (ArC); 131.1 (ArC); 130.4 (ArC); 130.2 (ArC); 129.9 (ArC); 129.7 (2 × ArC); 128.2 (ArC); 128.1 (2 × ArC); 127.1 (ArC); 126.9 (ArC); 126.4 (ArC); 124.0 (ArC); 122.4 (ArC); 121.1 (ArC); 70.2 (C3); 46.5 (C16); 31.0 (C23); 20.4 (C26); 19.3 (C27). HRMS: *m*/*z* calcd for $C_{28}H_{26}N_3O$ $[M+H]^+$; 420.2070, found: 420.2076. IR (cm^{-1}): 1680 (C=O); 1604 (C=N).

4.23. (*R*)-3-Isopropyl-1-methyl-5-phenyl-1*H*-benzo[*e*][1,4] diazepin-2(3*H*)-one (22)

This was prepared on a 15.40 mmol scale by the same method used to obtain **17**. Yellow oil (45%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.63–7.14 (9H, m); 3.45 (3H, s); 3.07 (1H, d); 2.75 (1H, m); 1.14 (3H, d); 1.02 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.6 (C2); 167.6 (C5); 143.9 (ArC); 138.9 (ArC); 131.1 (ArC); 130.2 (ArC); 130.1 (ArC); 129.6 (2 × ArC); 129.1 (ArC); 128.1 (2 × ArC); 123.5 (ArC); 121.3 (ArC); 69.7 (C3); 35.0 (C19); 29.4 (C16); 20.3 (C17); 19.1 (C18). HRMS: *m*/*z* calcd for $C_{19}H_{21}N_2O$ $[M+H]^+$: 293.1648, found: 293.1651. IR (ATR, cm^{-1}): 1680 (C=O); 1606 (C=N).

4.24. (*R*)-1-Benzyl-3-isopropyl-5-phenyl-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (23)

This was prepared on a 4.40 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (5:1 DCM/ethylacetate) yielded a white powder (43%). ^1H NMR (270 MHz) $CDCl_3$: δ 7.48–6.97 (14H, m); 5.60 (1H, d); 4.79 (1H, d); 3.17 (1H, d); 2.86 (1H, m); 1.14 (3H, d); 1.05 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 168.9 (C2); 168.3 (C5); 142.2 (ArC); 138.9 (ArC); 137.1 (ArC); 131.1 (ArC); 130.9 (ArC); 130.2 (ArC); 130.1 (ArC); 129.7 (2 × ArC); 128.6 (2 × ArC); 128.1 (2 × ArC), 127.5 (2 × ArC); 127.2 (ArC); 124.3 (ArC); 122.5 (ArC); 70.1 (C3); 50.2 (C23); 29.5 (C16); 20.3 (C24); 19.2 (C25). HRMS: *m*/*z* calcd for $C_{25}H_{25}N_2O$ $[M+H]^+$: 369.1961, found: 369.1964. IR (cm^{-1}): 1678 (C=O); 1603 (C=N). Anal. Calcd for $C_{25}H_{24}N_2O$: C, 81.49; H, 6.57; N, 7.60. Found: C, 81.11; H, 6.59; N, 7.98.

4.25. (*S*)-3-Benzyl-1-methyl-5-phenyl-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (24)

This was prepared on a 0.50 mmol scale by the same method used to obtain **17**. White solid (Quantitative). ^1H NMR (270 MHz) $CDCl_3$: δ 7.59–7.11 (14H, m); 3.82 (1H, t); 3.63 (2H, d), 3.43 (3H, s). ^{13}C NMR (75 MHz) $CDCl_3$: δ 170.5 (C2); 168.3 (C5); 143.8 (ArC); 139.6 (ArC); 138.9 (ArC); 131.4 (ArC); 130.5 (ArC); 130.4 (ArC); 130.0 (2 × ArC); 129.8 (2 × ArC), 129.1 (ArC); 128.3 (2 × ArC), 128.2 (2 × ArC); 126.1 (ArC); 123.8 (ArC); 121.3 (ArC); 65.2 (C3); 38.2 (C23); 35.3 (C16). HRMS: *m*/*z* calcd for $C_{23}H_{21}N_2O$ $[M+H]^+$: 341.1648, found: 341.1652. IR (cm^{-1}): 1682 (C=O);

1633 (C=N). Anal. Calcd for $C_{23}H_{20}N_2O \cdot 0.06\ CH_3COCH_3$: C, 80.96; H, 5.97; N, 8.15. Found: C, 80.43; H, 6.10; N, 8.65.

4.26. (*S*)-1,3-Dibenzyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (25)

This was prepared on a 0.50 mmol scale by the same method used to obtain **17**. Yellow oil (quantitative). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.44–6.97 (19H, m); 5.67 (1H, d); 4.77 (1H, d); 3.92 (1H, t); 3.69 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.6 (C2); 168.8 (C5); 141.8 (ArC); 139.5 (ArC); 138.8 (ArC); 136.9 (ArC); 131.3 (ArC); 130.9 (ArC); 130.3 (ArC); 130.2 (ArC); 130.0 (2 × ArC); 129.7 (2 × ArC); 128.6 (2 × ArC); 128.3 (2 × ArC); 128.2 (2 × ArC); 127.5 (2 × ArC); 127.3 (ArC); 126.2 (ArC); 124.4 (ArC); 122.5 (ArC); 65.3 (C3); 50.3 (C23); 38.1 (C16). HRMS: *m*/*z* calcd for $C_{29}H_{25}N_2O$ $[M+H]^+$: 417.1961, found: 417.1963. IR (cm^{-1}): 1682 (C=O); 1602 (C=N).

4.27. (*S*)-3-Benzyl-1-(biphenyl-4-ylmethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (26)

This was prepared on a 0.45 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM) yielded a yellow oil (83%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.46–7.03 (23H, m); 5.70 (1H, d); 4.79 (1H, d); 3.90 (1H, t); 3.63 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.5 (C2); 168.9 (C5); 141.8 (ArC); 140.7 (ArC); 140.2 (ArC); 139.5 (ArC); 138.8 (ArC); 135.9 (ArC); 131.3 (ArC); 131.0 (ArC); 130.4 (ArC); 130.3 (ArC); 130.0 (2 × ArC); 129.7 (2 × ArC); 128.8 (2 × ArC); 128.3 (2 × ArC); 128.2 (2 × ArC); 128.0 (2 × ArC); 127.3 (2 × ArC); 127.0 (2 × ArC); 126.2 (ArC); 124.5 (ArC); 123.5 (ArC); 122.7 (ArC); 65.3 (C3); 50.2 (C23); 38.1 (C16). HRMS: *m*/*z* calcd for $C_{35}H_{28}N_2O$ $[M]^+$: 492.2196, found: 492.2202. IR (cm^{-1}): 1677 (C=O); 1602 (C=N).

4.28. (*S*)-3-Benzyl-1-(biphenyl-3-ylmethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (27)

This was prepared on a 0.40 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM) yielded a yellow oil (82%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.49–7.00 (23H, m); 5.74 (1H, d); 4.87 (1H, d); 3.95 (1H, t); 3.69 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.8 (C2); 168.7 (C5); 141.8 (ArC); 141.6 (ArC); 140.6 (ArC); 139.5 (ArC); 138.6 (ArC); 131.3(ArC); 130.9 (ArC); 130.3 (ArC); 130.2 (ArC); 130.0 (2 × ArC); 129.6 (2 × ArC); 129.1 (2 × ArC); 128.7 (2 × ArC); 128.3 (2 × ArC); 128.1 (2 × ArC); 127.3 (ArC); 127.1 (2 × ArC); 126.5 (ArC); 126.2 (ArC); 126.1 (ArC); 126.0 (ArC); 124.4 (ArC); 122.4 (ArC); 65.3 (C3); 50.3 (C23); 38.2 (C16). HRMS: *m*/*z* calcd for $C_{35}H_{28}N_2O$ $[M]^+$: 492.2196, found: 492.2196. IR (cm^{-1}): 1678 (C=O); 1602 (C=N).

