Bioorganic & Medicinal Chemistry 18 (2010) 4928–4938

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Constrained peptidomimetics as antiplasmodial falcipain-2 inhibitors

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ARTICLE INFO

Article history:
Received 7 April 2010
Revised 28 May 2010
Accepted 4 June 2010
Available online 10 June 2010

Keywords:
Peptidomimetics
Falcipain-2 inhibitors
Rhodesain inhibitors
Cysteine proteases

ABSTRACT

Herein we report the synthesis of a series of novel constrained peptidomimetics 2–10 endowed with a dipeptide backbone (D-Ser-Gly) and a vinyl ester warhead, structurally related to a previously identified lead compound **1**, an irreversible inhibitor of falcipain-2, the main haemoglobinase of lethal malaria parasite *Plasmodium falciparum*. The new compounds were evaluated for their inhibition against falcipain-2, as well as against cultured *P. falciparum*. The inhibitory activity of the synthesized compounds was also evaluated against another protozoal cysteine protease, namely rhodesain of *Trypanosoma brucei rhodesiense*.

1. Introduction

Malaria, a disease caused by several species of protozoa belonging to the genus *Plasmodium*, remains to be one of the most deadly parasitic diseases affecting about 250 million people worldwide and leading to more than 1 million deaths each year.[1] Taking into consideration that current antimalarial therapeutics become increasingly ineffective because of parasitic resistance, there exists an urgent need to develop and pursue new therapeutic strategies.[2]

Many drug targets can be related to the functions of distinct organellar structures. Of particular interest is the lysosomal food vacuole of *P. falciparum* parasite, the site of extensive hemoglobin degradation. One of the main proteases involved in this metabolic process is named falcipain-2 (FP-2). Its primary functions include degradation of host hemoglobin, required for sustaining the metabolic needs of rapidly growing parasite,[3] together with the cleavage of host cytoskeletal proteins[4] to facilitate the invasion and rupture of erythrocytes.

In view of its key role in *P. falciparum* life cycle, in the last decades a lot of FP-2 inhibitors have been developed[5] and in this regard our research group has been actively involved in the design and synthesis of novel peptidomimetic FP-2 inhibitors. In this context, these FP-2 inhibitors are based on a 1,4-benzodiazepine scaffold, as β-turn mimetic, introduced internally to a peptide sequence with mimics the fragment D-Ser-Gly, and various α,β-unsaturated electrophilic functions such as vinyl-sulfones, -phosphonates, -ketones, -nitriles, -amides, -esters,[6–8] that react as classical 'Michael acceptors', leading to an alkylated enzyme by irreversible addition of the thiol group of the active site cysteine. Among all the synthesized inhibitors, a surprising inhibitory potency was observed for the vinyl ester **1** (Fig. 1) which showed an impressive value of second-order rate constant of inhibition (k_{2nd} = 3,570,000 M^{-1} min^{-1}), a potency two times higher than that of the standard inhibitor E-64 (k_{2nd} = 1,586,000 M^{-1} min^{-1}) and the highest enzymatic affinity (K_i = 17 nM). Furthermore, lead compound **1** displayed an excellent inner-class selectivity towards the target enzyme with respect to human cysteine proteases cathepsins B and L. Compound **1** was also tested against cultured *P. falciparum* strain FCBR, showing an IC_{50} value of 12 μM.[8]

In view of these consistent results, we designed novel peptidomimetic analogues of compound **1**, whereas the electrophilic warhead was left unmodified and recognition elements were added along the constrained dipeptide framework, in order to

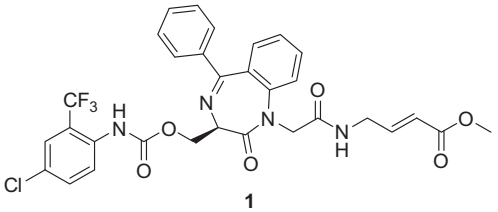

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Figure 1. Structure of FP-2 irreversible inhibitor **1**.

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doi:10.1016/j.bmc.2010.06.010

identify key interactions between inhibitor and the binding sites of the enzyme.

Specifically, at the P1 site the amino acid glycine was switched with leucine (i.e., **2**), useful to evaluate the relevance of the amino-acid chain in the process of recognition of the ligand by the enzyme. The P3 site was modified by introducing substituents with different electronic properties (i.e., Br, **4** or OCH_3, **5**) at C-4′ of the phenyl substituent on the benzodiazepine scaffold, and by introducing a chlorine atom (i.e., **3**) on the fused benzene ring. Moreover, the 4-chloro-2-trifluoromethylphenyl group appended to the side chain of serine at the P3 site by means of a carbamoyl moiety, was replaced by other aromatic rings (i.e., naphthyl, **7** or 4-methoxyphenyl, **6**) selected on the basis of our previous findings on related reversible inhibitors,[9] and (cyclo)alkyl groups (i.e., butyl **8**, cyclohexyl, **9** or adamantyl, **10**) in order to find out the capacity of this pocket in accommodating groups with different steric hindrance.

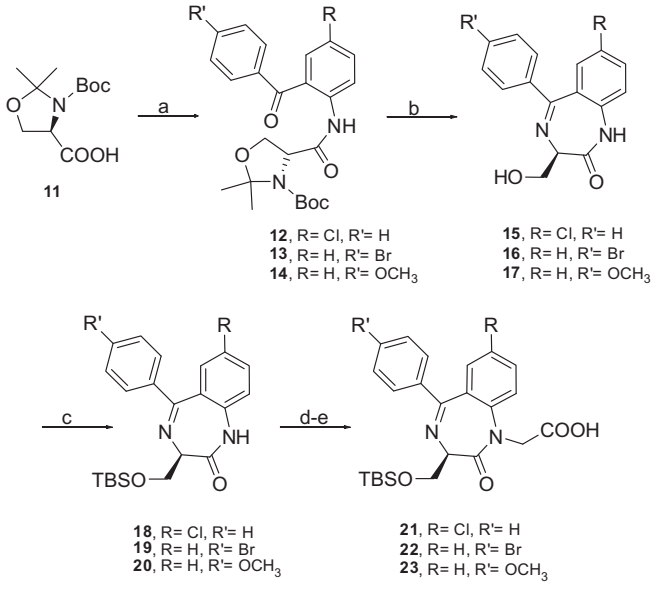
All compounds were tested on recombinant FP-2 and on cultured plasmodia in order to establish a structure–activity relationship for these recognition elements built on the lead structure **1**. Selectivity against the target enzymes was also estimated, testing inhibitors against papain-family human cysteine proteases such as cathepsins B and L. The most interesting compounds were also tested for their activity against rhodesain, another cysteine protease of papain-like family isolated from *T. brucei rhodesiense*, the causing agent of the sleeping sickness, spotting it as an alternative target.

2. Results and discussion

2.1. Chemistry

The constrained dipeptide framework substituted at the aromatic rings **21–23** (Scheme 1) were prepared according to a previously reported method for the unsubstituted analogue **24**.[9]

The oxazolidine derivative **11**[9] was converted into the corresponding mixed anhydride with *i*-butyl chloroformate and condensed in situ with the appropriate 2-aminobenzophenone to afford compounds **12–14**. Then, **12–14** underwent hydrolysis in acidic conditions, and the resultant 1,4-benzodiazepine derivatives 15–**17** were O-protected with TBS (**18–20**) and functionalized at N-1 with ethyl bromoacetate followed by hydrolysis of the ester moiety to give **21–23**.



Scheme 1. Reagents and conditions: (a) *i*-BuOCOCl, *N*-methylmorpholine, CH_2Cl_2, 0 °C to room temperature, 30 min, then appropriate 2-aminobenzophenone, reflux, 20 min, room temperature, 12 h; (b) HCl/MeOH, reflux, 5 h, $NaHCO_3$, then MeOH, room temperature, 12 h; (c) TBS-Cl, imidazole, CH_2Cl_2, 0 °C to room temperature, 12 h; (d) NaH, $BrCH_2COOEt$, 0 °C to room temperature, 5 h; (e) LiOH, MeOH, 0 °C to room temperature, 6 h.

The synthons **21–23** were coupled with commercially available allylamine **25** by means of HATU to afford intermediates **27–29**, whereas the unsubstituted synthon **24** was coupled with 3-methyl-1-vinyl-butylamine **26** (synthesized according to Albeck's work),[10] to give the intermediate **30**. Then, fluoride promoted desilylation of **27–30**, followed by treatment with the 4-chloro-2-trifluoromethyl isocyanate, afforded carbamates **31–34**.

The introduction of the required methyl ester warhead has been realized by cross-metathesis reaction using Hoveyda–Grubbs second generation catalyst,[11] a phosphine-free *N*-heterocyclic carbene–ruthenium complex.

Thus, cross-metathesis reactions between the tripeptides **31–34** and methyl acrylate proceeded under microwave irradiation[12] in an efficient and rapid manner to afford compounds **2–5** (Scheme 2).

By using the same synthetic procedure as for compounds **2–5**, compound **24** was coupled with allylamine and deprotected to give **35**[6] which was in sequence reacted with the suitable isocyanate, and conjugated through cross-metathesis to methyl acrylate to afford compounds **6–10** (Scheme 3).

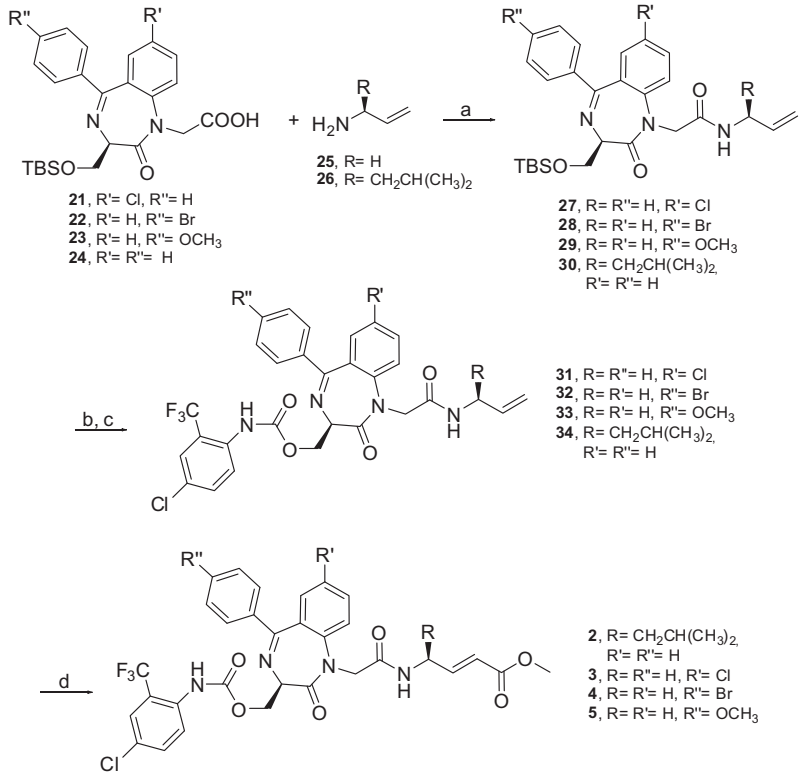
2.2. Pharmacology

Compounds **2–10** (Table 1) were tested for their inhibitory activity against recombinant FP-2[13] using Cbz-Phe-Arg-AMC as fluorogenic substrate.[14] First, a preliminary screening with inhibitor concentrations of 100 μM was performed. An equivalent volume of DMSO was used as negative control, and the irreversible standard inhibitor of clan CA, family C1 cysteine proteases (papain-family), namely E-64,[15] was used as positive control. For compounds which passed the initial screening, continuous assays (progress curve method)[16] were performed to determine the first-order rate constants of inhibition k_{inac} (min^{-1}), the dissociation constants K_i (μM), and the second-order rate constants of inhibition k_{2nd} ($M^{-1}\ min^{-1}$), with $k_{2nd} = k_{inac}/K_i$. In the case of time independent inhibition, K_i was determined using a Dixon plot.[17]

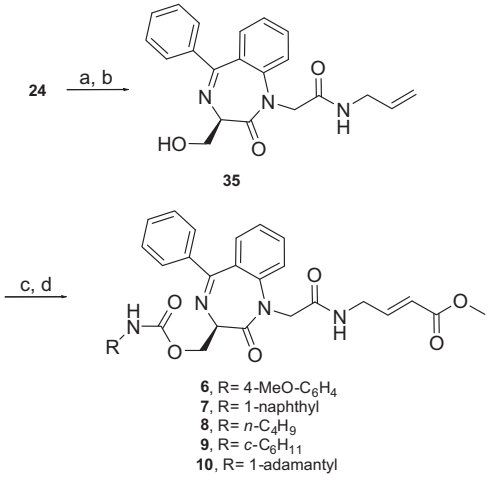
Among the novel synthesized compounds, only **5** and **8** didn't pass the preliminary screening. When a Leu residue was introduced at the P1 site (**2**) instead of the Gly residue of lead compound **1**, a strong decrease both in term of affinity and inhibitory potency was noticed (K_i = 17 nM and k_{2nd} = 3,570,000 $M^{-1}\ min^{-1}$ for **1** vs K_i = 2.22 μM and k_{2nd} = 18,000 $M^{-1}\ min^{-1}$ for **2**). This upshot is in disagreement to what is observed with other papain-like cysteine protease inhibitors, which usually accommodate bulky side chains closely associated with the electrophilic moiety.[18] Still, we observed a similar trend in our previous work, when we switched the Gly residue at the P1 site of **1** with a homoPhe residue.[6] So, we can assess for this class of FP-2 inhibitors endowed with a vinyl ester warhead, that the insertion of side chains at the P1 site is not a favorable design to construct a suitable recognition motif.

