Notes

3-[2-(*N*-Phenylacetamide)]-1,5-benzodiazepines: Orally Active, Binding Selective CCK-A Agonists

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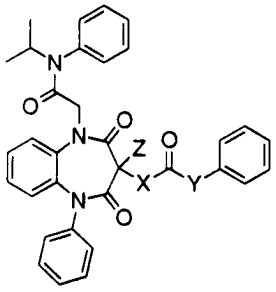
A series of modifications were made to the C-3 substituent of the 1,5-benzodiazepine CCK-A agonist **1**. Replacement of the inner urea NH and addition of a methyl group to generate a C-3 quaternary carbon resulted in acetamide **6**, which showed CCK-A receptor binding selectivity and sub-micromolar agonist activity *in vitro.* Benzodiazepine **6** was active in an *in vivo* mouse gallbladder emptying assay and represents a novel orally active, binding selective CCK-A agonist.

Introduction

Cholecystokinin (CCK) is the major gastrointestinal hormone that coordinates food digestion and satiety signals in response to feeding. CCK circulates as a series of C-terminal truncated peptides of 58, 33, 8, and 4 amino acids in length, with sulfated CCK-8 being the minimum sequence for biological activity.[1] The peripheral effects of CCK include contraction of the gallbladder, stimulation of enzyme secretion from the pancreas, and contraction of the pyloric sphincter to slow gastric emptying.[1] In conjunction with these effects as a digestive hormone, CCK functions as a satiety signal by direct stimulation of vagal afferents that, in turn, signal feeding centers in the brain.[2] These peripheral effects of CCK are mediated through the seven transmembrane G-protein-coupled CCK-A receptor.[1,3] A related CCK-B receptor subtype is located primarily within the central nervous system (CNS), where CCK functions as a peptide neurotransmitter.[4] CCK-A selective agonists have potential for use in obese patients as satiety agents[5] and for prevention of gallbladder stasis on low fat diets.[6]

Therapeutic applications of CCK ligands have been aggressively pursued following the identification of benzodiazepine antagonists for CCK-A and CCK-B receptors.[7] By comparison, the development of CCK agonists has been hampered by the lack of orally active small molecule ligands. We recently reported the identification of the first benzodiazepine CCK-A agonist (**1**).[8] However, benzodiazepine **1** shows relatively poor selectivity toward CCK-A receptors and lacks oral activity *in vivo.* With the aim of increasing the *in vivo* activity, we set out to synthesize compounds with binding selectivity for the CCK-A receptor subtype. We report that systematic modification of the C-3 ureido substituent has led to the identification of benzodiazepine CCK-A agonists with both binding selectivity and *in vivo* oral activity in mice.



1-9

Figure 1.

Chemistry

Four benzodiazepines were synthesized in which the urea nitrogens of **1** (X = NH, Y = NH, Figure 1) were sequentially replaced with either carbon or oxygen. The carbamate **2** (X = NH, Y = O) and the amide **4** (X = NH, Y = CH_2) were synthesized from the 3-amino-1,5-benzodiazepine **10** (Scheme 1). Conversion of **10** to the alcohol **11** allowed for generation of the isomeric carbamate **3** (X = O, Y = NH). Synthesis of the amide **5** (X = CH_2, Y = NH) started with the 1,5-benzodiazepine **12** (Scheme 2). The C-3 substituent was introduced by allylation to **13a** followed by oxidation to **14a** and amide bond formation to yield **5**. Three analogs of **5** were synthesized in which the 1,5-benzodiazepine contained a quaternary carbon at C-3 (Scheme 2). Deprotonation of **13a** and reaction with alkyl halides generated the C-3 dialkyled 1,5-benzodiazepines **13b**−**d**. Oxidation to acids **14b-d** and amide bond formation yielded the amides **6**−**8** (X = CH_2, Y = NH, Z = CH_3, CH_2CH_3, or CH_2Ph). Addition of a C-3 substituent to the urea **1** was achieved by sequential methylation of **12**, followed

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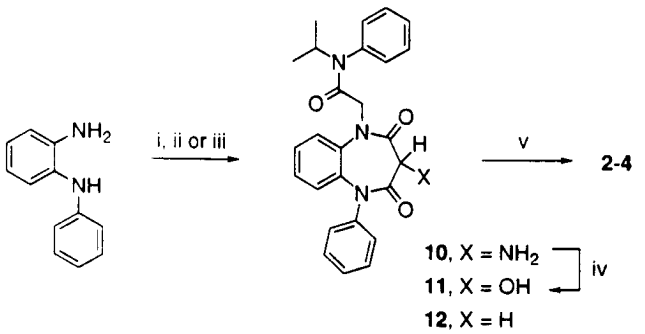
Scheme 1[a]



