Eur. J. Med. Chem. 33 (1998) 471–488

Design, syntheses, and activity of new 3-[(sulfonylaryl)-amino]-1,4-benzodiazepin-2-one derivatives as α-thrombin inhibitors

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(Received 6 October 1997; accepted 3 February 1998)

Abstract — Thrombin plays a central role in thrombosis. Because of the medical need for novel antithrombotic drugs, a search for structurally novel thrombin inhibitors was undertaken. In the absence of a crystal structure, a class was designed based on a modeling approach which involved placing the essential functional groups of the thrombin antagonist MD-805 [1] (Argatroban) into the benzodiazepine nucleus. The best superposition was obtained with a 1,4-benzodiazepin-2-one containing a 1,2,3,4-tetrahydro quinolylsulfonyl moiety in the 3-position, a guanidino-phenyl at the 5-position, and N^1-substituted with an acetic acid. Synthesis of these molecules provided compounds with an inhibitory activity with K_i in the range of 40–1000 μM. A report on the crystal structure of thrombin*hirudin(55–65)*MD-805 complex [2] suggested subsequent molecular modeling investigations to rationalize the pharmacological results. © Elsevier, Paris

thrombin inhibitors / molecular modeling / Argatroban / benzodiazepinones

1. Introduction

Thrombotic disorders are a major cause of mortality in industrialized countries. Thrombin, a trypsin-like serine protease, plays a central role in thrombosis and hemostasis [3]. As a key enzyme in the blood coagulation cascade, its major function is to convert fibrinogen into the fibrin that forms irreversible clots after Factor XIIIa induced cross-linking. Thrombin also activates the blood coagulation factors V, VIII, XIII, and protein C; stimulates platelet secretion and aggregation; and mediates other nonhemostatic cellular events [4–6]. In cases requiring anticoagulants, treatment with selective thrombin inhibitors might offer an attractive means of therapy. There is a real medical need for novel antithrombotic drugs which are more efficacious than the currently administered heparins and coumarins [7, 8].

Consequently, considerable effort has recently been directed toward the development of synthetic, low molecular weight inhibitors of thrombin. Hirudin, an extremely potent and specific protein inhibitor from the medicinal leech [9–13], has been used to suggest small peptidyl inhibitors. Various synthetic thrombin inhibitors have been modeled after natural peptide substrates [14–17] based on arginine [1, 18] or benzamidine derivatives [19, 20]. PPACK, shown in *figure 1*, is an example. A number of heterocyclic compounds have also been reported as thrombin inhibitors [21, 22].

With the help of quantitative structure activity relationships, Kikumoto et al. [18] suggested that potent inhibitors of thrombin could be obtained from arginine derivatives having two hydrophobic moieties. NAPAP and MD-805, shown in *figure 1*, are examples of potent thrombin inhibitors.

From the structures of these ligands it has been suggested that thrombin possesses three discrete binding sites which recognize the guanidino group, the aromatic substituent, and the hydrophobic carboxamide portion.

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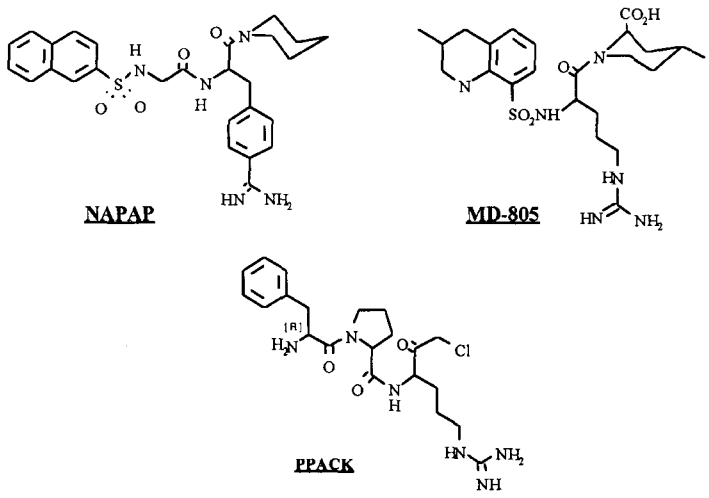