A comparable drop both in term of affinity and inhibitory potency can be also observed in compounds with substituents at the aromatic rings of the benzodiazepine scaffold, that is, **3–5,** suggesting that these aromatic portions of the skeleton are very sensitive to structural changes.

With compound **1** as a reference point and with these additional binding motif clues, we began an exploration of the group appended to the terminal serine hydroxyl by means of a carbamoyl moiety. The substitution of the 4-chloro-2-trifluoromethylphenyl



Scheme 2. Reagents and conditions: (a) HATU, CH_2Cl_2, room temperature, 12 h; (b) TBAF, THF, room temperature, 5 h; (c) 4-Cl-CF_3–C_6H_3NCO, CH_2Cl_2, room temperature, 12 h; (d) methyl acrylate, Hoveyda catalyst, CH_2Cl_2, 100 °C, MW, 2 h.



Scheme 3. Reagents and conditions: (a) allylamine, HATU, CH_2Cl_2, room temperature, 12 h; (b) TBAF, THF, rt, 5 h; (c) RNCO, CH_2Cl_2, room temperature, 12 h; (d) methyl acrylate, Hoveyda catalyst, CH_2Cl_2, 100 °C, MW, 2 h.

ring of **1** with other aromatic rings, that is, **6** and **7**, or with (cyclo)alkyl groups, that is, **8** and **9**, reduces the affinity for the target enzyme, in particular for the (cyclo)alkyl derivatives. In addition, the inhibition is reversible, and this can be probably due to a not proper orientation of the electrophilic warhead, preventing an irreversible alkylation of the thiol group of the target enzyme.

Surprisingly, the introduction of a bulkier hydrophobic group at the same site (i.e., compound **10**) gave an impressive irreversible inhibition with the same affinity and potency degree of lead compound **1**.

After determining the inhibition potencies of these compounds, the best candidates (**2**, **3**, **4**, **6**, **7**, and **10**) were tested against *P. falciparum* strain FCBR (Table 1). The dose-dependent effects of the compounds on parasite development were quantified using a previously published assay.[19]

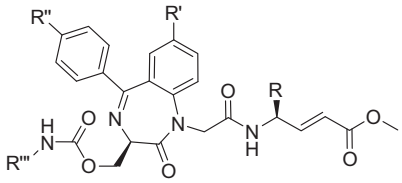
As shown in Table 1, all tested compounds displayed similar extent (micromolar range) of antiplasmodial activity against the test parasites, comparable to that of the reference compound **1**. In view of these data, a SAR discussion turns out to be a difficult task. However compounds **4** and **10** were found to exhibit the better antiplasmodial profile with IC_{50} values of 6.33 and 10.7 μM, respectively. This may be due to inhibition of the related protease falcipain-3 which also is involved in hemoglobin degradation.

This set of compounds which showed significant enzymatic inhibition and antiplasmodial activity (**2**, **3**, **4**, **6**, **7**, and **10**), as well as lead compound **1**, were considered for an assay against another papain-like protozoan cysteine protease, that is, rhodesain from *T. brucei rhodesiense* (Table 2).

Kinetic measurements against rhodesain showed the same trend of reaction mechanism and inhibitory activity of that observed for FP-2 for all compounds except **1**. Most importantly, all new compounds showed a relevant affinity toward rhodesain with K_i values three to fivefold lower than that of FP-2 (e.g.. **10**, K_i = 2.6 nM for rhodesain vs K_i = 14 nM for FP-2). The inhibitory potencies of new compounds toward rhodesain also resulted higher comparing to that observed toward FP-2.

The most representative compound **10** showed a remarkable value of second-order rate constant of inhibition (k_{2nd} = 4,620,000 M^{-1} min^{-1}). These results indicate that the adamantyl group anchors to a hollow pocket of rhodesain by means of extended hydrophobic interactions and ensures proper alignment of the peptide framework to the binding sites of the enzyme.

Table 1
Inhibition of FP-2 and antiplasmodial activity of compounds **2–10**[a]



Compd	R	R′	R″	R‴	k_{2nd} (M^{-1} min^{-1})	k_{inac} (min^{-1})	K_i (μM)	*P. falciparum* IC_{50} (μM)
2	*i*-C_4H_9	H	H	4-Cl-2-$CF_3C_6H_4$	18,000 ± 2000	0.040 ± 0.001	2.22 ± 0.37	26.9
3	H	Cl	H	4-Cl-2-$CF_3C_6H_4$	76,000 ± 26,000	0.030 ± 0.002	0.46 ± 0.18	28.9
4	H	H	Br	4-Cl-2-$CF_3C_6H_4$	24,500 ± 900	0.038 ± 0.002	1.56 ± 0.15	6.33
5	H	H	OCH_3	4-Cl-2-$CF_3C_6H_4$			>100	
6	H	H	H	4-MeO-C_6H_4	[b]	[b]	15.1 ± 1.4	33.1
7	H	H	H	1-Naphthyl	[b]	[b]	3.48 ± 0.21	17.6
8	H	H	H	*n*-C_4H_9			>100	
9	H	H	H	*c*-C_6H_{11}	[b]	[b]	89.0 ± 1.6	
10	H	H	H	1-Adamantyl	1,160,000 ± 570,000	0.013 ± 0.001	0.014 ± 0.007	10.7
1	H	H	H	4-Cl-2-$CF_3C_6H_4$	3,570,000 ± 400,000	0.06 ± 0.01	0.017 ± 0.004	12.0
E-64					1,586,000 ± 400,000	0.46 ± 0.07	0.29 ± 0.09	

[a] All results include standard deviations from two independent measurements, each performed in duplicate.
[b] Time independent inhibition.

Table 2
Inhibition of rhodesain of compounds **2–10**[a]

Compd	k_{2nd} (M^{-1} min^{-1})	k_{inac} (min^{-1})	K_i (μM)
2	36,000 ± 1000	0.031 ± 0.001	0.87 ± 0.05
3	220,000 ± 8000	0.019 ± 0.001	0.087 ± 0.003
4	46,000 ± 4000	0.022 ± 0.001	0.47 ± 0.03
6	[b]	[b]	4.3 ± 0.4
7	[b]	[b]	0.93 ± 0.02
10	4,620,000 ± 530,000	0.012 ± 0.001	0.0026 ± 0.0003
1	34,600 ± 200	0.028 ± 0.001	0.81 ± 0.02

[a] All results include standard deviations from two independent measurements, each performed in duplicate.
[b] Time independent inhibition.

Selectivity against the target enzymes (protozoan cysteine proteases) was also estimated, testing inhibitors against papain-family human cysteine proteases such as cathepsins B and L (Table 3).

In most cases, the compounds inhibit the enzymes in a time independent manner, showing K_i values one to two orders of magnitude higher than that for FP-2 inhibition, except for compounds **5**, **8**, and **9** that inhibit cathepsins B and L with K_i values lower comparing to that observed toward FP-2. The most representative compound **10** displayed an excellent selectivity toward FP-2 and rhodesain with respect to the inhibition of cathepsins B and L.

3. Conclusion

In conclusion, the structural modifications realized on lead compound **1** led to a drop in FP-2 inhibitory activity, with the exception of compound **10** which, besides a good FP-2 inhibitory properties, proved to inhibit rhodesain with an impressive potency and, when tested against human cysteine proteases, to possess a good selectivity towards the target enzymes. In view of the obtained results, compound **10** can be considered as a promising trypanocidal agent.

4. Experimental

4.1. Chemistry

4.1.1. Instruments and analyses

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were carried out on a C. Erba Model 1106 (Elemental Analyzer for C, H, and N) and the results are within ±0.4% of the theoretical values. Merck Silica Gel 60 F_{254} plates were used as analytical TLC; flash column chromatography was performed using Merck Silica Gel (200–400 mesh) on a

Table 3
Inhibition of human cathepsins B and L of compounds **2–10**[a]

Compd	Cathepsin B			Cathepsin L		
	k_{2nd} (M^{-1} min^{-1})	k_{inac} (min^{-1})	K_i (μM)	k_{2nd} (M^{-1} min^{-1})	k_{inac} (min^{-1})	K_i (μM)
2	[b]	[b]	39.1 ± 2.7			>150
3	[b]	[b]	6.1 ± 0.5	[b]	[b]	29.8 ± 3.4
4			>150			>150
5	[b]	[b]	10.9 ± 0.3	[b]	[b]	59.9 ± 2.7
6	[b]	[b]	2.23 ± 0.01	12,500 ± 3300	0.15 ± 0.01	12.0 ± 1.5
7	2000 ± 500	0.037 ± 0.001	25 ± 9	6400 ± 1400	0.079 ± 0.005	12.2 ± 3.4
8	[b]	[b]	20.4 ± 0.4	9400 ± 500	0.025 ± 0.001	2.7 ± 0.2
9	[b]	[b]	12.4 ± 0.1	[b]	[b]	76.5 ± 11.8
10	[b]	[b]	13.7 ± 2.5	550 ± 50	0.036 ± 0.001	65.0 ± 8.6
1	6000 ± 1000	0.044 ± 0.004	7.3 ± 3.2	[b]	[b]	8.4 ± 0.8

[a] All results include standard deviations from two independent measurements, each performed in duplicate.
[b] Time independent inhibition.

MP-LC BUCHI system. Preparative HPLC was performed on a Varian PrepStar SD-1 instrument equipped with a ProStar 325 UV–vis detector. The stationary phase was XTerra C_{18} column (5 μm, 19 × 150 mm); the mobile phase was a binary mixture of MeCN (containing 0.1% TFA) and water (containing 0.1% TFA) 55:45 at the flow rate of 5 mL min^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 300 spectrometer. ^1H chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hertz. ^{13}C chemical shifts are referenced to $CDCl_3$ (central peak, δ = 77.0 ppm).

4.1.1.1. (*R*)-*tert*-Butyl 4-(2-benzoyl-4-chlorophenylcarbamoyl)-2,2-dimethyloxazolidine-3-carboxylate (12). To a solution of acid **11**[9] (3.0 g, 12.2 mmol) in 40 mL of dry CH_2Cl_2 at 0 °C *N*-methyl morpholine (1.48 mL, 13.45 mmol) was added followed by *i*-butyl chloroformate (1.75 mL, 13.45 mmol). After 30 min., a solution of 2-amino-5-chloro-benzophenone (2.83 g, 12.2 mmol) in CH_2Cl_2 was added dropwise to refluxing reaction mixture over 20 min. After being stirred for 12 h at room temperature, the reaction mixture was washed with dilute HCl, aqueous $NaHCO_3$, and water. The organic layer was dried and concentrated to a residue and, after purification by column chromatography (petroleum ether/EtOAc 9:1) gave the product **12** (4.27 g, 76%). R_f = 0.31 (petroleum ether/EtOAc 9:1). ^1H NMR (300 MHz, $CDCl_3$): 1.23–1.85 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$), 4.10–4.24 (m, 2H, CH_2CH), 4.41–4.53 (m, 1H, CH_2CH), 7.46–7.72 (m, 7H, Ar), 8.69 (m, 1H, Ar), 11.16 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CHCl_3$): 24.42 ($C(CH_3)_2$), 28.51 ($C(CH_3)_2$), 62.07 (CH_2CH), 67.03 (CH_2CH), 76.23 ($C(CH_3)_3$), 106.31 ($C(CH_3)_2$), 123.17 (Ar), 123.27 (Ar), 125.62 (Ar), 127.96 (Ar), 128.69 (Ar), 130.21 (Ar), 132.62 (Ar), 133.18 (Ar), 133.82 (Ar), 147.91 (CO), 155.62 (CO), 171.08 (CO). Anal. Calcd for $C_{24}H_{27}ClN_2O_5$: C, 68.31; H, 5.93; N, 6.10. Found: C, 67.93; H, 6.28; N, 6.33.

4.1.1.2. (*R*)-*tert*-Butyl 4-(2-(4-bromobenzoyl)phenylcarbamoyl)-2,2-dimethyloxazolidine-3-carboxylate (13). With the same procedure employed for synthesis of **12**, intermediate **13** was obtained using 2-amino-4′-bromo-benzophenone (3.34 g, 12.2 mmol). Yield: 5.46 g (90%). R_f = 0.34 (petroleum ether/EtOAc 9:1). ^1H NMR (300 MHz, $CDCl_3$): 1.22–1.86 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$), 4.26–4.43 (m, 2H, CH_2CH), 4.50 (m, 1H, CH_2CH), 7.12 (m, 1H, Ar), 7.42–7.70 (m, 6H, Ar), 8.72 (d, 1H, J = 7.9 Hz, Ar), 11.25 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CHCl_3$): 22.98 ($C(CH_3)_2$), 23.98 ($C(CH_3)_2$), 28.18 ($C(CH_3)_3$), 66.73 (CH_2CH), 67.00 (CH_2CH), 77.37 ($C(CH_3)_3$), 108.32 ($C(CH_3)_2$), 121.41 (Ar), 122.46 (Ar), 124.33 (Ar), 128.16 (Ar), 129.95 (Ar), 131.67 (Ar), 132.35 (Ar), 133.17 (Ar), 133.88 (Ar), 151.36 (CO), 173.18 (CO), 198.51 (CO). Anal. Calcd for $C_{24}H_{27}BrN_2O_5$: C, 57.26; H, 5.41; N, 5.56. Found: C, 57.53; H, 5.20; N, 5.19.