[a] Reagents: (i) 2-bromo-*N*-isopropyl-*N*-phenylacetamide (ref 8), K_2CO_3, DMF, 18 h; (ii) 2-(phenylhydrazono)propanedioyl dichloride (ref 8), THF, 0 °C to room temperature, 18 h; Zn dust, AcOH, room temperature, 3 h; (iii) malonyl dichloride, THF, 0 °C to room temperature, 18 h; (iv) $NaNO_2$, HCl, H_2O, 50 °C, 1 h; Cu^0, MeCN, H_2O, 80 °C, 1 h; (v) PhOC(O)Cl, Et_3N, CH_2Cl_2, room temperature, 1 h; or PhNCO, pyridine, room temperature, 1 h; or $PhCH_2CO_2H$, EDC, HOBT, DMF, room temperature, 18 h.

by electrophilic amination at C-3 to give the amine **15** (Scheme 3). Urea formation yielded the urea **9** (X = NH, Y = NH, Z = CH_3) with a methyl group attached to the quaternary carbon at C-3.

Results and Discussion

Compounds were screened for functional CCK-A agonist activity in inducing contraction of isolated guinea pig gallbladder[8] (Table 1). Contractions which were reversed by addition of the receptor antagonist MK-329[7] were deemed to be CCK-A specific. In this assay, the urea **1** gave a maximal contraction of 80% relative to CCK-8. We chose to focus on the C-3 urea substituent since previous studies in 1,4-benzodiazepines had shown that this site affected CCK-A/CCK-B selectivity,[9] and initial studies with **1** had demonstrated that the N-1 isopropylphenylacetamide was essential for agonist activity.[8] Replacement of either urea NH with O, in carbamates **2** and **3**, led to a loss in efficacy. Similarly, replacement of the urea outer NH (Y, Figure 1) with CH_2 in amide **4** led to a loss in efficacy. By comparison, replacement of the urea inner NH (X, Figure 1) with CH_2 in amide **5** gave a partial agonist with sub-micromolar potency. In an effort to build on this lead, the effect of additional substitution at C-3 of the benzodiazepine ring was studied. Addition of a methyl group gave **6** which had increased efficacy. The amide **7** with a larger ethyl substituent was a partial agonist, and the amide **8** with a benzyl substituent lacked measurable agonist activity. Thus, in **6**, substitution of the C-3 carbon with a combination of the phenyl acetamide and the additional methyl group generated a sub-micromolar CCK-A agonist with efficacy comparable to that of **1**. To examine whether incorporation of a quaternary carbon at C-3 increased efficacy in other series, the C-3 methylated urea **9** was synthesized. Unfortunately, the urea **9** showed a drop in efficacy and a large drop in potency compared to those in **1**. Our initial studies suggest that differences in the SAR between the acetamide and urea series were due to conformational differences in the C-3 substituents.[10]

Compounds **1** and **5**−**9** were examined for their binding selectivity between the human CCK-A[11] and CCK-B[12] receptors. The binding assay employed CHO-K1 cell lines that had been engineered to stably express either the human CCK-A or human CCK-B receptors.[8] IC_{50}'s were determined using competitive radioligand binding assays (Table 1). Urea **1** showed higher affinity for the CCK-B receptor subtype compared to the CCK-A receptor subtype. Changing the inner NH for CH_2 resulted in a reversal in selectivity, with amide **5** showing a 5-fold higher affinity for the CCK-A receptor subtype over the CCK-B receptor subtype. The reversal in selectivity was due to a decrease in affinity for the CCK-B receptor subtype. Addition of a methyl substituent at C-3, with amide **6**, resulted in an additional increase in binding selectivity, due primarily to a further decrease in affinity for the CCK-B receptor subtype. Significantly, amide **6** showed >100-fold selectivity for the CCK-A receptor subtype over the CCK-B receptor subtype. Amides **7** and **8**, with ethyl and benzyl substituents at C-3, had slightly lower selectivity for the CCK-A receptor subtype. The C-3 methylated urea **9** showed higher affinity for the CCK-B receptor subtype, and affinity for both receptor subtypes was diminished compared to the unmethylated urea **1**. Overall, substitution of X = NH to X = CH_2 resulted in an increase in binding selectivity for the CCK-A receptor subtype. The effect was most pronounced in the series with the C-3 quaternary carbon, where this substitution (comparison of urea **9** with amide **6**) resulted in a 500-fold increase in selectivity for the CCK-A receptor subtype.