4.29. (*S*)-3-Benzyl-1-(biphenyl-2-ylmethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (28)

This was prepared on a 0.50 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM) yielded a yellow solid (85%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.46–6.84 (23H, m); 5.66 (1H, d); 4.88 (1H, d); 3.91 (1H, t); 3.70 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.8 (C2); 168.9 (C5); 141.6 (ArC); 141.5 (ArC); 140.6 (ArC); 139.5 (ArC); 138.9 (ArC); 134.1 (ArC); 131.2 (ArC); 130.4 (ArC); 130.2 (ArC); 130.0 (2 ArC); 129.9 (2 × ArC); 129.5 (2 × ArC); 128.4 (2 × ArC); 128.3 (2 × ArC); 128.2 (2 × ArC); 128.0 (ArC); 127.5 (2 × ArC); 127.1 (2 × ArC); 126.9 (ArC); 126.2 (ArC); 124.2 (ArC); 122.1 (ArC); 65.4 (C3); 46.9 (C23); 38.2 (C16). HRMS: *m*/*z* calcd for $C_{35}H_{29}N_2O$ $[M+H]^+$: 493.2274, found: 493.2270. IR (cm^{-1}): 1680 (C=O); 1603 (C=N). Anal. Calcd for $C_{35}H_{28}N_2O \cdot 0.1CHCl_3$: C, 83.54; H, 5.63; N, 5.55. Found: C, 83.99; H, 5.74; N, 6.05.

4.30. (*S*)-3-Benzyl-5-phenyl-1-(quinolin-6-ylmethyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (29)

This was prepared on a 0.50 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (10:1 DCM/acetone) yielded a red oil (85%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.82 (1H, dd); 8.04 (1H, dd); 7.61–6.97 (18H, m); 5.97 (2H, t); 4.01 (1H, t); 3.70 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.8 (C2); 169.0 (C5); 149.5 (ArC); 146.1 (ArC); 142.5 (ArC); 139.6 (ArC); 139.0 (ArC); 136.2 (ArC); 134.8 (ArC); 131.3 (ArC); 130.3 (ArC); 130.3 (ArC); 130.3 (2 × ArC); 130.1 (2 × ArC); 130.0 (ArC); 129.7 (ArC); 128.3 (2 × ArC); 128.2 (2 × ArC); 127.1 (ArC); 126.9 (ArC); 126.4 (ArC); 126.2 (ArC); 124.1 (ArC); 122.4 (ArC); 121.1 (ArC); 65.5 (C3); 46.6 (C16); 38.2 (C23). HRMS: *m*/*z* calcd for $C_{32}H_{26}N_3O$ $[M+H]^+$; 468.2070. Found: 468.2073. IR (cm^{-1}): 1679 (C=O); 1602 (C=N).

4.31. (*S*)-2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetonitrile (30)

This was made on a 3.50 mmol scale by the same method used to obtain **17**. An ash coloured powder precipitated from Et_2O (67%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.64–7.17 (14H, m); 4.75 (2H, q); 3.84 (1H, t), 3.59 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.9 (C2); 168.7 (C5); 140.6 (ArC); 138.8 (ArC); 138.4 (ArC); 132.0 (ArC); 130.9 (ArC); 130.6 (ArC); 129.8 (2 × ArC); 129.7 (ArC); 129.6 (2 × ArC), 128.3 (2 × ArC), 128.2 (2 × ArC); 126.3 (ArC); 125.4 (ArC); 120.7 (ArC); 115.2 (ArC); 64.7 (C3); 37.8 (C23); 35.4 (C16). HRMS: *m*/*z* calcd for $C_{24}H_{20}N_3O$ $[M+H]^+$: 366.1601, found: 366.1605. IR (cm^{-1}): 2259 (CN); 1684 (C=O); 1604 (C=N). Anal. Calcd for $C_{24}H_{19}N_3O$: C, 78.88; H, 5.24; N, 11.50. Found: C, 78.64; H, 5.30; N, 11.93.

4.32. (*S*)-2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetic acid (32)

Compound 32 was made on a 3.06 mmol scale by the same method used to obtain **17**. Thereafter, the crude product **31** was deprotected in an H-cube using 10% Pd on C as a catalyst. Then pressure was adjusted to full H_2 mode, the temperature to 40 °C and the flow rate to 1 mL min^{-1}. The crude product was dissolved in EtOH/AcOH (7:3) and passed through the H-cube. The collected mixture was concentrated under vacuum. Purification with flash chromatography (2:1 DCM/MeOH) afforded a white solid **32** (83%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 12.33 (1H, br s); 7.62–7.13 (14H, m); 4.62 (1H, d); 4.47 (1H, d); 4.03 (1H, t); 3.56 (2H, d). HRMS: *m*/*z* calcd for $C_{24}H_{20}N_2O_3$ $[M+H]^+$: 385.1547, found: 385.1550.

4.33. (*R*)-3-Benzyl-1-methyl-5-phenyl-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (33)

This was synthesised on a 0.50 mmol scale by the same method used to obtain **17**. White solid (92%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.57–7.11 (14H, m); 3.81 (1H, t); 3.61 (2H, d), 3.42 (3H, s). HRMS: *m*/*z* calcd for $C_{23}H_{21}N_2O$ $[M+H]^+$: 341.1648, found: 341.1653.

4.34. (*R*)-1,3-Dibenzyl-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (34)

This was made on a 0.50 mmol scale by the same method used to obtain **17**. Yellow oil (quantitative). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.45–6.98 (19H, m); 5.71 (1H, d); 4.81 (1H, d); 3.92 (1H, t); 3.67

(2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.4 (C2); 168.7 (C5); 141.6 (ArC); 139.3 (ArC); 138.6 (ArC); 136.7 (ArC); 131.1 (ArC); 130.7 (ArC); 130.1 (ArC); 130.0 (2 × ArC); 129.8 (2 × ArC); 129.5 (2 × ArC); 128.4 (2 × ArC); 128.1 (2 × ArC); 128.0 (2 × ArC); 127.3 (ArC); 127.1 (ArC); 126.0 (ArC); 124.2 (ArC); 122.3 (ArC); 65.1 (C3); 50.1 (C23); 38.0 (C16). HRMS: *m*/*z* calcd for $C_{29}H_{25}N_2O$ $[M+H]^+$: 417.1961, found: 417.1957. IR (cm^{-1}): 1674 (C=O); 1602 (C=N).