4.1.1.3. (*R*)-*tert*-Butyl 4-(2-(4-methoxybenzoyl)phenyl-carbamoyl)-2,2-dimethyloxazolidine-3-carboxylate (14). With the same procedure employed for synthesis of **12**, intermediate **14** was obtained using 2-amino-4′-methoxy-benzophenone (2.76 g, 12.2 mmol). Yield: 3.77 g (63%). In this case 2-amino-4′-methoxy-benzophenone was synthesized according to Frye et al.[20] R_f = 0.25 (petroleum ether/EtOAc 8:2). ^1H NMR (300 MHz, $CDCl_3$): 1.32–1.56 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$), 3.83 (s, 3H, OCH_3), 3.87–4.13 (m, 2H, CH_2CH), 4.57 (m, 1H, CH_2CH), 7.10 (m, 2H, Ar), 7.29–7.76 (m, 5H, Ar), 8.52 (d, 1H, J = 7.9 Hz, Ar), 11.25 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 24.98 ($C(CH_3)_2$), 28.98 ($C(CH_3)_3$), 55.96 (OCH_3), 63.91 (CH_2CH), 69.18 (CH_2CH), 78.73 ($C(CH_3)_3$), 106.10 ($C(CH_3)_2$), 111.41 (Ar), 119.46 (Ar), 124.16 (Ar), 125.95 (Ar), 127.35 (Ar), 130.17 (Ar), 131.88 (Ar), 132.64 (Ar), 133.50 (Ar), 164.36 (CO), 172.75 (CO), 198.51 (CO). Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 5.41; N, 5.56. Found: C, 66.37; H, 5.61; N, 5.23.

4.1.1.4. (*R*)-7-Chloro-3-(hydroxymethyl)-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (15). To a solution of **12** (4.27 g, 9.3 mmol) in MeOH (10 mL) was added 6 N HCl (4 mL). The mixture was refluxed for 5 h, after this time the solvent was concentrated under reduced pressure and the crude was extracted with EtOAc and washed with $NaHCO_3$. The organic phase was dried and again concentrated. The mixture was dissolved in MeOH and stirred until complete conversion of the starting material into the desired product (12 h). The crude product was purified by flash chromatography (petroleum ether/EtOAc 4:6) (1.96 g, 70%). R_f = 0.32 (petroleum ether/EtOAc 4:6). ^1H NMR (300 MHz, $CDCl_3$): 3.05 (br s, 1H, CH_2OH), 3.72 (dd, 1H, J = 6.3 and J = 6.0 Hz, $CHCH_2$), 4.24 (dd, 1H, J = 9.9 and J = 6.0 Hz, CH_AOH), 4.43 (dd, 1H, J = 9.9 and J = 6.3 Hz, CH_BOH), 7.14–7.46 (m, 8H, Ar), 10.30 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 62.97 (CH_2OH), 64.18 ($CHCH_2$), 123.22 (Ar), 128.58 (Ar), 128.91 (Ar), 129.17 (Ar), 129.98 (Ar), 130.72 (Ar), 131.93 (Ar), 137.45 (Ar), 138.91 (Ar), 168.72 (C=N), 171.41 (CO). Anal. Calcd for $C_{16}H_{13}ClN_2O_2$: C, 63.90; H, 4.36; N, 11.79. Found: C, 64.09; H, 4.05 N, 12.13.

4.1.1.5. (*R*)-5-(4-Bromophenyl)-3-(hydroxymethyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (16). In analogy to **15**, compound **16** was obtained from **13** (5.46 g, 11.0 mmol) by acidic hydrolysis in methanol (3.46 g, 90.4%). R_f = 0.34 (petroleum ether/EtOAc 4:6). ^1H NMR (300 MHz, $CDCl_3$): 3.07 (br s, 1H, CH_2OH), 3.78 (dd, J = 7.1 and J = 5.5 Hz, 1H, $CHCH_2OH$), 4.24 (dd, 1H, J = 11.5 and J = 5.5 Hz, CH_AOH), 4.42 (dd, 1H, J = 11.5 and J = 7.1 Hz, CH_BOH), 7.15–7.626 (m, 8H, Ar), 9.42 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 62.97 (CH_2OH), 64.18 ($CHCH_2OH$), 121.71 (Ar), 124.02 (Ar), 125.46 (Ar), 127.39 (Ar), 131.21 (Ar), 131.62 (Ar), 132.23 (Ar), 132.29 (Ar), 138.07 (Ar), 138.31 (Ar), 168. 30 (C=N), 170.20 (CO). Anal. Calcd for $C_{16}H_{13}BrN_2O_2$: C, 55.67; H, 3.80; N, 8.12. Found: C, 55.39; H, 4.07 N, 7.81.

4.1.1.6. (*R*)-3-(Hydroxymethyl)-5-(4-methoxyphenyl)-1*H*-benzo[*e*] [1,4]diazepin-2(3*H*)-one (17). In analogy to **15**, compound **17** was obtained from **14** (3.77 g, 8.3 mmol) by acidic hydrolysis in methanol (1.82 g, 74%). R_f = 0.40 (petroleum ether/EtOAc 3:7) ^1H NMR (300 MHz, $CDCl_3$): 3.08 (br s, 1H, CH_2OH), 3.78 (dd, 1H, J = 7.1 and J = 5.5 Hz, $CHCH_2$), 3.81 (s, 3H, OCH_3), 4.24 (dd, 1H, J = 11.5 Hz and J = 5.5 Hz, CH_AOH), 4.41 (dd, 1H, J = 11.5 Hz and J = 7.1 Hz, CH_BOH), 6.86 (d, 2H, J = 8.5 Hz, Ar), 7.12–7.27 (m, 2H, Ar), 7.34 (d, 1H, J = 7.7 Hz, Ar), 7.46–7.49 (m, 3H, Ar), 9.55 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 55.80 (OCH_3), 62.97 ($CHCH_2$), 64.18 ($CHCH_2$), 121.71 (Ar), 124.02 (Ar), 125.46 (Ar), 127.39 (Ar), 131.21 (Ar), 131.62 (Ar), 132.23 (Ar), 132.29 (Ar), 138.07 (Ar), 138.31 (Ar), 168.11 (C=N), 170.20 (CO). Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 68.91; H, 5.44; N, 9.45. Found: C, 69.07; H, 5.18; N, 9.21.

4.1.1.7. (*R*)-3-((*tert*-Butyldimethylsilyloxy)methyl)-7-chloro-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2-(3*H*)-one (18). To a solution of **15** (1.96 g, 6.5 mmol) in CH_2Cl_2 (40 mL) were added imidazole (0.66 g, 9.75 mmol) and TBS-Cl (1.47 g, 9.75 mmol) at 0 °C. After stirring 12 h at room temperature the mixture was washed with water and dried. The crude product was purified by flash chromatography to afford the title compound (petroleum ether/EtOAc 8:2) (2.43 g, 89.8%). R_f = 0.35 (petroleum ether/EtOAc 8:2). ^1H NMR (300 MHz, $CDCl_3$): 0.14 (s, 6H, $Si(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 3.69 (dd, 1H, J = 6.3 and J = 6.0 Hz, $CHCH_2$), 4.25 (dd, 1H, J = 10.0 and J = 6.0 Hz, $CHCH_A$), 4.56 (dd, 1H, J = 10.0 and J = 6.3 Hz, $CHCH_B$), 7.14–7.46 (m, 8H, Ar), 10.30 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): −4.83 ($Si(CH_3)_2$), 17.20 ($C(CH_3)_3$), 26.34 ($C(CH_3)_3$), 63.92 ($CHCH_2$), 65.69 ($CHCH_2$), 123.22 (Ar), 128.58 (Ar), 128.91 (Ar), 129.17 (Ar), 129.98 (Ar), 130.72 (Ar), 131.93 (Ar), 137.45 (Ar), 138.91 (Ar), 168.72 (C=N), 171.41 (CO). Anal. Calcd for

$C_{22}H_{27}ClN_2O_2Si$: C, 63.67; H, 6.56; N, 8.54. Found: C, 64.01; H, 6.87; N, 8.19.

4.1.1.8. (*R*)-5-(4-Bromophenyl)-3-((*tert*-butyldimethylsilyloxy) methyl)-1*H*-benzo[*e*][1,4]diazepin-2-(3*H*)-one (19). With a similar procedure, **19** was prepared from **16** (3.46 g, 10.0 mmol), imidazole (1.0 g, 14.8 mmol), and TBS-Cl (2.26 g, 14.8 mmol). Yield: 3.92 g (84.7%). R_f = 0.32 (petroleum ether/EtOAc 8:2). ^{1}H NMR (300 MHz, $CDCl_3$): 0.09 (s, 3H, $Si(CH_3)_2$), 0.10 (s, 3H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 3.64 (dd, 1H, J = 6.3 and J = 6.0 Hz, $CHCH_2$), 4.20 (dd, 1H, J = 10.1 and J = 6.3 Hz, $CHCH_2$), 4.52 (dd, 1H, J = 10.1 and J = 6.0 Hz, $CHCH_2$), 7.03 (t, 1H, J = 7.4 Hz, Ar), 7.17 (d, 2H, J = 7.9 Hz, Ar), 7.29 (d, 2H, J = 8.5 Hz, Ar), 7.35–7.42 (m, 3H, Ar), 10.26 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): −4.83 ($Si(CH_3)_3$), 26.28 ($C(CH_3)_3$), 43.34 ($C(CH_3)_3$), 63.99 ($CHCH_2$), 65.65 ($CHCH_2$), 121.95 (Ar), 123.55 (Ar), 125.17 (Ar), 127.39 (Ar), 131.01 (Ar), 131.61 (Ar), 132.0 (Ar), 138.37 (Ar), 138.90 (Ar), 168.88 (Ar), 171.50 (CO). Anal. Calcd for $C_{22}H_{27}BrN_2O_2Si$: C, 57.51; H, 5.92; N, 6.10. Found: C, 57.90; H, 5.61; N, 6.44.

4.1.1.9. (*R*)-3-((*tert*-Butyldimethylsilyloxy)methyl)-5-(4-methoxyphenyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (20). With a similar procedure, **20** was prepared from **17** (1.82 g, 6.14 mmol), imidazole (0.62 g, 9.2 mmol), and TBS-Cl (1.39 g, 9.2 mmol). Yield: 1.97 g (78.5%). R_f = 0.75 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 0.09 (s, 3H, $Si(CH_3)_2$), 0.10 (s, 3H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 3.10 (s, 3H, OCH_3), 3.66 (dd, 1H, J = 6.3 and J = 6.0 Hz, $CHCH_2$), 4.24 (dd, 1H, J = 10.1 and J = 6.3 Hz, $CHCH_2$), 4.41 (dd, 1H, J = 10.1 and J = 6.0 Hz, $CHCH_2$), 6.86 (d, 2H, J = 8.5 Hz, Ar), 7.12–7.27 (m, 2H, Ar), 7.34 (d, 1H, J = 7.7 Hz, Ar), 7.46–7.49 (m, 3H, Ar), 9.55 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): −4.83 ($Si(CH_3)_3$), 26.28 ($C(CH_3)_3$), 43.34 ($C(CH_3)_3$), 62.97 ($CHCH_2$), 64.18 ($CHCH_2$), 121.71 (Ar), 124.02 (Ar), 125.46 (Ar), 127.39 (Ar), 131.21 (Ar), 131.62 (Ar), 132.23 (Ar), 138.07 (Ar), 138.31 (Ar), 170.23 (CO). Anal. Calcd for $C_{23}H_{30}N_2O_3Si$: C, 67.28; H, 7.36; N, 6.82. Found: C, 67.50; H, 7.59; N, 6.51.