These studies identified compounds **5**−**7** as meeting our *in vitro* criteria of CCK-A agonist activity with increased CCK-A/CCK-B selectivity. To examine whether these changes translated to increased *in vivo* CCK-A agonist activity, we chose to test compounds **1** and **5**−**7** in a mouse gallbladder emptying assay.[13] This assay provided a robust measure of peripheral CCK agonist activity mediated *via* CCK-A receptors[13] and avoided some of the problems associated with behavioral feeding assays in rodents.[14] The compounds were administered to mice, and after 30 min the extent of CCK-A-mediated gallbladder emptying was measured (Table 2). All four compounds showed full agonist activity when administered at a dose of 0.1 μmol kg^{-1} (~0.05 mg kg^{-1}) ip. However, when administered at a dose of 1.0 μmol kg^{-1} (~0.5 mg kg^{-1}) po, only amides **5**−**7** showed significant agonist activity. Additional testing at a dose of 10 μmol kg^{-1} (~5 mg kg^{-1}) po, showed that **6** was a full agonist, but **7** was only a partial agonist. Thus, with benzodiazepine **6** we have identified an orally active, binding selective CCK-A agonist.

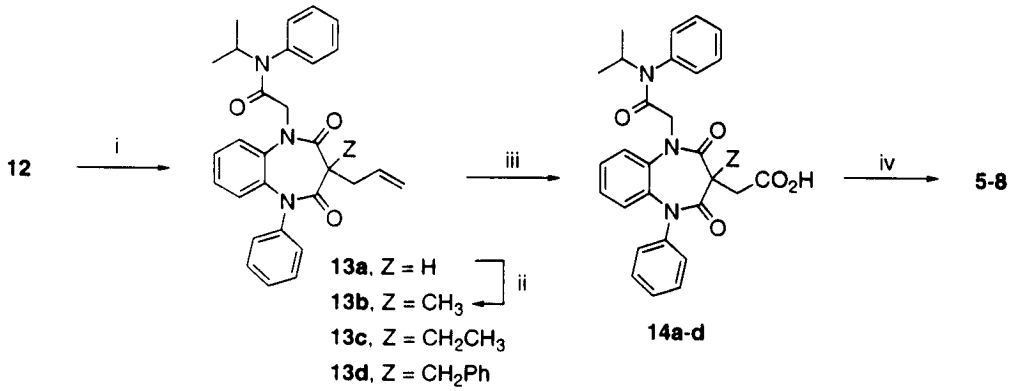
Conclusion

We have identified modifications of 1,5-benzodiazepine **1** that lead to an increase in selectivity for the CCK-A receptor while maintaining agonist activity. The resulting 3-methyl-3-[(*N*-phenylcarbamoyl)methyl]-1,5-benzodiazepine (**6**) is a novel orally active, binding selective CCK-A agonist.[15] This compound, GW 7178, represents an important step toward development of drug therapies for obesity that act through peripheral CCK receptors.

Experimental Section

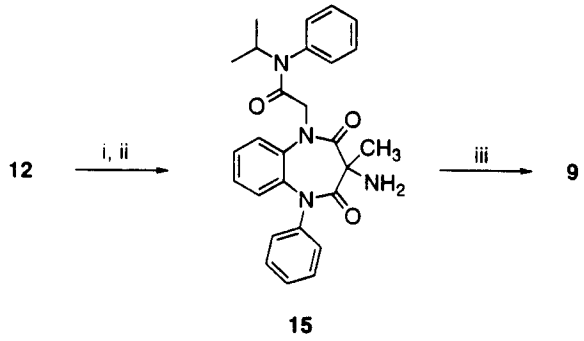
Melting points were recorded on a Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. ^{1}H-NMR spectra were recorded using a Varian VXR-300 or Varian

Scheme 2[a]



[a] Reagents: (i) NaH, DMF, 0 °C, 15 min; allyl bromide, DMF, 0 °C to room temperature, 18 h; (ii) NaH, DMF, 0 °C, 15 min; MeI, or EtI, or $PhCH_2Br$, DMF, room temperature 3 h; (iii) $RuCl_3$, $NaIO_4$, CCl_4, H_2O, room temperature, 1 d; (iv) $PhNH_2$, PyBroP, Hunig's base, DMF, 50 °C, 2 d.