4.35. (*S*)-1-(Biphenyl-4-ylmethyl)-3-(phenoxymethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (35)

(*S*)-3-(Benzyloxymethyl)-5-phenyl-*1H*-benzo[*e*][1,4]diazepin-2(*3H*)-one **10** was synthesised on a 2.00 mmol scale by the same method used to obtain **5**. Purification with flash chromatography (neat DCM followed by neat acetone) yielded a colourless oil (63%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.60–7.04 (23H, m); 5.69 (1H, d); 5.24 (1H, d); 4.94 (2H, s); 4.62 (1H, q); 4.29 (1H, t); 4.02 (1H, t). ^{13}C NMR (75 MHz) $CDCl_3$: δ 170.2 (C2); 167.4 (C5); 149.3 (ArC); 141.2 (ArC); 140.6 (ArC); 140.2 (ArC); 138.0 (ArC); 135.7 (ArC); 131.3 (ArC); 131.2 (ArC); 131.0 (ArC); 130.1 (ArC); 129.8 (2 × ArC); 129.7 (2 × ArC); 128.8 (2 × ArC); 128.3 (2 × ArC); 128.0 (2 × ArC); 127.8 (ArC); 127.4 (2 × ArC); 127.3 (2 × ArC); 126.8 (2 × ArC); 124.4 (ArC); 122.7 (ArC); 105.2 (ArC); 50.1 (C3); 31.9 (C25); 31.0 (C18); 22.7 (C16). HRMS: *m*/*z* calcd for $C_{36}H_{31}N_2O_2$ $[M+H]^+$; 523.2380, found: 523.2386. IR (cm^{-1}): 1668 (C=O); 1605 (C=N).

4.36. (*S*)-1-(Biphenyl-3-ylmethyl)-3-(phenoxymethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (36)

This was made on a 0.40 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM followed by neat acetone) yielded a colourless oil (88%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.55–6.99 (23H, m,); 5.75 (1H, d); 4.81 (1H, d); 4.73 (2H, s); 4.62 (1H, q); 4.25 (1H, t); 3.98 (1H, t). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.2 (C2); 168.8 (C5); 141.5 (ArC); 141.4 (ArC); 140.4 (ArC); 138.3 (ArC); 138.2 (ArC); 137.2 (ArC); 131.1 (ArC); 130.9 (ArC); 130.2 (ArC); 130.1 (ArC); 129.4 (2 × ArC); 128.9 (ArC); 128.5 (2 × ArC); 128.3 (2 × ArC); 128.0 (2 × ArC); 127.9 (2 × ArC); 127.5 (ArC); 127.1 (ArC); 126.9 (2 × ArC); 126.4 (ArC); 126.0 (ArC); 125.9 (ArC); 124.3 (ArC); 122.4 (ArC); 73.8 (C18); 70.8 (C16); 63.6 (C25); 49.8 (C3). HRMS: *m*/*z* calcd for $C_{36}H_{31}N_2O_2$ $[M+H]^+$; 523.2380, found: 523.2384. IR (cm^{-1}): 1673 (C=O); 1605 (C=N).

4.37. (*S*)-3-(Phenoxymethyl)-5-phenyl-1-(quinolin-6-ylmethyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (37)

This was prepared on a 0.37 mmol scale by the same method used to obtain **17** but instead of **5**, **10** (0.13 g, 0.37 mmol) was used and instead of iodomethane, 8-(bromomethyl)quinoline (0.10 g, 0.44 mmol) was used. Purification with flash chromatography (neat DCM followed by neat acetone) yielded a yellow oil (60%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.81 (1H, dd); 8.05 (1H, d); 7.63–6.99 (18H, m); 5.96 (2H, t); 4.74 (2H, s); 4.58 (1H, q); 4.30 (1H, t); 4.09 (1H, t). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.6 (C2); 169.0 (C5); 149.3 (ArC); 146.1 (ArC); 142.3 (ArC); 138.8 (ArC); 138.5 (ArC); 136.2 (ArC); 135.0 (ArC); 131.3 (ArC); 130.5 (ArC); 130.4 (ArC); 130.1 (ArC); 129.8 (2 × ArC); 129.7 (2 × ArC); 128.5 (2 × ArC); 128.2 (2 × ArC); 128.1 (2 × ArC); 127.7 (ArC); 127.3 (ArC); 127.0 (ArC); 126.4 (ArC); 124.2 (ArC); 122.7 (ArC); 74.0 (C18); 71.2 (C16); 63.9 (C3); 46.3 (C25). HRMS: *m*/*z* calcd for $C_{33}H_{28}N_3O_2$ $[M+H]^+$; 498.2176, found: 498.2177. IR (cm^{-1}): 1677 (C=O); 1604 (C=N).

4.38. 5-Isopropyl-1-methyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (38)

This was synthesised on a 1.00 mmol scale by the same method used to obtain **17**. Pale yellow oil (96%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.40 (2H, m); 7.15 (2H, m); 3.51 (1H, d); 3.25 (3H, s); 3.06 (1H, m); 1.14 (3H, d); 0.85 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 176.6 (C2); 170.7 (C5); 142.8 (ArC), 130.7 (ArC); 130.2 (ArC); 127.0 (ArC); 124.5 (ArC); 121.0 (ArC); 56.1 (C3); 35.6 (C10); 34.6 (C11); 21.6 (C12); 19.7 (C13). HRMS: *m*/*z* calcd for $C_{13}H_{17}N_2O$ $[M+H]^+$: 217.1335, found: 217.1337. IR (KBr, cm^{-1}): 1673 (C=O); 1621 (C=N).

4.39. (*S*)-3-Benzyl-5-isopropyl-1-methyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (39)

This was prepared on a 1.09 mmol scale by the same method used to obtain **17**. Red oil (98%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.42 (2H, t); 7.29–7.08 (7H, m); 3.59 (1H, t); 3.45 (2H, m); 3.35 (3H, s); 3.08 (1H, m); 1.27 (3H, d); 0.92 (3H, d). ^{13}C NMR (75 MHz) $CDCl_3$: δ 173.7 (C2); 170.7 (C5); 142.2 (ArC); 139.5 (ArC); 130.3 (2 × ArC); 129.8 (2 × ArC); 127.7 (2 × ArC); 126.7 (ArC); 125.7 (ArC), 124.1 (ArC); 121.1 (ArC); 64.0 (C3); 37.8 (C10); 35.3 (C17); 34.8 (C18); 21.3 (C19); 19.5 (C20). HRMS: *m*/*z* calcd for $C_{20}H_{23}N_2O$ $[M+H]^+$: 307.1805, found: 307.1804. IR (KBr, cm^{-1}): 1679 (C=O); 1626 (C=N).

4.40. (*S*)-1,3-Dibenzyl-5-cyclohexyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (40)

This was prepared on a 0.67 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM) yielded a yellow oil (38%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.40–7.05 (14H, m); 5.47 (1H, d); 4.83 (1H, d); 3.66 (1H, q), 3.50 (2H, m); 2.65 (1H, t); 1.90–0.72 (10H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 173.8 (C2); 169.9 (C5); 140.9 (ArC); 139.7 (ArC); 137.0 (ArC); 131.8 (ArC); 130.5 (ArC); 130.0 (2 × ArC); 128.5 (2 × ArC), 128.1 (2 × ArC), 128.0 (2 × ArC); 127.3 (ArC); 127.1 (ArC); 126.0 (ArC); 124.7 (ArC); 122.3 (ArC); 64.4 (C3); 50.4 (C23); 45.4 (C16); 38.1 (C10); 32.2 (C11); 30.0 (C15), 26.8 (C13); 26.1 (2 × C, C12, C14). HRMS: *m*/*z* calcd for $C_{29}H_{31}N_2O$ $[M+H]^+$: 423.2431, found: 423.2426. IR (cm^{-1}): 1674 (C=O); 1622 (C=N).