4.1.1.10. (*R*)-2-(3-((*tert*-Butyldimethylsilyloxy)methyl)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetic acid (21). To a suspension of NaH (153 mg, 6.38 mmol) in dry DMF (10 mL) was added a solution of **18** (2.43 g, 5.85 mmol) in DMF (10 mL) at 0 °C under nitrogen and then the reaction mixture was slowly brought to room temperature. After stirring 30 min. at room temperature, ethyl bromoacetate (0.8 mL, 7.3 mmol) was added via syringe and further stirred for 5 h. The reaction mixture was quenched with satd aqueous NH_4Cl (30 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with water, dried, concentrated, and the resulting residue was purified on silica gel column chromatography (petroleum ether/EtOAc 8:2) to obtain the ester derivative as a yellowish oil (2.64 g, 90.3%). R_f = 0.51 (petroleum ether/EtOAc 8:2). ^{1}H NMR (300 MHz, $CDCl_3$): 0.02 (s, 3H, $Si(CH_3)_2$), 0.03 (s, 3H, $Si(CH_3)_2$), 0.78 (s, 9H, $C(CH_3)_3$), 1.11 (t, 3H, J = 6.8 Hz, OCH_2CH_3), 3.69 (dd, 1H, J = 6.3 J = 6.0 Hz, $CHCH_2$), 3.89–4.06 (m, 3H, OCH_2CH_3 and $CHCH_A$), 4.16–4.21 (m, 1H, $CHCH_B$), 4.31–4.48 (m, 3H, NCH_2), 7.11–7.33 (m, 7H, Ar), 7.49 (d, 1H, J = 7.7 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −4.95 ($Si(CH_3)_2$), 14.22 (OCH_2CH_3), 23.22 ($C(CH_3)_3$), 26.90 ($C(CH_3)_3$), 49.54 (NCH_2), 61.65 (OCH_2CH_3), 64.15 ($CHCH_2$), 65.48 ($CHCH_2$), 123.56 (Ar), 128.49 (Ar), 129.77 (Ar), 130.02 (Ar), 130.75 (Ar), 131.59 (Ar), 131.77 (Ar), 132.87 (Ar), 138.44 (Ar), 141.20 (Ar), 168.56 (C=N), 168.73 (CO), 170.92 (CO). To a solution of the ester (2.64 g, 5.3 mmol) in a mixture of methanol/water 1:1 (20 mL) was added LiOH (251 mg, 10.6 mmol) keeping the temperature at 0 °C and stirred at room temperature until the disappearance of the starting material (TLC monitoring). The solvent was concentrated under reduced pressure and the mixture was treated with 10% citric acid and extracted with ethyl acetate (3 × 30 mL), dried and concentrated to give the acid **21** (1.78 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): 0.02 (s, 3H, $Si(CH_3)_2$), 0.03 (s, 3H, $Si(CH_3)_2$), 0.78 (s, 9H, $C(CH_3)_3$), 3.69 (dd, 1H, J = 6.3 and J = 6.0 Hz, $CHCH_2O$), 4.31 (dd, 1H, J = 10.0 and J = 6.3 Hz, $CHCH_A$), 4.16–4.30 (m, 3H, $CHCH_B$ and NCH_2), 7.11–7.33 (m, 7H, Ar), 7.49 (d, 1H, J = 7.7 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −4.95 ($Si(CH_3)_2$), 23.22 ($C(CH_3)_3$), 26.90 ($C(CH_3)_3$), 49.54 (NCH_2), 64.15 ($CHCH_2$), 65.48 ($CHCH_2$), 123.56 (Ar), 128.49 (Ar), 129.77 (Ar), 130.02 (Ar), 130.75 (Ar), 131.59 (Ar), 131.77 (Ar), 132.87 (Ar), 138.44 (Ar), 141.20 (Ar), 168.56 (C=N), 168.73 (CO), 170.92 (CO). Anal. Calcd for $C_{24}H_{29}ClN_2O_4Si$: C, 60.94; H, 6.18; N, 5.92. Found: C, 61.28; H, 5.87; N, 5.63.

4.1.1.11. (*R*)-2-(5-(4-Bromophenyl)-3-((*tert*-butyldimethyl-silyloxy)methyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl) acetic acid (22). Compound **22** was obtained from **19** (3.92 g, 8.5 mmol) employing the procedure described for compound **21**. The ethyl ester intermediate was obtained in 90.1% yield (4.16 g), after purification by flash chromatography (petroleum ether/EtOAc 8:2). R_f = 0.54 (petroleum ether/EtOAc 8:2). ^{1}H NMR (300 MHz, $CDCl_3$): 0.00 (s, 3H, $Si(CH_3)_2$), 0.02 (s, 3H, $Si(CH_3)_2$), 0.78 (s, 9H, $C(CH_3)_3$), 1.03 (t, 3H, J = 6.8 Hz, OCH_2CH_3), 3.70 (dd, 1H, J = 6.3 and J = 5.8 Hz, $CHCH_2$), 3.90–4.08 (m, 3H, OCH_2CH_3 and $CHCH_A$), 4.10–4.21 (m, 1H, $CHCH_B$), 4.36–4.52 (m, 3H, NCH_2), 7.05–7.42 (m, 8H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −4.90 ($Si(CH_3)_2$), 14.33 (OCH_2CH_3), 21.73 ($C(CH_3)_3$), 25.57 ($C(CH_3)_3$), 49.66 (NCH_2), 61.67 (OCH_2CH_3), 64.19 ($CHCH_2$), 65.46 ($CHCH_2$), 122.06 (Ar), 124.82 (Ar), 125.16 (Ar), 128.52 (Ar), 129.79 (Ar), 130.22 (Ar), 131.46 (Ar), 131.58 (Ar), 131.99 (Ar), 137.93 (Ar) 142.61 (Ar), 168.28 (C=N), 168.99 (CO), 170.92 (CO). Compound **22** was obtained in 65.6% yield (2.61 g,). ^{1}H NMR (300 MHz, $CDCl_3$): 0.05 (s, 3H, $Si(CH_3)_2$), 0.03 (s, 3H, $Si(CH_3)_2$), 0.78 (s, 9H, $C(CH_3)_3$), 3.70 (dd, 1H, J = 6.3 and J = 5.8 Hz, $CHCH_2$), 4.16–4.22 (m, 1H, $CHCH_A$), 4.38–4.54 (m, 3H, $CHCH_B$ and NCH_2), 6.70 (br s, 1H, COOH), 7.09–7.46 (m, 8H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −4.95 ($Si(CH_3)_2$), 23.22 ($C(CH_3)_3$), 26.90 ($C(CH_3)_3$), 49.54 (NCH_2), 64.15 ($CHCH_2$), 65.48 ($CHCH_2$), 123.56 (Ar), 128.49 (Ar), 129.77 (Ar), 130.02 (Ar), 130.75 (Ar), 131.59 (Ar), 131.77 (Ar), 132.87 (Ar), 138.44 (Ar), 141.20 (Ar), 168.56 (C=N), 168.73 (CO), 170.92 (CO). Anal. Calcd for $C_{24}H_{29}BrN_2O_4Si$: C, 55.70; H, 5.65; N, 5.41. Found: C, 55.45; H, 5.99; N, 5.06.

4.1.1.12. (*R*)-2-(3-((*tert*-butyldimethylsilyloxy)methyl)-5-(4-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl) acetic acid (23). Compound **23** was obtained from **20** (1.97 g, 4.8 mmol) employing the procedure described for compound **21**. The ethyl ester intermediate was obtained in 97.1% yield (2.24 g), after purification by flash chromatography (petroleum ether/EtOAc 7:3). R_f = 0.63 (petroleum ether/EtOAc 7:3). ^{1}H NMR (300 MHz, $CDCl_3$): 0.07 (s, 3H, $Si(CH_3)_2$), 0.08 (s, 3H, $Si(CH_3)_2$), 0.84 (s, 9H, $C(CH_3)_3$), 1.10 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 3.76 (s, 3H, OCH_3), 3.73–3.87 (m, 1H, $CHCH_2$), 4.01–4.26 (m, 2H, OCH_2CH_3 and NCH_2), 4.42–4.61 (m, 2H, $CHCH_2$). 6.82 (d, 2H, J = 8.8 Hz, Ar), 7.14 (t, 1 H, J = 7.1 Hz, Ar), 7.23–7.28 (m, 2H, Ar), 7.46 (t, 1 H, J = 7.1 Hz, Ar), 7.53 (d, 2H, J = 8.8 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −4.90 ($Si(CH_3)_2$), 14.33 (OCH_2CH_3), 21.73 ($C(CH_3)_3$), 25.57 ($C(CH_3)_3$), 49.66 (NCH_2), 55.81 (OCH_3), 61.67 (OCH_2CH_3), 64.19 ($CHCH_2$), 65.46 ($CHCH_2$), 122.06 (Ar), 124.82 (Ar), 125.44 (Ar), 128.52 (Ar), 129.79 (Ar), 130.22 (Ar), 131.46 (Ar), 131.58 (Ar), 132.99 (Ar), 137.93 (Ar) 142.61 (Ar), 168.28 (C=N), 168.99 (CO), 170.92 (CO). Compound **23** was obtained in 84% yield (1.75 g,). ^{1}H NMR (300 MHz, D_2O): −0.03 (s, 3H, $Si(CH_3)_2$), 0.00 (s, 3H, $Si(CH_3)_2$), 0.76 (s, 9H, $C(CH_3)_3$), 3.68 (s, 3H, OCH_3), 3.72 (dd, 1H, J = 6.3 and J = 5.8 Hz, $CHCH_2$), 4.16–4.22 (m, 1H, $CHCH_A$), 4.24–4.46 (m, 3H, $CHCH_B$ and NCH_2), 7.13–7.53 (m, 8H, Ar). ^{13}C NMR

(75 MHz, D_2O): −4.97 ($Si(CH_3)_2$), 23.09 ($C(CH_3)_3$), 26.82 ($C(CH_3)_3$), 50.71 (NCH_2), 55.54 (OCH_3), 64.15 ($CHCH_2$), 65.79 ($CHCH_2$), 120.61 (Ar), 124.54 (Ar), 129.76 (Ar), 130.05 (Ar), 130.90 (Ar), 131.62 (Ar), 131.81 (Ar), 131.94 (Ar), 138.44 (Ar), 148.52 (Ar), 168.56 (C=N), 168.73 (CO), 170.92 (CO). Anal. Calcd for $C_{25}H_{32}N_2O_5Si$: C, 64.08; H, 6.88; N, 5.98. Found: C, 64.40; H, 6.57; N, 6.20.

4.1.1.13. (*R*)-*N*-Allyl-2-(3-((*tert*-butyldimethylsilyloxy)methyl)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-1-yl)acetamide (27). To a solution **21** (300 mg, 0.63 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added HATU (361 mg, 0.95 mmol). After 5 min, the ice bath was removed and a solution of amine **25** (72 mg, 1.26 mmol) in CH_2Cl_2 (5 mL) was added. The resulting mixture was stirred at room temperature for 12 h and washed with water (2 × 50 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with petroleum ether/EtOAc (5:5) to give title compound **27** (300 mg, 92.4%) as a yellow oil. R_f = 0.31 (petroleum ether/EtOAc 5:5). ^1H NMR (300 MHz, $CDCl_3$): 0.02 (s, 3H, $Si(CH_3)_2$), 0.04 (s, 3H, $Si(CH_3)_2$), 0.80 (s, 9H, $C(CH_3)_3$), 3.67–3.72 (m, 3H, $NHCH_2$ and $CHCH_2$), 4.07 (d, 1H, J = 15.4 Hz, NCH_A), 4.16 (dd, 1H, J = 10.2 and 6.3 Hz, $CHCH_A$), 4.47 (dd, 1H, J = 10.2 and 6.3 Hz, $CHCH_B$), 4.54 (d, 1H, J = 15.4 Hz, NCH_B), 4.91–4.95 (m, 2H, $CH=CH_2$), 5.56–5.65 (m, 1H, $CH=CH_2$), 6.38 (br t, 1H, NH), 7.15–7.60 (m, 8H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −5.75 ($Si(CH_3)_2$), 25.84 ($C(CH_3)_3$), 30.62 ($C(CH_3)_3$), 41.76 ($NHCH_2$), 54.06 (NCH_2), 62.97 ($CHCH_2$), 68.19 ($CHCH_2$), 116.24 ($CH=CH_2$), 121.77 (Ar), 124.59 (Ar), 127.32 (Ar), 128.93 (Ar), 130.50 (Ar), 131.02 (Ar), 131.17 (Ar), 133.24 ($CH=CH_2$), 138.06 (Ar), 168.34 (C=N), 171.17 (CO), 171.21 (CO). Anal. Calcd for $C_{27}H_{34}ClN_3O_3Si$: C, 63.62; H, 6.69; N, 8.20. Found: C, 63.40; H, 7.01; N, 8.03.

4.1.1.14. (*R*)-*N*-Allyl-2-(5-(4-bromophenyl)-3-((*tert*-butyldimethylsilyloxy)methyl)-2-oxo-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-1-yl)acetamide (28). Compound **28** was obtained from **22** (250 mg, 0.48 mmol) and amine **25** (55 mg, 0.96 mmol) employing the procedure described for compound **27** The residue was purified by flash chromatography, eluting with petroleum ether/EtOAc (5:5), to afford compound **28** (240 mg, 89.3%). R_f = 0.33 (petroleum ether/EtOAc 5:5). ^1H NMR (300 MHz, $CDCl_3$): 0.00 (s, 3H, $Si(CH_3)_2$), 0.02 (s, 3H, $Si(CH_3)_2$), 0.78 (s, 9H, $C(CH_3)_3$), 3.60–3.69 (m, 3H, $NHCH_2$ and $CHCH_2$), 4.13 (d, 1H, J = 15.1 Hz, NCH_A), 4.10–4.15 (m, 1H, $CHCH_A$), 4.46 (dd, 1H, J = 10.1 and 7.4 Hz, $CHCH_B$), 4.55 (d, 1H, J = 15.3 Hz, NCH_B), 4.89–4.98 (m, 2H, $CH=CH_2$), 5.52–5.63 (m, 1H, $CH=CH_2$), 6.38 (br t, 1H, NH), 7.09–7.18 (m, 2H, Ar), 7.34–7.57 (m, 6H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −6.31 ($Si(CH_3)_2$), 26.22 ($C(CH_3)_3$), 28.70 ($C(CH_3)_3$), 42.21 ($NHCH_2$), 64.05 (NCH_2), 65.71 ($CHCH_2$), 52.72 ($CHCH_2$), 116.77 ($CH=CH_2$), 123.00 (Ar), 125.19 (Ar), 125.53 (Ar), 1289.19 (Ar), 130.06 (Ar), 130.20 (Ar), 131.46 (Ar), 131.76 (Ar), 132.43 (Ar), 133.71 ($CH=CH_2$), 137.58 (Ar), 167.42 (C=N), 170.09 (CO), 171.20 (CO). Anal. Calcd for $C_{27}H_{34}BrN_3O_3Si$: C, 58.27; H, 6.16; N, 7.55. Found: C, 57.60; H, 6.47; N, 7.86.