Scheme 3[a]



[a] Reagents: (i) NaH, DMF, 0 °C, 20 min; MeI, DMF, 0 °C, 1 h; (ii) KHMDS, THF, 0 °C, 20 min; $Ph_2P(O)ONH_2$, THF, 0 °C to room temperature, 18 h; (iii) PhNCO, pyridine, room temperature, 1 h.

Unity-300 spectrometer using tetramethylsilane as internal standard. Chemical shifts are expressed as δ (ppm) values for protons relative to the internal standard. Mass spectra were recorded on a JEOL JMS-AX505HA or a JEOL SX-102 spectrometer.

2-(2,4-Dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo[*b*][1,5]-diazepin-1-yl)-*N*-isopropyl-*N*-phenylacetamide (12). A 6.9 g (5.0 mmol) sample of potassium carbonate was added to a solution of 9.2 g (5.0 mmol) of *N*-phenyl-1,2-phenylenediamine and 12.7 g (5.0 mmol) of 2-bromo-*N*-isopropyl-*N*-phenylacetamide[8] in DMF (200 mL), and the mixture was allowed to stir overnight. The DMF was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate (400 mL) and washed exhaustively with aqueous 1 N HCl (4 × 250 mL). The organic layer was washed with water (2 × 200 mL), dried (Na_2SO_4), and evaporated to give 17.8 g of crude alkylated product. The oil was purified by chromatography on silica gel (600 g) using first $CHCl_3$ (8000 mL) and then hexane/ethyl acetate (2/1, 8000 mL) as eluents to give 10.0 g (56%) of *N*-isopropyl-*N*-phenyl-2-[[2-(phenylamino)phenyl]amino]acetamide as a brown oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.42−6.8 (m, 14 H), 6.36 (d, 1 H), 4.95 (m, 1 H), 3.22 (s, 2 H), 1.05 (d, 6 H); MS (FAB) *m/e* 360 (MH^+); TLC R_f = 0.18 ($CHCl_3$).

To 20 mL of THF at 0 °C was simultaneously added dropwise over 10 min a solution of 1.97 g (5.48 mmol) of *N*-isopropyl-*N*-phenyl-2-[[2-(phenylamino)phenyl]amino]acetamide in 20 mL of THF and 0.53 mL (5.48 mmol) of malonyl dichloride in 20 mL of THF. The resulting red-brown solution was stirred at room temperature for 5.5 h and the solvent removed *in vacuo*. Purification of the resulting brown oil by silica gel flash chromatography (50−75% ethyl acetate/petroleum ether) followed by recrystallization from ethyl acetate/petroleum ether gave 0.84 g (36%) of **12** as a white powder: mp 199−200 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.6−7.2 (m, 12 H), 7.09 (t, 1 H, J = 8), 6.90 (d, 1 H, J = 8), 5.05 (m, 1 H), 4.38 (d, 1 H, J = 17), 4.04 (d, 1 H, J = 17), 3.54 (dd, 2 H, J = 5, 22), 1.10 (d, 6 H, J = 7); MS (FAB) *m/e* 428 (MH^+). Anal. ($C_{26}H_{25}N_3O_3$) C, H, N.

2-(3-Allyl-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo-[*b*][1,5]diazepin-1-yl)-*N*-isopropyl-*N*-phenylacetamide (13a). To a stirring solution of 4.43 g (10.4 mmol) of **12** in 20 mL of DMF at 0 °C was added in one portion 456 mg (11.4 mmol, 1.1 equiv) of sodium hydride (60% dispersion in oil). The resulting solution was stirred at 0 °C for 20 min, during which time gas evolution was observed, and then a solution of 0.90 mL (10.4 mmol) of allyl bromide in 10 mL of DMF was added dropwise over 20 min. The resulting brown solution was stirred for 30 min at 0 °C and then at room temperature for 18 h. The reaction was quenched by careful addition of 10 mL of H_2O, and the solvent was removed *in vacuo*. The residue was poured into 30 mL of H_2O and extracted with EtOAc (3 × 30 mL). The organic layers were washed with brine (1 × 30 mL) and dried ($MgSO_4$), and the solvent was removed *in vacuo*. Purification of the brown residue by silica gel flash column chromatography using petroleum ether/EtOAc (7/3) as eluent afforded an off-white solid. Recrystallization from EtOAc/petroleum ether (1/1) gave 2.33 g (48%) of **13a** as a white powder: mp 192−3 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46−7.08 (m, 13 H), 6.94 (d, 1 H, J = 8.1), 5.92 (m, 1 H), 5.02 (m, 2 H), 4.34 (d, 1 H), 4.04 (d, 1 H), 3.43 (t, 1 H), 2.78 (m, 2 H), 1.11 (m, 6 H); MS (FAB) *m/e* 468 (MH^+). Anal. ($C_{29}H_{29}N_3O_3$) C, H, N.