4.41. (*S*)-3-Benzyl-1-(4-nitrobenzyl)-5-phenyl-1*H*-benzo[*e*] [1,4]-diazepin-2(3*H*)-one (41)

This was prepared on a 4.50 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM followed by 15:1 DCM/acetone) yielded a yellow oil (Quantitative). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.94 (2H, d); 7.47–7.09 (16H, m); 5.71 (1H, d); 4.90 (1H, d); 3.93 (1H, t); 3.66 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.7 (C2); 168.7 (C5); 147.3 (ArC); 144.4 (ArC); 141.4 (ArC); 139.1 (ArC); 138.4 (ArC); 131.6 (ArC); 130.7 (ArC); 130.5 (ArC); 130.0 (2 × ArC); 129.5 (2 × ArC); 128.3 (4 × ArC); 128.1 (2 × ArC); 126.3 (ArC); 124.9 (ArC); 123.9 (2 × ArC); 122.1 (ArC); 65.3 (C3); 49.9 (C23); 38.0 (C16). HRMS: *m*/*z* calcd for $C_{29}H_{24}N_3O_3$ $[M+H]^+$: 462.1812, found: 462.1818. IR (cm^{-1}): 1680 (C=O); 1603 (C=N); 1522 and 1346 (NO_2).

4.42. (*S*)-3-Benzyl-1-(3-nitrobenzyl)-5-phenyl-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (42)

This was prepared on a 3.60 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM followed by 15:1 DCM/acetone) yielded a yellow oil (79%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.80 (2H, d); 7.34–6.94 (16H, m);

5.58 (1H, d); 4.86 (1H, d); 3.92 (1H, t); 3.56 (2H, m). ^{13}C NMR (75 MHz) $CDCl_3$: δ 169.7 (C2); 168.6 (C5); 148.3 (ArC); 141.4 (ArC); 139.2 (ArC); 139.1 (ArC); 138.4 (ArC); 133.6 (ArC); 131.7 (ArC); 130.7 (ArC); 130.6 (ArC); 130.5 (ArC); 129.9 (2 × ArC); 129.8 (ArC); 129.5 (2 × ArC); 128.3 (4 × ArC); 126.3 (ArC); 124.9 (ArC); 122.5 (ArC); 122.3 (ArC); 122.2 (ArC); 65.2 (C3); 50.0 (C23); 38.0 (C16). HRMS: *m*/*z* calcd for $C_{29}H_{24}N_3O_3$ $[M+H]^+$: 462.1812, found: 462.1811. IR (cm^{-1}): 1666 (C═O); 1599 (C═N); 1524 and 1349 (NO_2).

4.43. (*S*)-3-Benzyl-1-(2-nitrobenzyl)-5-phenyl-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (43)

This was prepared on a 1.50 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM followed by 15:1 DCM/acetone) yielded a yellow oil (quantitative). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.9 (1H, d); 7.50–6.94 (17H, m); 5.68 (1H, d); 5.45 (1H, d); 3.99 (1H, t); 3.61 (2H, m). HRMS: *m*/*z* calcd for $C_{29}H_{24}N_3O_3$ $[M+H]^+$: 462.1812, found: 462.1809.

4.44. (*S*)-5-Cyclohexyl-3-isopropyl-1-(4-nitrobenzyl)-1*H*-benzo-[*e*][1,4]diazepin-2(3*H*)-one (44)

This was prepared on a 0.35 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (20:1 DCM/acetone) yielded a colourless oil (70%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.04 (2H, d), 7.45 (2H, m); 7.29–7.18 (4H, m); 5.43 (1H, d); 4.92 (1H, d); 2.94 (1H, d); 2.64 (2H, m); 1.93–1.01 (10H, m); 0.98 (6H, t). HRMS: *m*/*z* calcd for $C_{25}H_{30}N_3O_3$ $[M+H]^+$: 420.2282, found: 420.2286.

4.45. (*S*)-5-Cyclohexyl-3-isopropyl-8-nitro-1-(4-nitrobenzyl)-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one (45)

This was prepared on a 0.31 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (20:1 DCM/acetone) yielded a yellow oil (69%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.36 (1H, d); 8.24 (1H, dd); 8.06 (2H, d); 7.43 (1H, d); 7.25 (2H, d); 5.50 (1H, d); 4.98 (1H, d); 2.92 (1H, d); 2.66 (2H, m); 1.97–1.13 (10H, m); 0.98 (6H, dd). HRMS: *m*/*z* calcd for $C_{25}H_{29}N_4O_5$ $[M+H]^+$: 465.2132, found: 465.2133.

4.46. (*S*)-3-Benzyl-5-cyclohexyl-1-(4-nitrobenzyl)-1*H*-benzo[*e*]-[1,4]diazepin-2(3*H*)-one (46)

This was prepared on a 0.45 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM followed by 20:1 DCM/acetone) yielded a pale yellow solid (22%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.04 (2H, d), 7.41 (2H, m); 7.29–7.12 (9H, m); 5.48 (1H, d); 4.93 (1H, d); 3.68 (1H, t), 3.47 (2H, m); 2.66 (1H, t); 1.90–0.74 (10H, m). HRMS: *m*/*z* calcd for $C_{29}H_{30}N_3O_3$ $[M+H]^+$: 468.2282, found: 468.2274.

4.47. (*S*)-3-Benzyl-5-cyclohexyl-1-(3-nitrobenzyl)-1*H*-benzo [*e*]-[1,4]diazepin-2(3*H*)-one (47)

This was prepared on a 0.86 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (neat DCM followed by 20:1 DCM/acetone) yielded a colourless oil (38%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.04 (1H, d), 7.90 (1H,s); 7.50–7.11 (11H, m); 5.53 (1H, d); 4.90 (1H, d); 3.69 (1H, t), 3.48 (2H, m); 2.64 (1H, t); 1.90–0.69 (10H, m). HRMS: *m*/*z* calcd for $C_{29}H_{30}N_3O_3$ $[M+H]^+$: 468.2282, found: 468.2273.

4.48. (*S*)-3-Benzyl-1-(4-nitrobenzyl)-5-(pyridin-2-yl)-1*H*-benzo-[*e*][1,4]diazepin-2(3*H*)-one (48)

This was made on a 1.00 mmol scale by the same method used to obtain **17**. Purification with flash chromatography (9:1 DCM/acetone) yielded a red oil (84%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.56 (1H, d); 8.01 (1H, d); 7.93 (2H, d); 7.81 (1H, t); 7.48–7.14 (12H, m); 5.41 (1H, d); 5.02 (1H, d); 4.01 (1H, t); 3.63 (2H, m). HRMS: *m*/*z* calcd for $C_{28}H_{23}N_4O_3$ $[M+H]^+$: 463.1765, found: 463.1766.

4.49. (*S*)-1-(4-Aminobenzyl)-3-benzyl-5-phenyl-1*H*-benzo[*e*]-[1,4]diazepin-2(3*H*)-one (49)

This was synthesised in a microwave at 300 W initial power. **41** (0.60 g, 1.30 mmol), $Mo(CO)_6$ (0.35 g, 1.30 mmol), DBU (593 μL, 4.00 mmol) and ethanol (5 mL) were charged into a microwave tube with a stir bar. The tube was sealed and the temperature was ramped until 150 °C, and then kept at this temperature for 15 min. After cooling, the solvent was reduced under reduced pressure. The resulting mixture was purified by flash chromatography (10:1 DCM/acetone). Purification with flash chromatography (10:1 DCM/acetone) yielded a red oil (79%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.29–6.82 (14H, m); 6.64 (2H, d); 6.23 (2H, d); 5.50 (1H, d); 4.53 (1H, d); 3.83 (1H, t); 3.64–3.49 (4H, m). HRMS: *m*/*z* calcd for $C_{29}H_{26}N_3O$ $[M+H]^+$: 432.2070, found: 432.2065.