4.1.1.15. (*R*)-*N*-Allyl-2-(3-((*tert*-butyldimethylsilyloxy)methyl)-5-(4-methoxyphenyl)-2-oxo-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-1-yl)acetamide (29). Compound **29** was obtained from **23** (200 mg, 0.42 mmol) and amine **25** (48 mg, 0.86 mmol) employing the procedure described for compound **27** The residue was purified by flash chromatography, eluting with petroleum ether/EtOAc (4:6), to afford compound **29** (212 mg, 97.8%). R_f = 0.53 (petroleum ether/EtOAc 4:6). ^1H NMR (300 MHz, $CDCl_3$): 0.01 (s, 3H, $Si(CH_3)_2$), 0.03 (s, 3H, $Si(CH_3)_2$), 0.79 (s, 9H, $C(CH_3)_3$), 3.68–3.72 (m, 3H, $NHCH_2$ and $CHCH_2$), 3.73 (s, 3H, OCH_3), 4.09–4.20 (m, 2H, NCH_A and $CHCH_A$), 4.41–4.63 (m, 2H, $CHCH_B$ and NCH_B), 4.85–4.95 (m, 2H, $CH=CH_2$), 5.51–5.61 (m, 1H, $CH=CH_2$), 6.38 (br t, 1H, NH), 6.78 (d, 2H, J = 8.8 Hz, Ar), 7.15–7.52 (m, 6H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −5.23 ($Si(CH_3)_2$), 25.92 ($C(CH_3)_3$), 30.61 ($C(CH_3)_3$), 43.61 ($NHCH_2$), 55.88 (OCH_3), 56.10 (NCH_2), 65.71 ($CHCH_2$), 67.32 ($CHCH_2$), 117.41 ($CH=CH_2$), 121.00 (Ar), 123.20 (Ar), 124.70 (Ar), 128.22 (Ar), 129.64 (Ar), 130.20 (Ar), 131.20 (Ar), 131.46 (Ar), 132.74 (Ar), 134.26 ($CH=CH_2$), 137.58 (Ar), 168.42 (N=C), 170.09 (CO), 171.20 (CO). Anal. Calcd for $C_{28}H_{37}N_3O_4Si$: C, 66.24; H, 7.35; N, 8.28. Found: C, 66.00; H, 7.66; N, 7.93.

4.1.1.16. 2-((*R*)-3-((*tert*-Butyldimethylsilyloxy)methyl)-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)-*N*-(5-methylhex-1-en-3-yl)acetamide (30). Compound **30** was obtained from **24** (200 mg, 0.60 mmol) and amine **26** (0.14 g, 1.2 mmol) employing the procedure described for compound **27**. The residue was purified by flash chromatography, eluting with petroleum ether/EtOAc (8:2), to afford compound **30** (0.31 g, 80%). R_f = 0.35 (petroleum ether/EtOAc 5:5). ^1H NMR (300 MHz, $CDCl_3$): 0.07 (s, 3H, $Si(CH_3)_2$), 0.08 (s, 3H, $Si(CH_3)_2$), 0.73–0.82 (m, 6H, $CH(CH_3)_2$), 0.89 (s, 9H, $C(CH_3)_3$), 1.27–1.52 (m, 3H, CH_2CH and $CH(CH_3)_2$), 4.17–4.30 (m, 3H, $CHCH_A$, $CHCH_2$ and $NHCH$), 4.38–4.43 (m, 2H, $CHCH_B$ and NCH_A), 4.57 (d, 1H, J = 15.9 Hz, NCH_B), 4.85–5.22 (m, 2H, $CH=CH_2$), 5.51–5.60 (m, 1H, $CH=CH_2$), 6.55 (br s, 1H, NH), 7.11–7.51 (m, 9H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): −5.23 ($Si(CH_3)_2$), 20.70 ($CH(CH_3)_2$), 21.15 ($C(CH_3)_3$), 24.83 ($CH(CH_3)_2$), 25.99 ($CH(CH_3)_2$), 31.45 ($CHCH_2$), 46.80 ($CHCH_2$), 57.30 (NCH_2), 62.34 ($CHCH_2$), 63.87 ($CHCH_2$), 114.90 ($CH=CH_2$), 122.68 (Ar), 125.15 (Ar), 128.55 (Ar), 129.65 (Ar), 129.94 (Ar), 130.45 (Ar), 130.97 (Ar), 132.36 ($CH=CH_2$), 138.44 (Ar), 138.56 (Ar), 142.34 (Ar), 167.82 (C=N), 170.15 (CO), 171.39 (CO). Anal. Calcd for $C_{31}H_{43}N_3O_3Si$: C, 69.75; H, 8.12; N, 7.87. Found: C, 69.99; H, 7.92; N, 7.54.

4.1.1.17. (*R*)-(1-(2-Allylamino)-2-oxoethyl)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-3-yl)methyl 4-chloro-2-(trifluoromethyl)phenylcarbamate (31). To a solution of **27** (300 mg, 0.58 mmol) in dry THF (10 mL) was added TBAF (1 M solution in THF, 0.87 mL, 0.87 mmol) dropwise. The mixture was stirred at room temperature until disappearance of the starting material (TLC monitoring), and then it was diluted with EtOAc (20 mL) and washed with water (3 × 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the deprotected compound, which was purified by flash chromatography eluting with petroleum ether/EtOAc 2:8 (150 mg, 64.4%). ^1H NMR (300 MHz, $CDCl_3$): 3.95–3.99 (m, 3H, $NHCH_2$ and $CHCH_2$), 4.34–4.39 (m, 2H, NCH_A and $CHCH_A$), 4.51 (m, 1H, $CHCH_B$), 4.72 (d, 1H, J = 15.4 Hz, NCH_B), 5.18–5.33 (m, 2H, $CH=CH_2$), 5.88 (m, 1H, $CH=CH_2$), 6.62 (br t, 1H, NH), 7.40–7.96 (m, 8H, Ar). To a solution of *N*-allyl-2-(7-chloro-3-hydroxymethyl-2-oxo-5-phenyl-2,3-dihydro-benzo[*e*][1,4]diazepin-1-yl-acetamide (50 mg, 0.12 mmol) in dry CH_2Cl_2 (5 mL) under N_2 was added 4-Cl-2-$CF_3C_6H_3NCO$ (56 mg, 37 μL, 0.25 mmol) and the resulting mixture was stirred for 12 h at room temperature. After this time, the mixture was washed with water (2 × 10 mL). The residue was purified by flash chromatography, eluting with petroleum ether/EtOAc 6:4, to afford compound **31** (55 mg, 71%). R_f = 0.40 (petroleum ether/EtOAc 4:6). ^1H NMR (300 MHz, $CDCl_3$): 3.84 (t, 2H, J = 5.7 Hz, $NHCH_2$), 4.04 (t, 1H, J = 6.5 Hz, $CHCH_2$), 4.24 (d, 1H, J = 15.1 Hz, NCH_A), 4.61 (d, 1H, J = 15.1 Hz, NCH_B), 4.89–5.13 (m, 4H, $CHCH_2$ and $CH=CH_2$), 5.72 (m, 1H, $CH=CH_2$), 6.37 (br t, 1H, NH), 6.96 (s, 1H, NH), 7.26–7.62 (m, 9H, Ar), 7.75 (d, 1H, J = 8.8 Hz,), 8.11 (d, 1H, J = 9.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 41.75 ($NHCH_2$), 54.03 (NCH_2), 60.38 ($CHCH_2$), 65.86 ($CHCH_2$), 114.62 (Ar), 116.25 ($CH=CH_2$), 121.75 (Ar), 121.98 (Ar), 123.35

(Ar), 124.58 (CF_3), 125.25 (Ar), 127.34 (Ar), 128.97 (Ar), 129.08 (Ar), 129.24 (Ar), 129.46 (Ar), 130.27 (Ar), 131.17 (Ar), 131.32 (Ar), 132.45 (Ar), 134.38 (*CH*=CH_2), 139.04 (Ar), 140.75 (Ar), 153.97 (CO), 168.67 (C=N), 171.12 (CO), 171.24 (CO). Anal. Calcd for $C_{29}H_{23}Cl_2F_3N_4O_4$: C, 56.25; H, 3.74; N, 9.04. Found: C, 56.03; H, 4.04; N, 8.71.

4.1.1.18. (*R*)-(1-(2-Allylamino)-2-oxoethyl)-5-(4-bromophenyl)-2-oxo-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-3-yl)methyl 4-chloro-2-(trifluoromethyl)phenylcarbamate (32). Compound **32** was obtained from **28** (250 mg, 0.45 mmol) employing the procedure described for compound **31**. Intermediate *N*-allyl-2-(5-(4-bromo-phenyl)-3-hydroxymethyl-2-oxo-2,3-dihydro-benzo[*e*][1,4] diazepin-1-yl-acetamide was obtained in 90.6% yield (180 mg), after purification by flash chromatography (petroleum ether/EtOAc 2:8). ^{1}H NMR (300 MHz, $CDCl_3$): 3.72–3.93 (m, 3H, NH*CH*$_2$ and *CH*CH_2), 4.18–4.37 (m, 3H, NCH_A and CH*CH*$_2$), 4.57 (d, 1H, J = 15.6 Hz, NCH_B), 5.00–5.09 (m, 2H, CH=*CH*$_2$), 5.70 (m, 1H, *CH*=CH_2), 6.45 (br t, 1H, NH), 7.19–7.64 (m, 8H, Ar). Title compound **32** was purified by flash chromatography, eluting with petroleum ether/EtOAc (4:6) (50 mg, 72.5%). R_f = 0.42 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 3.70–3.84 (m, 2H, NH*CH*$_2$), 4.03 (dd, 1H, J = 6.3 and J = 5.8 Hz, *CH*CH_2), 4.31 (d, 1H, J = 15.4 Hz, NCH_A), 4.64 (d, 1H, J = 15.4 Hz, NCH_B), 4.87–5.12 (m, 4H, CH*CH*$_2$ and CH=*CH*$_2$), 5.73 (m, 1H, *CH*=CH_2), 6.38 (br t, 1H, NH), 6.94 (s, 1H, NH), 7.27–7.63 (m, 9H, Ar), 7.74 (d, 1H, J = 8.0 Hz, Ar), 8.09 (d, 1H, J = 8.8 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 41.25 ($NHCH_2$), 54.02 (NCH_2), 60.35 (*CH*CH_2), 64.86 (CH*CH*$_2$), 114.60 (Ar), 116.15 (CH=*CH*$_2$), 121.62 (Ar), 121.99 (Ar), 123.32 (Ar), 124.59 (CF_3), 125.27 (Ar), 127.31 (Ar), 127.93 (Ar), 129.12 (Ar), 129.27 (Ar), 129.49 (Ar), 131.27 (Ar), 131.33 (Ar), 131.35 (Ar), 132.45 (Ar), 134.39 (*CH*=CH_2), 139.10 (Ar), 140.73 (Ar), 154.97 (CO), 168.68 (C=N), 171.21 (CO), 171.24 (CO). Anal. Calcd for $C_{29}H_{23}BrClF_3N_4O_4$: C, 52.47; H, 3.49; N, 8.44. Found: C, 52.77; H, 3.80; N, 8.75.

4.1.1.19. (*R*)-(1-(2-Allylamino)-2-oxoethyl)-5-(4-methoxyphenyl)-2-oxo-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-3-yl)methyl 4-chloro-2-(trifluoromethyl)phenylcarbamate (33). Compound **33** was obtained from **29** (106 mg, 0.21 mmol) employing the procedure described for compound **31**. Intermediate *N*-allyl-2-(3-hydroxymethyl-5-(4-methoxy-phenyl)-2-oxo-2,3-dihydro-benzo[*e*] [1,4]diazepin-1-yl-acetamide was obtained in 61% yield (50 mg), after purification by flash chromatography (petroleum ether/EtOAc 1:9). ^{1}H NMR (300 MHz, $CDCl_3$): 3.68–3.75 (m, 3H, NH*CH*$_2$ and *CH*CH_2), 3.77 (s, 3H, OCH_3), 4.12–4.28 (m, 3H, NCH_A and CH*CH*$_2$), 4.53 (d, 1H, J = 15.4 Hz, NCH_B), 4.94–5.02 (m, 2H, CH=*CH*$_2$), 5.63 (m, 1H, *CH*=CH_2), 6.37 (br t, 1H, NH), 6.82 (d, 2H, J = 8.5 Hz, Ar), 7.15–7.59 (m, 6H, Ar). Title compound **33** was purified by flash chromatography, eluting with petroleum ether/EtOAc (5:5) (40 mg, 51.2%). R_f = 0.36 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 3.86–3.92 (m 2H, NH*CH*$_2$), 3.89 (s, 3H, OCH_3), 4.05 (dd, 1H, J = 6.3 and J = 5.8 Hz, *CH*CH_2), 4.38 (d, 1H, J = 15.4 Hz, NCH_A), 4.85 (d, 1H, J = 15.4 Hz, NCH_B), 4.90–5.13 (m, 4H, CH*CH*$_2$ and CH=*CH*$_2$), 5.73 (m, 1H, *CH*=CH_2), 6.41 (br t, 1H, NH), 6.93–6.99 (m, 3H, NH and Ar), 7.30–7.66 (m, 6H, Ar), 7.76 (d, 1H, J = 8.2 Hz, Ar), 8.13 (d, 1H, J = 8.6 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 41.28 ($NHCH_2$), 55.12 (NCH_2), 55.82 (OCH_3), 60.25 (*CH*CH_2), 64.73 (CH*CH*$_2$), 115.60 (Ar), 116.35 (CH=*CH*$_2$), 122.62 (Ar), 123.97 (Ar), 124.31 (Ar), 124.69 (CF_3), 125.33 (Ar), 126.44 (Ar), 127.88 (Ar), 129.34 (Ar), 129.46 (Ar), 129.51 (Ar), 131.27 (Ar), 131.33 (Ar), 131.38 (Ar), 132.56 (Ar), 134.44 (*CH*=CH_2), 139.17 (Ar), 142.38 (Ar), 155.12 (CO), 168.34 (C=N), 171.22 (CO), 171.25 (CO). Anal. Calcd for $C_{30}H_{26}ClF_3N_4O_5$: C, 58.59; H, 4.26; N, 9.11. Found: C, 58.77; H, 4.57; N, 8.98.