2-(3-Allyl-3-methyl-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo[*b*][1,5]diazepin-1-yl)-*N*-isopropyl-*N*-phenylacetamide (13b). To a stirring solution of 1.50 g (3.20 mmol) of **13a** in 15 mL of DMF at 0 °C was added 192 mg (4.81 mmol, 1.5 equiv) of sodium hydride (60% dispersion in mineral oil). The resulting solution was stirred 15 min, and then 0.36 mL (5.76 mmol, 1.8 equiv) of methyl iodide was added. The reaction mixture was stirred for 3 h at room temperature, and then tje reactopm was quenched with 10 mL of H_2O. The DMF was removed *in vacuo*, and the residue was dissolved in 100 mL of Et_2O and washed with 100 mL of H_2O. The organic layer was dried ($MgSO_4$), and the solvents were removed *in vacuo* to afford 1.61 g (99%) of **13b** as a white solid which was used without further purification: ^{1}H NMR (DMSO-d_6, 300 MHz, mixture of conformers) δ 7.56−7.11 (m, 13 H), 6.74 (m, 1 H), 5.85 (m, 0.34 H), 5.61 (m, 0.66 H), 5.10−4.69 (m, 2.34 H), 4.20 (m, 1.66 H), 1.94 (d, 2 H, J = 7.3), 1.21 (s, 2 H), 0.98 (m, 6 H), 0.82 (s, 1 H); R_f = 0.66 in hexane/EtOAc (1/1).

[1-[(Isopropylphenylcarbamoyl)methyl]-3-methyl-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]-diazepin-3-yl]acetic Acid (14b). To a rapidly stirring biphasic solution of 1.03 g (2.14 mmol) of **13b** in 50 mL of CCl_4 and 25 mL of H_2O was added 44 mg (0.21 mmol, 0.1 equiv) of ruthenium(III) chloride hydrate, followed by 4.58 g (21.40 mmol, 10.0 equiv) of sodium periodate. The resulting black solution was stirred rapidly at room temperature for 24 h, then diluted with 100 mL of H_2O, and extracted with EtOAc (3 × 200 mL). The organics were washed with brine (1 × 150 mL) and saturated $NaHSO_3$ (1 × 150 mL) and dried ($MgSO_4$), and

Table 1. *In Vitro* Activity of 1,5-Benzodiazepine CCK-A Agonists

	structures[a]			functional assay[b]		binding assay[c]		
no.	X	Y	Z	ED_{50} (μM)	RE	CCK-A (pIC_{50})	CCK-B (pIC_{50})	sel
CCK-8	AspTyr(SO_3H)MetGlyTrpMetAspPheNH_2			0.002 ± 0.001 (5)	1.0	8.88 ± 0.22 (8)	9.46 ± 0.04 (8)	0.3
1	NH	NH	H	1.6 ± 0.9 (2)	0.8	7.26 ± 0.05 (4)	7.62 ± 0.02 (3)	0.4
2	NH	O	H	−	0.2			
3	O	NH	H	−	0.2			
4	NH	CH_2	H	−	0.3			
5	CH_2	NH	H	0.43 (1)	0.5	6.81 ± 0.08 (3)	6.08 ± 0.04 (3)	5
6	CH_2	NH	CH_3	0.20 ± 0.11 (5)	0.7	7.12 ± 0.02 (3)	5.08 ± 0.04 (3)	110
7	CH_2	NH	CH_2CH_3	0.78 (1)	0.6	7.44 ± 0.04 (2)	6.10 ± 0.19 (2)	22
8	CH_2	NH	CH_2Ph	−	ns	6.41 ± 0.06 (2)	5.05 ± 0.09 (2)	23
9	NH	NH	CH_3	>10 (1)	0.5	5.88 ± 0.02 (2)	6.55 ± 0.27 (2)	0.2