4.50. (*S*)-1-(3-Aminobenzyl)-3-benzyl-5-phenyl-1*H*-benzo[*e*]-[1,4]diazepin-2(3*H*)-one (50)

This was prepared on a 1.02 mmol scale by the same method used to obtain **49**. Purification with flash chromatography (10:1 DCM/acetone) yielded an orange oil (99%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.43–7.01 (14H, m); 6.88 (1H, t); 6.38 (2H, d); 6.16 (1H, s); 5.54 (1H, d); 4.73 (1H, d); 3.92 (1H, t); 3.68 (2H, m); 3.33 (2H, br s). HRMS: *m*/*z* calcd for $C_{29}H_{26}N_3O$ $[M+H]^+$: 432.2070, found: 432.2061.

4.51. (*S*)-1-(2-Aminobenzyl)-3-benzyl-5-phenyl-1*H*-benzo[*e*]-[1,4]diazepin-2(3*H*)-one (51)

This was synthesised on a 0.77 mmol scale by the same method used to obtain **49**. Purification with flash chromatography (25:1 DCM/acetone) yielded a yellow oil (72%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.48–6.99 (14H, m); 6.91 (1H, t); 6.67 (1H, d); 6.41 (1H, t); 6.38 (1H, d); 5.76 (1H, d); 4.56 (1H, d); 3.93 (2H, br s); 3.85 (1H, t); 3.61 (2H, m). HRMS: *m*/*z* calcd for $C_{29}H_{26}N_3O$ $[M+H]^+$: 432.2070, found: 432.2070.

4.52. (*S*)-1-(4-Aminobenzyl)-5-cyclohexyl-3-isopropyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (52)

The reaction was carried out on 0.18 mmol scale by the same method used to obtain **49** but instead of **41**, **44** (0.07 g, 0.18 mmol) was used. Purification with flash chromatography (20:1 DCM/acetone) yielded a colourless oil (94%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.38 (3H, m); 6.85 (1H, t), 6.47 (2H, d), 7.45 (2H, m); 5.29 (1H, d); 4.67 (1H, d); 3.50 (2H, br s); 2.88 (1H, d); 2.64 (2H, m); 1.90–1.01 (10H, m); 0.94 (6H, dd). HRMS: *m*/*z* calcd for $C_{25}H_{32}N_3O$ $[M+H]^+$: 390.2540, found: 390.2540.

4.53. (*S*)-8-Amino-1-(4-aminobenzyl)-5-cyclohexyl-3-isopropyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (53)

The reaction was carried out using a H-cube hydrogenation reactor (Thalesnano) in continuous flow mode, and RaNi (CarCart™

cartridge) was used as a catalyst. To prime the system, the catalyst bed was washed continuously with ethyl acetate (10 mL) at a flow rate of 1 mL min^{-1}. The pressure was adjusted to full H_2 mode and the temperature was set at 60 °C. **45** (0.08 g, 0.17 mmol) was dissolved in ethyl acetate (30 mL) and allowed to flow through the H-cube at 1 mL/min. The collected mixture was concentrated under reduced pressure. Colourless oil (61%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.09 (1H, d); 6.80 (2H, d); 6.68 (1H, dd); 6.63 (1H, d); 6.40 (2H, d); 5.24 (1H, d); 4.27 (1H, d); 3.73 (4H, br s); 2.88 (1H, d); 2.55 (2H, m); 1.98–0.99 (10H, m); 0.90 (6H, d). HRMS: *m*/*z* calcd for $C_{25}H_{33}N_4O$ $[M+H]^+$: 405.2649, found: 405.2650.

4.54. (*S*)-1-(4-Aminobenzyl)-3-benzyl-5-cyclohexyl-1*H*-benzo-[*e*][1,4]diazepin-2(3*H*)-one (54)

This was synthesised on a 0.13 mmol scale by the same method used to obtain **49** but instead of **41**, **46** (0.06 g, 0.13 mmol) was used. Purification with flash chromatography (20:1 DCM/acetone) yielded a colourless oil (quantitative). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.37–7.08 (9H, m); 6.84 (2H, d), 6.48 (2H, d); 5.35 (1H, d); 4.63 (1H, d); 3.60 (1H, t), 3.48 (4H, m); 2.61 (1H, t); 1.91–0.72 (10H, m). HRMS: *m*/*z* calcd for $C_{29}H_{32}N_3O$ $[M+H]^+$: 438.2540, found: 438.2539.

4.55. (*S*)-1-(3-Aminobenzyl)-3-benzyl-5-cyclohexyl-1*H*-benzo-[*e*][1,4]diazepin-2(3*H*)-one (55)

This was made on a 0.24 mmol scale by the same method used to obtain **49**. Purification with flash chromatography (20:1 DCM/acetone) yielded colourless oil (quantitative). ^{1}H NMR (270 MHz) $CDCl_3$: δ 7.41–7.10 (9H, m); 6.94 (1H, t), 6.47 (2H, t); 6.35 (1H, s); 5.20 (1H, d); 4.81 (1H, d); 3.69–3.40 (5H, m); 2.67 (1H, t); 1.92–0.85 (10H, m). HRMS: *m*/*z* calcd for $C_{29}H_{32}N_3O$ $[M+H]^+$: 438.2540, found: 438.2535.

4.56. (*S*)-1-(4-Aminobenzyl)-3-benzyl-5-(pyridin-2-yl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (56)

This was prepared on a 0.42 mmol scale by the same method used to obtain **53**. Purification with flash chromatography (9:1 DCM/acetone) resulted in an orange oil (39%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.58 (1H, d); 7.83 (1H, d); 7.73 (1H, t); 7.40–7.07 (10H, m); 6.82 (2H, d); 6.41 (2H, d); 5.08 (1H, d); 4.94 (1H, d); 3.95 (1H, t); 3.68 (2H, m). HRMS: *m*/*z* calcd for $C_{28}H_{25}N_4O$ $[M+H]^+$: 433.2023, found: 433.2025.

4.57. (*S*)-8-Amino-5-cyclohexyl-3-isopropyl-1*H*-benzo[*e*][1,4]-diazepin-2(3*H*)-one (57)

This was prepared on a 0.28 mmol scale by the same method used to obtain **53**. Grey oil (88%). ^{1}H NMR (270 MHz) $CDCl_3$: δ 8.57 (1H, br s), 6.77 (3H, m); 3.75 (2H, br s); 2.88 (1H, d); 2.64 (1H, t); 2.52 (1H, m); 1.94–1.14 (10H, m); 0.97 (6H, dd). HRMS: *m*/*z* calcd for $C_{18}H_{26}N_3O$ $[M+H]^+$: 300.2070, found: 300.2067.

4.58. (*S*)-1-(4-((3-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl)guanidine (58)

Step 1: $HgCl_2$ (0.08 g, 0.29 mmol) was added to a solution of **49** (0.11 g, 0.25 mmol), NEt_3 (115 μL, 0.83 mmol) and 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.08 g, 0.29 mmol) in DCM (10 mL). The mixture was stirred for 16 h at rt and insoluble salts were removed by filtration over Celite. The filtrate was washed with water and dried ($MgSO_4$). After filtration, the solvent was removed under reduced pressure to afford a white solid (0.14 g).