4.1.1.20. (*R*)-(1-(2-(5-methylhex-1-en-3-ylamino)-2-oxoethyl)-2-oxo-5-phenyl-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-3-yl)methyl 4-chloro-2-(trifluoromethyl)phenylcarbamate (34). Compound **34** was obtained from **30** (0.31 g, 0.58 mmol) employing the procedure described for compound **31**. The intermediate 2-(3-hydroxymethyl-2-oxo-5-phenyl-2,3-dihydro-benzo[*e*][1,4]diazepin-1-yl)-*N*-3-methyl-1-vinyl-butyl)-acetamide was obtained in 63% yield (155 mg), after purification by flash chromatography (petroleum ether/EtOAc 2:8). ^{1}H NMR (300 MHz, $CDCl_3$): 0.74 (d, 3H, J = 6.0 Hz, CH(*CH*$_3$)$_2$), 0.76 (d, 3H, J = 6.0 Hz, CH(*CH*$_3$)$_2$), 1.27–1.52 (m, 3H, *CH*$_2$CH and *CH*(CH$_3$)$_2$), 3.94–4.07 (m, 3H, *CH*$_A$OH, *CH*CH$_2$OH and NH*CH*), 4.30–4.35 (m, 2H, *CH*$_B$OH and NCH_A), 4.57 (d, 1H, J = 15.9 Hz, NCH_B), 4.85–5.22 (m, 2H, CH=*CH*$_2$), 5.51–5.60 (m, 1H, *CH*=CH_2), 6.55 (d, 1H, J = 8.5 Hz, NH), 7.11–7.51 (m, 9H, Ar). Title compound **34** was purified by flash chromatography, eluting with petroleum ether/EtOAc (5:5) (150 mg, 63.4%). R_f = 0.31 (petroleum ether/EtOAc 6:4). ^{1}H NMR (300 MHz, $CDCl_3$): 0.85 (d, 3H, J = 6.3 Hz, CH(*CH*$_3$)$_2$), 0.87 (d, 3H, J = 6.0 Hz, CH(*CH*$_3$)$_2$), 1.46–1.62 (m, 3H, *CH*$_2$CH and *CH*(CH$_3$)$_2$), 4.07 (dd, 1H, J = 6.6 and J = 6.0 Hz, *CH*CH$_2$O), 4.30 (d, 1H, J = 15.1 Hz, NCH_A), 4.41–4.58 (m, 1H, NH*CH*), 4.71 (d, 1H, J = 15.1 Hz, NCH_B), 4.85–5.17 (m, 4H, CH*CH*$_2$O and CH=*CH*$_2$), 5.66–5.77 (m, 1H, *CH*=CH_2), 6.36 (d, 1H, J = 8.5 Hz, *NH*CH), 7.0 (br s, 1H, NHCO), 7.27–7.64 (m, 11H, Ar), 8.15 (d, 1H, J = 9.3 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 22.90 (CH(*CH*$_3$)$_2$), 23.45 (CH(*CH*$_3$)$_2$), 25.01 (*CH*(CH$_3$)$_2$), 43.65 (CH*CH*$_2$), 51.98 (*CH*CH_2), 53.34 (NCH_2), 62.38 (CH*CH*$_2$O), 65.80 (*CH*CH_2O), 111.13 (Ar), 115.36 (Ar), 116.61 (CH=*CH*$_2$), 120.77 (Ar), 123.27 (Ar), 125.57 (CF_3), 126.70 (Ar), 128.92 (Ar), 130.20 (Ar), 131.28 (Ar), 132.35 (Ar), 132.65 (*CH*=CH_2), 133.29 (Ar), 134.60 (Ar), 138.48 (Ar), 139.0 (Ar), 148.28 (Ar), 152.57 (CO), 161.75 (C=N), 168.10 (CO), 170.15 (CO). Anal. Calcd for $C_{33}H_{32}ClF_3N_4O_4$: C, 61.83; H, 5.03; N, 8.74. Found: C, 61.63; H, 4.89; N, 9.02.

4.1.1.21. Methyl 4-(2-((*R*)-3-((4-chloro-2-(trifluoromethyl)phenylcarbamoyloxy)methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetamido)-6-methylhept-2-enoate (2). To a solution of **34** (41 mg, 0.063 mmol) in dry CH_2Cl_2 (5 mL) was added methyl acrylate (55 mg, 0.64 mmol) followed by Hoveyda–Grubbs 2nd generation catalyst [(1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)-dichloro-(*o*-isopropoxyphenylmethylene)ruthenium] (4.5 mg, 0.0072 mmol). The resulting mixture was heated under microwave irradiation at 100 °C for 2 h. The solvent was then removed under reduced pressure and the residue was purified by preparative HPLC to give the title compound as a white solid (34 mg, 76.1%). HPLC: retention time 8.76 min. R_f = 0.24 (petroleum ether/EtOAc 6:4). ^{1}H NMR (300 MHz, $CDCl_3$): 0.81 (d, 3H, J = 6.3 Hz, CH(*CH*$_3$)$_2$), 0.84 (d, 3H, J = 6.0 Hz, CH(*CH*$_3$)$_2$), 1.33–1.59 (m, 3H, *CH*$_2$CH and *CH*(CH$_3$)$_2$), 3.65 (s, 3H, OCH_3), 4.04 (dd, 1H, J = 6.6 and J = 6.0 Hz, *CH*CH$_2$O), 4.25 (d, 1H, J = 15.1 Hz, NCH_A), 4.58–4.68 (m, 2H, NCH_B and NH*CH*), 4.94 (dd, 1H, J = 11.5 and J = 6.6 Hz, CH*CH*$_A$O), 5.08 (dd, 1H, J = 11.5 and J = 6.0 Hz, CH*CH*$_B$O), 5.88 (d, 1H, J = 15.6 Hz, CH=*CH*CO), 6.54 (d, 1H, J = 8.2 Hz, *NH*CH), 6.80 (dd, 1H, J = 15.6 and 5.2 Hz, *CH*=CHCO), 7.20 (br s, 1H, NHCO), 7.37–7.63 (m, 10H, Ar), 7.77 (d, 1H, J = 8.2 Hz, Ar), 8.03 (d, 1H, J = 8.2 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 22.78 (CH(*CH*$_3$)$_2$), 23.41 (CH(*CH*$_3$)$_2$), 24.89 (*CH*(CH$_3$)$_2$), 43.55 (CH*CH*$_2$), 48.95 (OCH_3), 51.89 (*CH*CH_2), 53.29 (N*CH*$_2$), 62.26 (*CH*CH_2O), 65.70 (CH*CH*$_2$O), 120.67 (Ar), 123.07 (Ar), 125.50 (Ar), 126.54 (Ar), 128.66 (Ar), 129.48 (CF_3), 129.59 (Ar), 130.10 (Ar), 130.73 (Ar), 131.18 (Ar), 132.59 (Ar), 133.17 (Ar), 134.49 (Ar), 138.36 (Ar), 142.41 (Ar), 148.13 (*CH*=CHCO), 152.43 (CO), 153.57 (Ar), 161.62 (Ar), 167.95 (CH=*CH*CO), 166.52 (CO), 167.98 (C=N), 170.18 (CO). 170.76 (CO). Anal. Calcd for $C_{35}H_{34}ClF_3N_4O_6$: C, 60.13; H, 4.90; N, 8.01. Found: C, 60.33; H, 4.77; N, 7.90.

4.1.1.22. (*R*)-Methyl 4-(2-(7-chloro-3-((4-chloro-2-(trifluoromethyl) phenylcarbamoyloxy)methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (3). Compound **31** (30 mg, 0.048 mmol) was reacted with methyl acrylate (41 mg, 0.48 mmol) according to the procedure described for **2**. The title compound was obtained after purification by preparative HPLC as a white solid (25 mg, 76.2%). HPLC: retention time 51.35 min. R_f = 0.44 (petroleum ether/EtOAc 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (s, 3H, OCH_3), 3.94–4.06 (m, $NHCH_2$ and $CHCH_2$), 4.16 (d, 1H, J = 15.1 Hz, NCH_A), 4.55 (d, 1H, J = 15.1 Hz, NCH_B), 4.83–4.99 (m, 2H, $CHCH_2$) 5.78 (d, 1H, J = 15.6, CH=*CH*CO), 6.52 (br t, 1H, NH), 6.77 (dr t, 1H, J = 15.6 and J = 4.7 Hz, *CH*=CHCO), 6.97 (s, 1H, NH), 7.19–7.54 (m, 9H, Ar), 7.67 (d, 1H, J = 8.8 Hz, Ar), 7.99 (d, 1H, J = 8.8 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 40.51 ($NHCH_2$), 47.80 (OCH_3), 52.89 (NCH_2), 62.26 ($CHCH_2$), 65.63 ($CHCH_2$), 121.93 (Ar), 121.99 (Ar), 124.56 (CF_3), 124.79 (CH=*CH*CO), 126.56 (Ar), 128.73 (Ar), 128.89 (Ar), 129.79 (Ar), 130.18 (Ar), 130.83 (Ar), 131.17 (Ar), 131.19 (Ar), 131.48 (Ar), 132.67 (Ar), 133.24 (Ar), 137.69 (Ar), 143.34 (Ar), 143.51 (*CH*=CHCO), 158.24 (CO), 166.34 (CO), 168.10 (C=N), 172.22 (CO), 173.06 (CO). Anal. Calcd for $C_{31}H_{25}Cl_2F_3N_4O_6$: C, 54.96; H, 3.72; N, 8.27. Found: C, 55.22; H, 3.41; N, 8.48.

4.1.1.23. (*R*)-Methyl 4-(2-(5-(4-bromophenyl)-3-((4-chloro-2-(trifluoromethyl)phenylcarbamoyloxy) methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (4). Compound **32** (25 mg, 0.041 mmol) was reacted with methyl acrylate (35 mg, 0.41 mmol) according to the procedure described for **2**. The title compound was obtained after purification by preparative HPLC as a white solid (29 mg, 98.2%). HPLC: retention time 40.52 min. R_f = 0.42 (petroleum ether/EtOAc 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (s, 3H, OCH_3), 3.93–3.97 (m, $NHCH_2$ and $CHCH_2$), 4.27 (d, 1H, J = 15.1 Hz, NCH_A), 4.58 (d, 1H, J = 15.1 Hz, NCH_B), 4.87–4.96 (m, 2H, $CHCH_2$) 5.83 (d, 1H, J = 15.2, CH=*CH*CO), 6.56 (br t, 1H, NH), 6.78 (dt, 1H, J = 15.2 and J = 4.7 Hz, *CH*=CH_2CO), 6.96 (s, 1H, NH), 7.19–7.57 (m, 9H, Ar), 7.66 (d, 1H, J = 8.2 Hz, Ar), 7.97 (d, 1H, J = 9.06 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 40.51 ($NHCH_2$), 51.86 (OCH_3), 52.88 (NCH_2), 62.32 ($CHCH_2$), 65.64 ($CHCH_2$), 121.59 (Ar), 121.89 (Ar), 123.18 (CH=*CH*CO), 124.84 (Ar), 125.21 (Ar), 125.63 (Ar), 125.93 (CF_3), 126.48 (Ar), 129.00 (Ar), 129.51 (Ar), 130.42 (Ar), 131.50 (Ar), 131.87 (Ar), 132.86 (Ar), 133.23 (Ar), 134.39 (Ar), 137.21 (Ar), 142.46 (Ar), 143.70 (*CH*=CHCO), 153.39 (CO), 166.56 (CO), 168.43 (C=N), 169.30 (CO), 171.19 (CO). Anal. Calcd for $C_{31}H_{25}BrClF_3N_4O_6$: C, 51.58; H, 3.49; N, 7.76. Found: C, 51.73; H, 3.11; N, 7.60.