[a] Figure 1. [b] Functional activity in the isolated guinea pig gallbladder following incubation with the test ligand; ED_{50}, concentration at which 50% of the maximal contraction was observed ± SE (number of determinations); −, an ED_{50} could not be determined; RE, relative efficacy as determined by the maximal contraction observed at 30 μM standardized to CCK-8 (1 μM) = 1.0, all values ± 0.1, $n \geq 3$; ns, no significant contraction observed. [c] Binding affinity for human CCK-A and CCK-B receptors; pIC_{50}, −log of the concentration that displaced 50% of [^{125}I]Bolton−Hunter CCK-8 from membrane preparations isolated from CHO-K1 cells stably transfected with cDNA of human CCK-A and CCK-B receptors ± SE (number of determinations); sel, CCK-A receptor selectivity calculated from IC_{50} (B)/IC_{50} (A).

Table 2. *In Vivo* Activity of 1,5-Benzodiazepine CCK-A Agonists

	mouse gallbladder emptying[a]		
no.	0.1 μmol kg^{-1} ip (%)	1.0 μmol kg^{-1} po (%)	10 μmol kg^{-1} po (%)
CCK-8	95[b]	−	−
1	78	ns	−
5	63	34	−
6	73	36	73
7	68	45	42

[a] Following overnight food deprivation, male CD-1 mice (10 animals per dose) were treated (ip or po) with vehicle (ethanol/propylene glycol/water, 2/3/5, 1 mL kg^{-1}) or test compound dissolved in vehicle (1 mL kg^{-1}). Thirty minutes after drug treatment, animals were sacrificed (CO_2) and the gallbladders were dissected out and weighed. Gallbladder wet weights of the treated animals were normalized to the vehicle control group. Gallbladder emptying was inhibited by the CCK-A receptor antagonist MK-329 (0.5 μmol kg^{-1} ip); %, percent emptying $p < 0.05$; ns, not statistically significant; −, not determined. [b] CCK-8 at 1 nmol kg^{-1} ip.

the solvent was removed *in vacuo* to afford 0.98 g (92%) of **14b** as a dark gray foam: ^{1}H NMR ($CDCl_3$, 300 MHz, mixture of conformations) δ 7.53−7.09 (m, 13 H), 6.86 (m, 1 H), 5.02 (m, 1 H), 4.34 (d, 1 H), 4.41 (m, 1 H), 4.08 (m, 1 H), 3.27 (d, 1 H), 3.03 (d, 1 H), 1.24 (s, 3 H), 1.11 (m, 6 H); MS (FAB) *m/e* 500 (MH^+). The crude product was used directly in the next reaction.

Purification of a portion of the crude product by C-18 reverse phase MPLC using methanol/0.1% TFA−H_2O (13/2) as eluent gave **14b** as a white powder: mp 100−105 °C. Anal. ($C_{29}H_{29}N_3O_5 \cdot CF_3CO_2H \cdot H_2O$) C, H, N.

2-[2,4-Dioxo-5-phenyl-3-methyl-3-[(phenylcarbamoyl)-methyl]-2,3,4,5-tetrahydrobenzo[*b*][1,5]diazepin-1-yl]-*N*-isopropyl-*N*-phenylacetamide (6). A solution of 250 mg (0.50 mmol) of **14b**, 0.091 mL (1.0 mmol) of aniline, 0.47 g (1.0 mmol) of PyBroP, and 0.35 mL (2.0 mmol) of *N*,*N*-diisopropyl-*N*-ethylamine in 2 mL of DMF was stirred at 50 °C for 2 d. The reaction mixture was diluted with 50 mL of 1 N HCl and extracted with EtOAc (3×). The organic extract was washed with 1 N HCl, brine, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated *in vacuo* to a yellow oil. Purification by silica gel flash chromatography (50% EtOAc/petroleum ether) followed by recrystallization from MeCN/H_2O (2/1) gave 0.125 g (44%) of **6** as a white powder: mp 228−9 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, mixture of conformations) δ 9.73 (s, 1 H), 7.6−7.2 (m , 16 H), 7.05 (m, 2 H), 6.65 (d, 1 H, $J = 8$), 4.85 (m, 1 H), 4.22 (m, 2 H), 2.33 (m, 2 H), 1.20 (s, 3 H), 1.00 (m, 6 H); MS (FAB) *m/e* 575 (MH^+). Anal. ($C_{35}H_{34}N_4O_4$) C, H, N.

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