Step 2: The crude product from the previous step (0.13 g, 0.19 mmol) was stirred in HCl–dioxane (4 M, 5 mL) for 16 h. The solvent was removed under reduced pressure and the residue was reconcentrated with Et_2O (10 mL × 4). Et_2O was added and a solid was obtained via trituration, which was collected by filtration and dried to obtain a white powder (0.08 g, 74%). ^{1}H NMR (270 MHz) DMSO-d_6·D_2O: δ 7.65–7.16 (14H, m); 7.07 (1H, d); 6.94 (4H, q); 5.49 (1H, d); 4.93 (1H, d); 3.85 (1H, t); 3.46 (2H, m). D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-d_6·D_2O: δ 170.1 (C2); 169.8 (C5); 156.2 (C31); 142.0 (ArC); 139.3 (ArC); 138.0 (ArC); 136.3 (ArC); 134.5 (ArC); 133.4 (ArC); 132.1 (ArC); 130.7 (2 × ArC); 130.4 (2 × ArC); 130.2 (2 × ArC); 129.3 (2 × ArC); 129.2 (2 × ArC); 129.1 (2 × ArC); 127.3 (ArC); 124.4 (ArC); 125.4 (2 × ArC); 123.8 (ArC); 65.2 (C3); 49.7 (C23); 37.2 (C16). HRMS: *m*/*z* calcd for $C_{30}H_{28}N_5O$ $[M+H]^+$: 474.2288 found: 474.2291. IR (ATR, cm^{-1}): 3300 and 3171 (NH); 1660 (C=O); 1635 (C=N). Anal. Calcd for $C_{30}H_{27}N_5O{\cdot}3HCl{\cdot}2H_2O$: C, 58.21; H, 5.54; N, 11.31. Found: C, 58.04; H, 5.50; N, 11.50.

4.59. (*S*)-1-(3-((3-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl)guanidine (59)

This reaction was carried out on 0.99 mmol scale by the same method used to obtain **58** but instead of **49**, **50** (0.43 g, 0.99 mmol) was used in step1. Step 2 was carried out on a 0.30 mmol scale. Yellow solid (66%). ^{1}H NMR (270 MHz) DMSO-d_6·D_2O: δ 7.69–7.08 (15H, m); 7.00 (1H, t); 6.86 (2H, d); 6.74 (1H, s); 5.48 (1H, d); 4.98 (1H, d); 3.43 (2H, m); CH (H3); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-d_6·D_2O: δ 165.4 (C2); 164.5 (C5); 151.0 (C31); 147.2 (ArC); 134.5 (ArC); 134.2 (ArC); 132.5 (ArC); 130.8 (ArC); 128.6 (2 × ArC); 127.3 (ArC); 126.1 (ArC); 125.6 (2 × ArC); 125.4 (2 × ArC); 124.7 (2 × ArC); 124.3 (2 × ArC); 124.1 (2 × ArC); 122.2 (C ArC); 120.9 (C ArC); 118.9 (ArC); 118.7 (ArC); 118.3 (ArC); 62.1 (C3); 44.8 (C23); 32.3 (C16). HRMS: *m*/*z* calcd for $C_{30}H_{28}N_5O$ $[M+H]^+$: 474.2288, found: 474.2291. IR (ATR, cm^{-1}): 3299, 3154, 3062 and 2962 (NH); 1658 (C=O); 1612 (C=N).

4.60. (*S*)-1-(2-((3-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl)guanidine (60)

This was made on a 0.45 mmol scale by the same method used to obtain **58** but instead of **49**, **51** (0.20 g, 0.45 mmol) was used in step 1. Step 2 was carried out on a 0.19 mmol scale. White powder (92%). ^{1}H NMR (270 MHz) DMSO-d_6·D_2O: δ 7.56–7.15 (16H, m); 7.02 (1H, t); 6.71 (1H, d); 5.24 (1H, d); 5.06 (1H, d); 3.94 (1H, t); 3.43 (2H, br s); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-d_6·D_2O: δ 169.5 (C2); 169.2 (C5); 156.6 (C31); 142.2 (ArC); 139.2 (ArC); 138.0 (ArC); 134.6 (ArC); 133.0 (ArC); 132.5 (ArC); 131.5 (ArC); 130.5 (ArC); 130.1 (2 × ArC); 129.9 (2 × ArC); 129.5 (2 × ArC); 129.1 (ArC); 129.0 (2 × ArC); 128.7 (2 × ArC); 128.6 (ArC); 127.2 (ArC); 126.8 (ArC); 125.5 (ArC); 122.7 (ArC); 65.0 (C3); 46.5 (C23); 37.5 (C16). HRMS: *m*/*z* calcd for $C_{30}H_{28}N_5O$ $[M+H]^+$: 474.2288, found: 474.2291. IR (ATR, cm^{-1}): 3112 (NH); 1687 (C=O); 1609 (C=N).

4.61. (*S*)-1-(4-((5-Cyclohexyl-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl) guanidine (61)

This reaction was carried out on a 0.18 mmol scale by the same method used to obtain **58** but instead of **49**, **52** (0.07 g, 0.18 mmol) was used in step1. Step 2 was carried out on a 0.16 mmol scale. White powder (80%). ^{1}H NMR (270 MHz) DMSO-d_6·D_2O: δ 7.78 (3H, m); 7.42 (1H, t); 7.17–6.98 (4H, m); 5.48 (1H, d); 4.85 (1H, d, J = 15.4 Hz); 3.41 (2H, m); 2.60 (1H, m); 1.89–1.00 (10H, m); 0.89 (6H, d); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-d_6·D_2O: δ 185.6 (C2); 168.0 (C5); 156.5 (C24); 141.8 (ArC); 136.5

(ArC); 136.0 (ArC); 135.1 (ArC); 130.7 (ArC); 130.4 (2 × ArC); 123.7 (ArC); 127.7 (ArC); 125.8 (2 × ArC); 124.1 (ArC); 66.3 (C3); 50.5 (C16); 32.7 (C10); 29.6 (C27); 27.3 (2 × C, C11, C15); 26.7 (C13); 25.8 (2 × C, C12, C14); 20.4 (C28); 19.2 (C29). HRMS: *m*/*z* calcd for $C_{26}H_{34}N_5O$ $[M+H]^+$: 432.2758, found: 432.2761. IR (ATR, cm^{-1}): 3301, 3132, 2932 and 2854 (NH); 1672 (C=O); 1627 (C=N). Anal. Calcd for $C_{26}H_{33}N_5O{\cdot}3HCl{\cdot}3H_2O$: C, 52.48; H, 7.11; N, 11.77. Found: C, 52.42; H, 7.09; N, 11.54.

4.62. (*S*)-1-(1-Benzyl-5-cyclohexyl-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-7-yl)guanidine) phenylguanidine (62)

This reaction was carried out on a 0.07 mmol scale by the same method used to obtain **58** but instead of **49**, **53** (0.03 g, 0.07 mmol) was used in step1. Step 2 was carried out on a 0.06 mmol scale. White powder (highly hygroscopic) (quantitative). ^1H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 7.66 (1H, d); 7.52 (1H, d); 7.24 (1H, q); 7.06 (4H, s); 5.35 (1H, d); 4.89 (1H, d); 3.65 (1H, m); 3.45 (1H, m); 3.12 (1H, d); 1.87–1.05 (10H, m); 0.91 (6H, t); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 175.6 (C2); 168.6 (C5); 156.2 (C31); 155.9 (C24); 139.3 (ArC); 135.8 (ArC); 134.5 (ArC); 132.1 (ArC); 131.4 (ArC); 129.3 (2 × ArC); 123.3 (ArC); 124.8 (2 × ArC); 124.5 (ArC); 124.3 (ArC); 67.9 (C3); 49.1 (C16); 32.3 (C10); 30.0 (C27); 28.7 (2 × C, C11, C15); 25.9 (3 × C, C12, C13, C14); 20.1 (C28); 19.1 (C29). HRMS: *m*/*z* calcd for $C_{27}H_{37}N_8O$ $[M+H]^+$: 489.3085, found: 489.3078.IR (ATR, cm^{-1}): 3309, 3133, 2929 and 2856 (NH); 1678 (C=O); 1600 (C=N).