4.1.1.24. (*R*)-Methyl 4-(2-(3-((4-chloro-2-(trifluoromethyl) phenylcarbamoyloxy)-5-(methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (5). Compound **33** (15 mg, 0.024 mmol) was reacted with methyl acrylate (20 mg, 0.24 mmol) according to the procedure described for **2**. The title compound was obtained after purification by flash chromatography, eluting with petroleum ether/EtOAc 2:8 (16 mg, 97.8%). R_f = 0.35 (petroleum ether/EtOAc 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): 3.61 (s, 3H, OCH_3), 3.78 (s, 3H, $ArOCH_3$), 3.85–3.97 (m, $NHCH_2$ and $CHCH_2$), 4.26 (d, 1H, J = 15.4 Hz, NCH_A), 4.59 (d, 1H, J = 15.4 Hz, NCH_B), 4.81–5.01 (m, 2H, $CHCH_2$) 5.75 (d, 1H, J = 15.6, CH=*CH*CO), 6.52 (br t, 1H, NH), 6.74 (dt, 1H, J = 15.6 and 4.7 Hz, *CH*=CH_2CO), 6.83 (d, 2H, J = 8.8 Hz, Ar), 6.98 (s, 1H, NH), 7.19–7.56 (m, 7H, Ar), 7.65 (d, 1H, J = 8.5 Hz, Ar), 7.99 (d, 1H, J = 8.5 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 40.53 ($NHCH_2$), 51.30 (OCH_3), 52.84 (NCH_2), 55.75 ($ArOCH_3$), 62.03 ($CHCH_2$), 65.77 ($CHCH_2$), 114.05 (Ar), 121.75 (Ar), 121.85 (Ar), 122.77 (Ar), 123.02 (CH=*CH*CO), 124.23 (CF_3), 124.84 (Ar), 125.58 (Ar), 126.13 (Ar), 126.53 (Ar), 129.66 (Ar), 130.78 (Ar), 131.39 (Ar), 131.69 (Ar), 132.59 (Ar), 133.22 (Ar), 142.38 (Ar), 143.66 (*CH*=CHCO), 147.18 (Ar), 161.79 (CO), 169.01 (C=N), 182.26 (CO), 182.31 (CO), 192.70 (CO). Anal. Calcd for $C_{32}H_{28}ClF_3N_4O_7$: C, 55.11; H, 4.19; N, 8.32. Found: C, 55.35; H, 4.07; N, 8.40.

4.1.1.25. (*R*)-Methyl 4-(2-(3-((4-methoxyphenylcarbamoyloxy) methyl)-2-oxo-5-phenyl-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (6). To a solution of *N*-allyl-2-((3*R*)-2,3-dihydro-3-hydroxymethyl-2-oxo-5-phenyl-benzo[*e*][1,4]diazepin-1-yl-acetamide **35**[6] (100 mg, 0.27 mmol) in dry CH_2Cl_2 (15 mL) under N_2 was added 4-$OCH_3C_6H_4NCO$ (82 mg, 0.071 mL, 0.54 mmol) and the resulting mixture was stirred for 12 h at room temperature. After this time, the mixture was washed with water (2 × 15 mL). The residue was purified by flash chromatography, eluting with petroleum ether/EtOAc 4:6, to afford the intermediate (4-methoxy-phenyl)-carbamic acid 1-allylcarbamoylmethyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-ylmethyl ester (100 mg, 72.2%). R_f = 0.35 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 3.75 (s, 3H, OCH_3), 3.80–3.90 (m, 2H, $NHCH_2$), 4.01 (m, 1H, $CHCH_2$), 4.35 (d, 1H, J = 15.6 Hz, NCH_A), 4.62 (d, 1H, J = 15.6 Hz, NCH_B), 4.90–5.08 (m, 4H, CH=CH_2 and $CHCH_2$), 5.68 (m, 1H, *CH*=CH_2), 6.36 (br s, 1H, NH), 6.75–6.82 (m, 2H, Ar), 7.24–765 (m, 11H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 42.17 ($NHCH_2$), 52.56 (OCH_3), 55.76 (NCH_2), 62.70 ($CHCH_2$), 64.34 ($CHCH_2$), 116.62 (CH=CH_2), 122.84 (Ar), 123.11 (Ar), 125.23 (Ar), 128.20 (Ar), 128.53 (Ar), 129.61 (Ar), 129.98 (Ar), 130.56 (Ar), 130.95 (Ar), 132.35 (Ar), 133.76 (*CH*=CH_2), 138.51 (Ar), 114.53 (Ar), 142.47 (Ar), 144.04 (Ar), 157.25 (CO), 169.17 (C=N), 169.82 (CO), 170.02 (CO). To a solution of the intermediate (50 mg, 0.097 mmol) methyl acrylate (84 mg, 0.97 mmol) was added followed by Hoveyda–Grubbs 2nd generation catalyst (12 mg, 0.0195 mmol). The resulting mixture was heated under microwave irradiation at 100 °C for 2 h. The solvent was then removed under reduced pressure and the residue was purified by preparative HPLC as a white solid (40 mg, 81%). HPLC: retention time 20.66 min. R_f = 0.18 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 3.76 (s, 3H, $COOCH_3$), 3.80 (s, 3H, $ArOCH_3$), 4.07 (m, 1H, $CHCH_2$), 4.15–4.24 (m, 3H, $NHCH_2$ and NCH_A), 4.78 (d, 1H, J = 15.2 Hz, NCH_B), 4.98–5.11 (m, 2H, $CHCH_2$), 5.93 (d, 1H, J = 16.2 Hz, CH=*CH*CO), 6.87–7.04 (m, 5H, 2NH, *CH*=CHCO and Ar), 7.31–7.98 (m, 11H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 40.40 ($NHCH_2$), 53.37 (OCH_3), 53.56 (NCH_2), 55.76 ($ArOCH_3$), 62.75 ($CHCH_2$), 64.49 ($CHCH_2$), 114.41 (Ar), 114.53 (Ar) (CH=*CH*CO), 122.84 (Ar), 123.11 (Ar), 125.23 (Ar), 128.20 (Ar), 128.53 (Ar), 129.61 (Ar), 129.98 (Ar), 130.56 (Ar), 130.95 (Ar), 132.35 (Ar), 138.51 (Ar), 142.53 (Ar), 142.47 (Ar), 144.04 (*CH*=CHCO), 154.25 (CO), 166.57 (CO), 169.17 (C=N), 169.82 (CO), 170.2 (CO). Anal. Calcd for $C_{31}H_{30}N_4O_7$: C, 65.25; H, 5.30; N, 9.82. Found: C, 65.06; H, 5.52; N, 9.71.

4.1.1.26. (*R*)-Methyl 4-(2-(3-((naphthalen-1-ylcarbamoyloxy) methyl)-2-oxo-5-phenyl-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin -1-yl)acetamido)but-2-enoate (7). Compound **35** (100 mg, 0.27 mmol) was reacted with 1-naphthyl isocyanate (93 mg, 0.072 mL, 0.54 mmol) according to the same procedure described for compound **6**. The intermediate naphthalen-2-yl-carbamic acid 1-allylcarbamoylmethyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl-methyl ester was obtained in 76.5% yield (110 mg), after purification by flash chromatography (petroleum ether/EtOAc 4:6). R_f = 0.38 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 3.81 (dd, 1H, J = 6.0 and J = 5.5 Hz, $CHCH_2$), 4.06–4.12 (m, 2H, $NHCH_2$), 4.34 (d, 1H, J = 15.3 Hz, NCH_A), 4.64 (d, 1H, J = 15.3 Hz, NCH_B), 4.95–5.13 (m, 4H, $CHCH_2$ and CH=CH_2), 5.74 (m, 1H, *CH*=CH_2), 6.43 (br s, 1H, NH), 7.22–7.87 (m, 16H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 45.36 ($NHCH_2$), 53.19 (NCH_2), 62.66 ($CHCH_2$), 65.00 ($CHCH_2$), 109.42 (Ar), 119.88 (CH=CH_2), 121.19 (Ar), 122.84 (Ar), 123.14 (Ar), 124.70 (Ar), 125.53 (Ar), 126.01 (Ar), 126.31 (Ar), 127.50 (Ar), 128.67 (Ar), 128.89 (Ar), 129.38

(Ar), 130.07 (Ar), 130.70 (Ar), 131.15 (Ar), 132.60 (Ar), 134.30 (*CH*=CH_2), 136.53 (Ar), 142.51 (Ar), 144.01 (Ar), 153.90 (CO), 154.70 (C=N), 170.13 (CO), 171.14 (CO). This intermediate (50 mg, 0.093 mmol) was reacted with methyl acrylate (80 mg, 0.93 mmol). The title compound was obtained after purification by preparative HPLC (40 mg, 72.4%). HPLC: retention time 25.62 min. R_f = 0.21 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 3.64 (s, 3H, OCH_3), 4.01 (m, 1H, *CH*CH_2), 4.12–4.20 (m, 2H, NH*CH*$_2$), 4.29 (d, 1H, *J* = 15.1 Hz, NCH_A), 4.75 (d, 1H, *J* = 15.1 Hz, NCH_B), 5.06–5.17 (m, 2H, CH*CH*$_2$), 5.92 (d, 1H, *J* = 15.6 Hz, CH=*CH*CO), 6.85–6.91 (m, 2H, *CH*=CHCO and NH), 7.28–7.96 (m, 16H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 40.46 ($NHCH_2$), 52.01 (OCH_3), 53.19 (NCH_2), 62.66 (*CH*CH_2), 65.00 (CH*CH*$_2$), 109.42 (Ar), 119.88 (Ar), 121.29 (Ar), 121.58 (Ar), 123.14 (Ar), 124.70 (Ar), 125.53 (CH=*CH*CO), 126.01 (Ar), 126.31 (Ar), 128.67 (Ar), 128.89 (Ar), 129.38 (Ar), 130.07 (Ar), 130.70 (Ar), 131.15 (Ar), 132.60, 132.90 (Ar), 134.34 (Ar), 138.42 (Ar), 142.51 (Ar), 144.01 (*CH*=CHCO), 153.90 (Ar), 154.70 (CO), 166.53 (C=N), 170.13 (CO), 171.14 (CO), 171.25 (CO). Anal. Calcd for $C_{34}H_{30}N_4O_6$: C, 69.14; H, 5.12; N, 9.49. Found: C, 69.20; H, 4.90; N, 9.81.

4.1.1.27. (*R*)-Methyl 4-(2-(3-((butylcarbamoyloxy)methyl)-2-oxo-5-phenyl-2,3-dihydro-1*H* -benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (8). Compound **35** (100 mg, 0.27 mmol) was reacted with butyl isocyanate (54 mg, 0.062 mL, 0.54 mmol) according to the procedure described for compound **6**. The intermediate butyl-carbamic acid 1-allylcarbamoylmethyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl-methyl ester was obtained in 86.4% yield (108 mg), after purification by flash chromatography (petroleum ether/EtOAc 4:6). R_f = 0.36 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 0.94 (t, 3H, *J* = 7.1 Hz, *CH*$_3$CH$_2$), 1.27–1.36 (m, 2H, CH_3*CH*$_2$), 1.37–1.49 (m, 2H, CH_3CH_2*CH*$_2$), 3.10–3.17 (q, 2H, *J* = 6.5 Hz, NH*CH*$_2CH_2$), 3.79–3.83 (m, 2H, NH*CH*$_2$CH=CH_2), 3.96 (t, 1H, *J* = 6.8 Hz, $CHCH_2$), 4.34 (d, 1H, *J* = 15.6 Hz, NCH_A), 4.63 (d, 1H, *J* = 15.6 Hz, NCH_B), 4.78–5.09 (m, 4H, CH*CH*$_2$ and CH=*CH*$_2$), 5.70 (m, 1H, *CH*=CH_2), 6.44 (br s, 1H, NH), 7.21–7.69 (m, 9H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 14.51 (*CH*$_3$CH$_2$), 20.23 (CH_3*CH*$_2$), 34.21 (CH_3CH_2*CH*$_2$), 42.17 (NH*CH*$_2CH_2$), 42.3 (NH*CH*$_2$CH=CH_2), 52.56 (NCH_2), 62.70 (*CH*CH_2), 64.34 (CH*CH*$_2$), 116.62 (CH=*CH*$_2$), 122.24 (Ar), 125.23 (Ar), 128.53 (Ar), 129.61 (Ar), 129.98 (Ar), 130.56 (Ar), 130.95 (Ar), 132.35 (Ar), 133.76 (*CH*=CH_2), 138.51 (Ar), 142.47 (Ar), 156.21 (CO), 169.17 (C=N), 169.82 (CO), 170.02 (CO). This intermediate (50 mg, 0.108 mmol) was reacted with methyl acrylate (92 mg, 1.08 mmol). The title compound was obtained after purification by preparative HPLC (42 mg, 74.6%). HPLC: retention time 19.11 min. R_f = 0.20 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 1.02 (t, 3H, *J* = 7.4 Hz, CH_2*CH*$_3$), 1.31–1.55 (m, 4H, *CH*$_2$*CH*$_2$CH$_3$), 3.18 (q, 2H, *J* = 6.3 Hz, NH*CH*$_2CH_2$), 3.77 (s, 3H, OCH_3), 3.80–4.00 (m, 3H, *CH*CH_2O and $NHCH_2$), 4.27 (d, 1H, *J* = 15.1 Hz, N*CH*$_A$CH$_B$CO), 4.76 (d, 1H, *J* = 15.1 Hz, NCH_A*CH*$_B$CO), 4.86–4.97 (m, 2H, CH*CH*$_2$O), 5.25 (br t, 1H, NH), 5.90 (d, 1H, *J* = 15.6 Hz, CH=*CH*$COOCH_3$), 6.86–6.92 (m, 2H, *CH*=CHCOOCH_3 and NH), 7.31–7.79 (m, 9H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 13.06 (CH_2*CH*$_3$), 19.87 (*CH*$_2CH_3$), 32.36 (*CH*$_2CH_2$ CH_3), 40.41 (NH*CH*$_2(CH_2)_2CH_3$), 40.46 (NH*CH*$_2$), 52.01 (OCH_3), 53.19 (NCH_2CO), 62.66 (*CH*CH_2O), 65.00 (CH*CH*$_2$O), 124.70 (Ar), 125.53 (CH=*CH*$COOCH_3$), 128.67 (Ar), 129.38 (Ar), 130.07 (Ar), 130.70 (Ar), 131.15 (Ar), 132.60 (Ar), 132.90 (Ar), 138.42 (Ar), 142.51 (Ar), 144.01 (*CH*=CHCOOCH_3), 154.70 (CO), 166.53 (C=N), 170.13 (CO), 171.14 (CO), 171.25 (CO). Anal. Calcd for $C_{28}H_{32}N_4O_6$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.37; H, 6.33; N, 10.98.

4.1.1.28. (*R*)-Methyl 4-(2-(3-((cyclohexylcarbamoyloxy)methyl)-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (9). Compound **35** (100 mg, 0.27 mmol) was reacted with cyclohexyl isocyanate (67 mg, 0.068 mL, 0.54 mmol) according to the procedure described for compound **6**. The intermediate cyclohexyl-carbamic acid 1-allylcarbamoylmethyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl-methyl ester was obtained in 53% yield (70 mg), after purification by flash chromatography (petroleum ether/EtOAc 4:6). R_f = 0.42 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 1.04–1.91 (m, 2H, *c*-C_6H_{11}), 1.25–1.37 (m, 4H, *c*-C_6H_{11}), 1.59–1.68 (m, 2H, *c*-C_6H_{11}), 1.87–1.92 (m, 2H, *c*-C_6H_{11}), 3.45 (m, 1H, *c*-C_6H_{11}), 3.80–3.95 (m, 2H, NH*CH*$_2$), 4.01 (dd, 1H, *J* = 6.3 and *J* = 5.8 Hz, *CH*CH_2O), 4.34 (d, 1H, *J* = 15.3 Hz, NCH_A), 4.63 (d, 1H, *J* = 15.3 Hz, NCH_B), 4.72–5.08 (m, 4H, CH*CH*$_2$ and CH=*CH*$_2$), 5.74 (m, 1H, *CH*=CH_2), 6.48 (br s, 1H, NH), 7.24–7.69 (m, 9H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 24.97 (*c*-C_6H_{11}), 25.73 (*c*-C_6H_{11}), 33.54 (*c*-C_6H_{11}), 33.63 ($NHCH_2$), 42.17 (*c*-C_6H_{11}), 52.56 (NCH_2), 62.70 (*CH*CH_2), 64.34 (CH*CH*$_2$), 116.61 (CH=*CH*$_2$), 122.84 (Ar), 125.23 (Ar), 128.53 (Ar), 129.61 (Ar), 129.98 (Ar), 130.56 (Ar), 130.95 (Ar), 132.35 (Ar), 133.76 (*CH*=CH_2), 138.51 (Ar), 142.47 (Ar), 157.25 (CO), 169.17 (C=N), 169.82 (CO), 170.02 (CO). This intermediate (50 mg, 0.102 mmol) was reacted with methyl acrylate (88 mg, 1.02 mmol). The title compound was obtained after purification by flash chromatography, eluting with petroleum ether/EtOAc 2:8, (38 mg, 68%). R_f = 0.28 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 1.05–1.34 (m, 6H, *c*-C_6H_{11}), 1.57–1.64 (m, 2H, *c*-C_6H_{11}), 1.87–1.90 (m, 2H, *c*-C_6H_{11}), 3.45 (m, 1H, *c*-C_6H_{11}), 3.72 (s, 3H, OCH_3), 3.86–4.12 (m, 3H, NH*CH*$_2$ and *CH*CH_2), 4.24 (d, 1H, *J* = 15.1 Hz, NCH_A), 4.70 (d, 1H, *J* = 15.1 Hz, NCH_B), 4.96–5.05 (m, 2H, CH*CH*$_2$), 5.83 (d, 1H, *J* = 15.6 Hz, CH=*CH*CO), 6.81–6.87 (m, 2H, *CH*=CHCO and NH), 7.22–7.61 (m, 8H, Ar), 7.74 (d, 1H, *J* = 8.3 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 24.78 (*c*-C_6H_{11}), 29.91 (*c*-C_6H_{11}), 33.51 (*c*-C_6H_{11}), 40.41 ($NHCH_2$), 50.20 (OCH_3), 53.05 (*c*-C_6H_{11}), 55.76 (NCH_2), 62.81 (*CH*CH_2), 64.15 (CH*CH*$_2$), 122.14 (Ar), 122.97 (CH=*CH*CO), 125.53 (Ar), 128.50 (Ar), 128.64 (Ar), 128.92 (Ar), 130.07 (Ar), 131.11 (Ar), 132.55 (Ar), 138.46 (Ar), 142.62 (*CH*=CHCO), 143.65 (Ar), 166.50 (CO), 168.60 (C=N), 171.10 (CO), 171.27 (CO). Anal. Calcd for $C_{30}H_{34}N_4O_6$: C, 65.92; H, 6.27; N, 10.25. Found: C, 65.60; H, 6.48; N, 10.09.

4.1.1.29. (*R*)-Methyl 4-(2-(3-((adamantan-1-ylcarbamoyloxy)methyl)-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl)acetamido)but-2-enoate (10). To a solution of **35** (100 mg, 0.27 mmol) in dry CH_2Cl_2 (15 mL) adamantyl isocyanate (96 mg, 0.54 mmol) and Et_3N (54.6 mg, 0.075 mL, 0.54 mmol) were added under N_2. The resulting mixture was stirred for 48 h at room temperature. After this time, the mixture was washed with citric acid solution and water (2 × 15 mL). The residue was purified by flash chromatography, eluting with petroleum ether/EtOAc 5:5, to afford the intermediate adamantan-1-yl-carbamic acid 1-allylcarbamoylmethyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl-methyl ester (60 mg, 41.2%). R_f = 0.58 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 1.14–1.18 (m, 4H, Ad), 1.40–1.56 (m, 9H, Ad), 1.70–1.73 (m, 2H, Ad), 3.78–3.82 (m, 2H, NH*CH*$_2$), 3.94 (m, 1H, *CH*CH_2), 4.35 (d, 1H, *J* = 15.3 Hz, NCH_A), 4.64 (d, 1H, *J* = 15.3 Hz, NCH_B), 4.75–4.91 (m, 2H, CH*CH*$_2$), 4.98–5.08 (m, 2H, CH=*CH*$_2$), 5.67 (m, 1H, *CH*=CH_2), 6.38 (br s, 1H, NH), 7.22–7.70 (m, 9H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 29.68 (Ad), 36.54 (Ad), 42.07 (Ad), 42.85 ($NHCH_2$), 46.83 (Ad), 52.65 (NCH_2), 62.77 (*CH*CH_2), 63.30 (CH*CH*$_2$), 116.68 (CH=*CH*$_2$), 122.84 (Ar), 125.30 (Ar), 128.58 (Ar), 129.60 (Ar), 130.01 (Ar), 130.65 (Ar), 131.00 (Ar), 132.40 (*CH*=CH_2), 138.49 (Ar), 142.31 (Ar), 142.42 (Ar), 155.36 (CO), 168.62 (C=N), 178.20 (CO), 180.20 (CO). This intermediate (50 mg, 0.092 mmol) was reacted with methyl acrylate (71 mg, 0.92 mmol). The title compound was obtained after purification by preparative HPLC (34 mg, 61.7%).

HPLC: retention time 68.87 min. R_f = 0.38 (petroleum ether/EtOAc 4:6). ^{1}H NMR (300 MHz, $CDCl_3$): 1.22–1.40 (m, 4H, Ad), 1.58–1.79 (m, 9H, Ad), 1.87–2.00 (m, 2H, Ad), 3.75 (s, 3H, OCH_3), 3.97–4.15 (m, 3H, NHCH_2 and CHCH$_2$), 4.30 (d, 1H, J = 15.0 Hz, NCH_A), 4.71 (d, 1H, J = 15.3 Hz, NCH_B), 4.85–4.91 (m, 2H, CHCH_2), 5.70 (d, 1H, J = 15.6 Hz, CH=CHCO), 6.84–6.93 (m, 2H, CH=CHCO and NH), 7.25–7.50 (m, 7H, Ar) 7.64 (d, 1H, J = 7.1 Hz, Ar), 7.75 (d, 1H, J = 7.9 Hz, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): 29.69 (Ad), 36.74 (Ad), 40.47 (Ad), 42.85 (NHCH_2), 50.10 (Ad), 52.89 (OCH_3), 56.20 (NCH_2), 62.86 (CHCH$_2$), 63.84 (CHCH_2), 121.76 (Ar), 122.92 (CH=CHCO), 125.44 (Ar), 126.90 (Ar), 128.61 (Ar), 129.98 (Ar), 131.04 (Ar), 132.48 (Ar), 138.46 (Ar), 138.93 (Ar), 142.41 (Ar), 143.72 (CH=CHCO), 155.81 (CO), 166.52 (C=N), 169.87 (CO), 178.54 (CO), 179.20 (CO). Anal. Calcd for $C_{34}H_{38}N_4O_4$: C, 68.21; H, 6.40; N, 9.36. Found: C, 68.51; H, 6.77; N, 9.12.

4.2. Pharmacology

4.2.1. Enzyme assays

The preliminary screening was performed with 100 μM inhibitor concentrations using an equivalent amount of DMSO as negative control. Product release from substrate hydrolysis (Cbz-Phe-Arg-AMC, 40 μM for FP-2 and 10 μM for rhodesain) was determined continuously over a period of 10 min. Compounds showing at least 50% inhibition were subjected to detailed assays. These were performed in a 100 mM sodium acetate buffer, pH 5.5 containing 10 mM DTT with Cbz-Phe-Arg-AMC (40 or 10 μM) as substrate.[15] The K_m values used to correct K_{iapp} values was determined to 21.5 μM (FP-2) and 0.9 μM (rhodesain).[21] Inhibitor solutions were prepared from stocks in DMSO. Each assay was performed twice in 96-well plates in a total volume of 300 μL. A Varian Cary Eclipse spectrofluorometer Varian, Darmstadt, Germany with a microplate reader (excitation 365 nm, emission 460 nm) was used. Standard units of FP-2 were added to the reaction mixture containing the fluorogenic substrate and increasing from 0 to 25 or 50 μM concentrations of compounds. To determine first-order inactivation rate constants (k_{obs}) for the time dependent inhibition, progress curves (fluorescence (F) versus time) were analyzed by non-linear regression analysis using the equation $F = A(1 - \exp(-k_{obs}\ t)) + B$.[16] Product formation was monitored continuously for 15 min at room temperature. Fitting of the k_{obs} values against the inhibitor concentrations to the hyperbolic equation $k_{obs} = K_{inac}\ [I]/(K_{iapp} + [I])$ gave the individual values of K_{iapp} and k_{inac}.[16] The K_{iapp} values were corrected to zero substrate concentration by the term $(1 + [S]/K_m)$ in equation $K_i = K_{iapp}/(1 + [S]/K_m)$. The second-order rate constants $k_{2nd} = k_{inac}/K_i$ were directly calculated from the individual constants. K_i and k_{inac} values were calculated by non-linear regression analyses using the program GraFit.[22] In cases where the k_{obs} versus $[I]$ plots were restricted to the linear range k_{2nd} was calculated by using the equation $k_{2nd} \approx k_{obs}\ [I]^{-1}\ (1 + [S]\ K_m^{-1})$. In case of time independent inhibition K_i was obtained by a Dixon plot[17] using equation $[E]_0/[E]_a = 1 + [I]/K_{iapp}$ and correction to zero substrate concentration from $K_i = K_{iapp}/(1 + [S]\ K_m^{-1})$ with $[E]_0$ as enzyme activity in the absence, and $[E]_a$ as residual enzyme activities in the presence of the inhibitor. Assays with cathepsins B and L were performed as described previously.[23] Cbz-Phe-Arg-AMC was used as substrate (80 μM for cathepsin B, 5 μM for cathepsin L). The K_m values used to correct K_{iapp} values were 150 μM (cathepsin B) and 6.5 μM (cathepsin L).

4.2.2. Drug screening on *P. falciparum* cultures

The compounds were screened in quadruplicates against the human malaria pathogen *P. falciparum* at concentrations between 100–0.0488 μM. *P. falciparum* (strain FCBR) was maintained in a continuous culture basically according to Trager and Jensen.[24] Parasites were cultured in human red blood cells (RBC) (blood group A^{Rh+}) in RPMI 1640 medium supplemented with 25 mM HEPES (Molecular Probes, Invitrogen), 20 mM sodium bicarbonate, and 0.5% AlbuMAX I (Molecular Probes, Invitrogen) instead of human serum at 2.5% (v/v) hematocrit. Cultures were maintained at 37 °C with a gaseous phase of 94% N_2, 1% O_2, and 5% CO_2. Early staged parasites of strain FCBR were plated in 96-well plates at a parasitemia of 1%, in the presence of the compounds (dissolved in DMSO and diluted 10-fold in 50% ethanol before added to the cells). Incubation of parasites with an equal concentration of ethanol alone was used as a negative control. As a positive control, the parasites were incubated in presence of 2 μM chloroquine (0% viability). The viability of the parasites was assayed according to Evers et al. (2008).[19]

Acknowledgments

We would like to thank the DAAD Vigoni project (2009/10) for partial support of this work. T.S. thanks the German Research Society (DFG, SFB630) for financial support.

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