4.63. (*S*)-1-(4-((3-Benzyl-5-cyclohexyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl) guanidine (63)

This reaction was carried out on 0.10 mmol scale by the same method used to obtain **58** but instead of **49**, **54** (0.04 g, 0.10 mmol) was used in step 1. Step 2 was carried out on a 0.10 mmol scale. White powder (78%). ^1H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 7.58 (3H, m), 7.33–7.13 (6H, m); 7.04 (4H, br s); 5.39 (1H, d); 4.88 (1H, d); 3.69 (1H, t), 3.33 (2H, m); 2.83 (1H, t); 1.74–0.77 (10H, m); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 177.3 (C2); 169.2 (C5); 161.0 (C31); 155.9 (ArC); 140.7 (ArC); 138.8 (ArC); 135.7 (ArC); 134.5 (ArC); 132.8 (ArC); 130.0 (2 × ArC); 129.4 (2 × ArC); 128.7 (2 × ArC), 128.3 (ArC), 126.8 (ArC); 126.0 (ArC); 124.8 (2 × C ArC); 123.2 (ArC); 63.5 (C3); 49.1 (C23); 36.7 (C16); 32.4 (C10); 29.7 (2 × C, C11, C15); 26.4 (C13), 25.9 (C12); 25.6 (C14). HRMS: *m*/*z* calcd for $C_{30}H_{34}N_5O$ $[M+H]^+$: 480.2758, found: 480.2760. IR (KBr, cm^{-1}): 3323, 2931 and 2854 (NH); 1674 (C=O); 1600 (C=N). Anal. Calcd for $C_{30}H_{33}N_5O{\cdot}3HCl{\cdot}3H_2O$: C, 56.03; H, 6.58; N, 10.89. Found: C, 55.59; H, 6.31; N, 10.82.

4.64. (*S*)-1-(3-((3-Benzyl-5-cyclohexyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl) guanidine (64)

This reaction was carried out on a 0.21 mmol scale by the same method used to obtain **58**. Step 2 was carried out on a 0.20 mmol scale. White powder (79%). ^1H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 7.64 (3H, m), 7.35–7.17 (7H, m); 7.03 (1H, d); 6.94 (1H, d); 6.73 (1H, br s); 5.44 (1H, d); 4.84 (1H, d); 3.80 (1H, t), 3.33 (2H, m); 1.78–0.83 (10H, m); CH (H10) is not visible due to DMSO peak overlap and D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 181.0 (C2); 169.7 (C5); 156.3 (C31); 141.30 (ArC); 139.2 (ArC); 138.5 (ArC); 135.7 (ArC); 134.6 (ArC); 131.2 (ArC); 130.4 (2 × ArC); 129.6 (ArC); 129.5 (2 × ArC); 127.8 (ArC); 127.5 (ArC); 127.1 (ArC); 124.7 (ArC); 124.1 (ArC); 124.0 (2 × ArC); 63.4 (C3); 50.1 (C23); 36.1 (C16); 33.0 (C10); 30.2 (2 × C, C11, C15), 26.9 (C13); 26.2 (C12); 26.0 (C14). HRMS: *m*/*z* calcd for $C_{30}H_{34}N_5O$ $[M+H]^+$: 480.2758, found: 480.2760. IR (ATR, cm^{-1}): 3135, 2929 and 2855 (NH); 1666 (C=O); 1627 (C=N). Anal. Calcd for $C_{30}H_{33}N_5O{\cdot}3HCl{\cdot}3H_2O$: C, 56.03; H, 6.58; N, 10.89, found: C, 56.29; H, 6.40; N, 11.24.

4.65. (*S*)-1-(4-((3-Benzyl-2-oxo-5-(pyridin-2-yl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)methyl)phenyl) guanidine (65)

This reaction was carried out on a 0.16 mmol scale by the same method used to obtain **58** 1. Step 2 was carried out on a 0.16 mmol scale. White powder (85%). ^1H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 8.50 (1H, d); 7.97 (1H, t); 7.85 (1H, d); 7.52 (3H, br s); 7.26–7.16 (7H, m); 6.99 (2H, d); 6.90 (2H, d); 5.28 (1H, d); 4.98 (1H, d); 3.90 (1H, t); 3.43 (2H, m); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 169.3 (C2); 167.9 (C5); 155.9 (ArC); 155.2 (C31); 148.8 (ArC); 142.1 (ArC); 139.1 (ArC); 138.4 (ArC); 136.3 (ArC); 133.9 (ArC); 132.4 (ArC); 130.7 (ArC); 130.1 (2 × ArC); 129.3 (ArC); 128.8 (2 × ArC); 128.3 (2 × ArC); 126.9 (2 × ArC); 125.9 (ArC); 125.2 (2 × ArC); 124.2 (ArC); 122.7 (ArC); 65.6 (C3); 49.9 (C23); 37.5 (C16). HRMS: *m*/*z* calcd for $C_{29}H_{27}N_6O$ $[M+H]^+$: 475.2241, found: 475.2246. IR (ATR, cm^{-1}): 3147 and 2853 (NH); 1668 (C=O); 1633 (C=N). Anal. Calcd for $C_{29}H_{26}N_6O{\cdot}6HCl{\cdot}2H_2O$: C, 46.60; H, 5.12; N, 11.24. Found: C, 46.96; H, 5.22; N, 11.46.

4.66. (*S*)-1-(5-Cyclohexyl-3-isopropyl-2-oxo-2,3-dihydro-1*H*-*benzo[e]*[1,4]diazepin-7-yl)guanidine (66)

This reaction was carried out on a 0.21 mmol scale by the same method used to obtain **58**. Step 2 was carried out on a 0.20 mmol scale. White powder (highly hygroscopic, quantitative). ^1H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 7.65 (1H, br s); 7.45 (1H, d); 7.24 (1H, d); 3.67 (1H, br s); 3.46 (1H, m); 3.02 (1H, m); 1.94–0.81 (16H, m); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 177.6 (C2); 169.3 (C5); 156.4 (C20); 137.5 (ArC); 130.9 (ArC); 130.3 (ArC); 127.4 (ArC); 126.0 (ArC); 123.3 (ArC); 67.3 (C3); 45.0 (C16); 31.9 (C10); 30.1 (2 × C, C11, C15); 27.8 (C13); 25.92 (2 × C, C12, C14); 20.3 (C17); 19.1 (C18). HRMS: *m*/*z* calcd for $C_{19}H_{28}N_5O$ $[M+H]^+$: 342.2288, found: 342.2289. IR (ATR, cm $^{-1}$): 3112, 2924and 2853 (NH); 1710 (C=O); 1630 (C=N).

4.67. (*S*)-1-(3-(2-Oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]-diazepin-3-yl)propyl)guanidine (67)

Step 1: 2-Aminobenzophenone (0.49 g, 2.50 mmol), EEDQ (0.62 g, 2.50 mmol) and FMOC-Arg (Pbf)-OH (1.63 g, 2.50 mmol) were combined in DCM (20 mL) and stirred overnight at rt. The organic layer was washed successively with HCl (20 mL, 10% solution) and saturated Na_2CO_3 (100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was stirred with a 20% solution of diethylamine in acetonitrile (15 mL) at rt. After solvent concentration under reduced pressure followed by flash chromatography (DCM/ethylacetate 10:1), a yellow oil was obtained (0.62 g, 42%).

Step 2: The crude product from the previous step (0.20 g, 0.34 mmol) was stirred in TFA: H_2O: TIS (10 mL) 9.4:0.3:0.3 for 4 h. The resulting mixture was concentrated under reduced pressure and reconcentrated with Et_2O (20 mL × 2). When the crude product was triturated with Et_2O (20 mL), a white product **67** was obtained collected by filtration and dried in air (0.10 g, 88%).

^1H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 7.59–7.38 (6H, m); 7.19 (3H, m); 3.43 (1H, m); 3.14 (2H, t); 1.95 (2H, m); 1.64 (2H, m); D_2O exchanged NHs. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 171.5 (C2); 169.3 (C5); 157.0 (C20); 139.4 (ArC); 139.4 (ArC); 132.6 (ArC); 131.2 (2 × ArC); 130.0 (2 × ArC); 129.1 (2 × ArC); 127.3 (ArC); 123.9 (ArC); 121.8 (ArC); 65.7 (C3); 41.3 (C18); 28.5 (C16); 25.6 (C17). HRMS: *m*/*z* calcd for $C_{19}H_{22}N_5O$ $[M+H]^+$: 336.1819,

found: 336.1822. IR (ATR, cm^{-1}): 3180 (NH); 1666 (C=O); 1607 (C=N). CHN: calcd for $C_{19}H_{22}N_5O.1.4TFA{\cdot}0.04H_2O$ C, 52.81; H, 4.57; N, 14.13, found: C, 52.82; H, 5.04; N, 14.47.

4.68. (*S*)-3-Benzyl-1-(2-oxo-2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (68)

1-(2-Pyrimidyl)piperazine (54 μL, 0.38 mmol) was added to a solution of **32** (0.15 g, 0.38 mmol), HATU (0.21 g, 0.38 mmol), DMAP (0.003 g) and EDCI (0.11 g, 0.56 mmol) in DMF (5 mL). The solution was stirred at ambient temperature for 16 h. The resulting mixture was diluted with H_2O (30 mL), and extracted with ethyl acetate (20 mL × 2). The combined extracts were washed successively with a 5% $KHSO_4$ (20 mL) solution, saturated $NaHCO_3$ (20 mL) and brine (30 mL) and dried ($MgSO_4$), then filtered and concentrated. The crude product was purified by flash chromatography (10:1 DCM/acetone) to afford **68** as a white solid (44%).

^{1}H NMR (270 MHz) $CDCl_3$: δ 8.30 (2H, d); 7.54–7.10 (14H, m); 6.52 (1H, t); 4.86 (1H); 4.53 (1H, d); 3.93–3.48 (11H, m). ^{13}C NMR (270 MHz) $CDCl_3$: 170.20 (C2); 168.7 (C5); 166.2 (C24); 161.4 (C31); 157.7 (2 × ArC); 142.6 (ArC); 139.4 (ArC); 138.9 (ArC); 131.5 (ArC); 130.2 (2 × ArC); 129.8 (2 × ArC); 129.5 (2 × ArC); 129.4 (ArC); 128.1 (4 × ArC); 126.0 (ArC); 124.2 (ArC); 121.8 (ArC); 110.5 (ArC); 64.5 (C3); 49.7 (C23); 44.9 (C26); 43.5 (C27); 43.5 (C29); 42.0 (C30); 37.8 (C16). HRMS: *m*/*z* calcd for $C_{32}H_{31}N_6O_2$ $[M+H]^+$: 531.2503, found: 531.2496. IR (ATR, cm^{-1}): 1659 (C=O); 1602, 1583 and 1548 (3 × C=N).

4.69. (*S*)-3-Benzyl-1-(2-oxo-2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (69)

Compound **69** was made on a 0.42 mmol scale by the same method used to obtain **68**. Flash chromatography (10:1 DCM/acetone). Yield: white powder; 11%.

^{1}H NMR (270 MHz) $CDCl_3$: δ 8.17 (1H, d); 7.54–7.10 (15H); 6.60 (2H); 4.83 (1H); 4.55 (1H); 3.92 (1H, q, J= 4.8 Hz); 3.81–3.48 (10H, m, 5 × CH_2, H16, H26–27, H29–30). ^{13}C NMR (270 MHz) $CDCl_3$: δ 170.2 (C2); 168.7 (C5); 166.1 (C24); 158.8 (C31); 147.9 (ArC); 142.5 (ArC); 139.3 (ArC); 138.9 (ArC); 137.6 (ArC); 131.5 (ArC); 130.2 (2 × ArC); 129.8 (2 × ArC); 129.6 (2 × ArC); 129.5 (ArC); 128.1 (2 × ArC); 128.1 (2 × ArC); 126.0 (ArC); 124.2 (ArC); 121.8 (ArC); 113.9 (ArC); 107.2 (ArC); 64.5 (C3); 49.6 (C23); 45.0 (C26); 44.9 (C27); 44.8 (C29); 41.8 (C30); 37.8 (C16). HRMS: *m*/*z* calcd for $C_{33}H_{32}N_5O_2$ $[M+H]^+$: 530.2551, found: 530.2556. IR (ATR, cm^{-1}): 1658 (C=O); 1592 and 1564 (2 × C=N).

4.70. (*S*)-3-Benzyl-1-(2-oxo-2-(piperazin-1-yl)ethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (70)

Boc-**70** was prepared on a 0.34 mmol scale by the same method used to obtain **68**. Thereafter the crude was stirred with in HCl–dioxane (4 M, 10 mL) for 16 h. The solvent was removed at reduced pressure and the residue was reconcentrated with Et_2O (10 mL × 4). Et_2O was added and the mixture was triturated then dried to obtain a light yellow solid (71%).

^{1}H NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: δ 7.65–7.12 (14H, m); 4.89 (2H, m, CH_2); 3.68–3.40 (10H, m, 5 × CH_2). CH (H3) peak is concealed by H_2O peak. NH peaks are not visible. ^{13}C NMR (270 MHz) DMSO-$d_6{\cdot}D_2O$: 170.2 (C2); 169.2 (C5); 166.6 (C24); 142.9 (ArC); 138.9 (ArC); 137.8 (ArC); 133.1 (ArC); 131.7 (ArC); 130.6 (ArC); 130.2 (2 × ArC); 130.0 (2 × ArC); 129.1 (ArC); 128.9 (2 × ArC); 128.8 (2 × ArC); 126.9 (ArC); 125.3 (ArC); 123.1 (ArC); 66.8 (C3); 64.1 (C23); 49.3 (C26); 42.9 (C27); 41.6 (C29); 37.0 (C30); 31.2 (C16). HRMS: *m*/*z* calcd for $C_{28}H_{29}N_4O_2$ $[M+H]^+$: 453.2285, found: 453.2288. IR (ATR, cm^{-1}): 3387 (NH); 1695 and 1658 (2 × C=O); 1598 (C=N).

Acknowledgements

The School of Science, University of Greenwich, and GRE are thanked for a Ph.D. studentship (to RPR) and for providing a CHN analysis apparatus. Swansea University (EPSRC Mass Spectrometry Service Centre) are gratefully acknowledged for HRMS measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.010.

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