Figure 1. The inhibitors studied: **NAPAP**, N^{α}-(2-naphthyl-sulfonyl-glycyl)-D-para-amidinophenylalanyl piperidine, K_i = 6.6 nM (racemic form); **MD-805**, (2*R*,4*R*)-4-methyl-[N^{α}–[(3-methyl-1,2,3,4-tetrahydro-8-quinolyl)sulfonyl]-L-arginyl)]-2-piperidine carboxylic acid (also called MQPA, Argipidine or Argatroban, K_i = 39 nM); **PPACK**, D-Phe-Pro-Arg-CH_2Cl which binds covalently with $K_{inact} = 1.2 \times 10^7\ M^{-1}S^{-1}$.

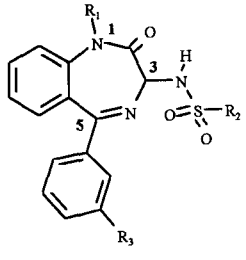
On the basis of this information a new class of thrombin inhibitors was designed using a molecular modeling approach.

Even though crystallographic information on the thrombin PPACK structure had been published when this work was initiated it was not made publicly available. Consequently, initial comparisons were ligand-to-ligand as a guide for new molecule design. We decided to use the nonpeptide MD-805 because the compounds of this class have the potential to be bioavailable, resersible inhibitors of thrombin, a profile which is essential for a therapeutically useful agents. Using compounds like PPACK that interact with thrombin's serine hydroxy group could possibly lead to a biased and narrow view of interaction sites along the surface of the catalytic cavity.

Consequently, model ligands containing the essential functional groups present in the linear thrombin antagonist MD-805 (see *figure 2*) were mapped onto a more rigid template. We sought to mimic the natural peptidal structure of the substrate by choosing the benzodiazepine scaffold, a template often found in peptidomimetics [23, 24]. Three published patent applications appeared after the initiation of this work [25–27]. These cases used alternate templates to achieve some conformational restriction. After numerous computational investigations, the benzodiazepine comparisons appeared to give satisfactory overlaps, suggesting that this more rigid scaffold might be a suitable skeleton for derivatization into thrombin inhibitors. Since the linear compound MD-805 can adopt a number of different conformations several were selected as models for the conformationally restricted benzodiazepine. From this study the most appropriate points for the incorporation of the acid, the amidino, and the sulfamoyl group were identified (see *figure 2*).

The best superposition was obtained with a 1,4-benzodiazepin-2-one containing a 1,2,3,4-tetrahydro quinolylsulfonyl moiety in the 3 position, a meta-amidino-phenyl or meta-guanidino-phenyl group in the 5 position, and N^1-substituted with an acetic acid (see *figure 3*). It should be noted that while the syntheses of such compounds were underway, the report of a similar exercise appeared [28]. In this study a different location for the amidino group and variation of the N^1-substituents were suggested in order to separate factor Xa inhibitory activity from that of thrombin.

This paper reports the synthesis and the evaluation of 1,4-benzodiazepin-2-one derivatives differently substituted in the 1,3 and 5-positions as represented by the generic structure:



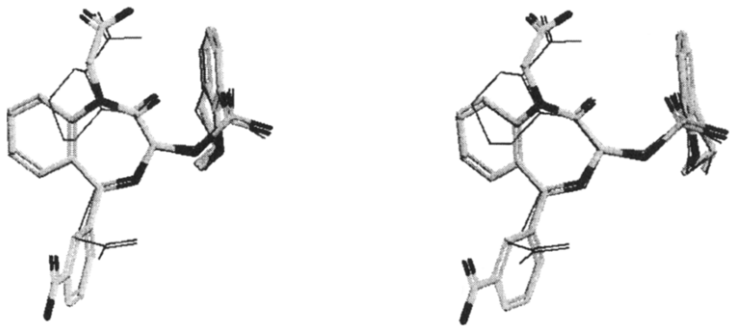


Figure 2. Stereoview of the superposition of the linear compound MD-805 (drawn as thin wires) and a benzodiazepine compound (drawn as thicker sticks with N and O atoms in dark gray).

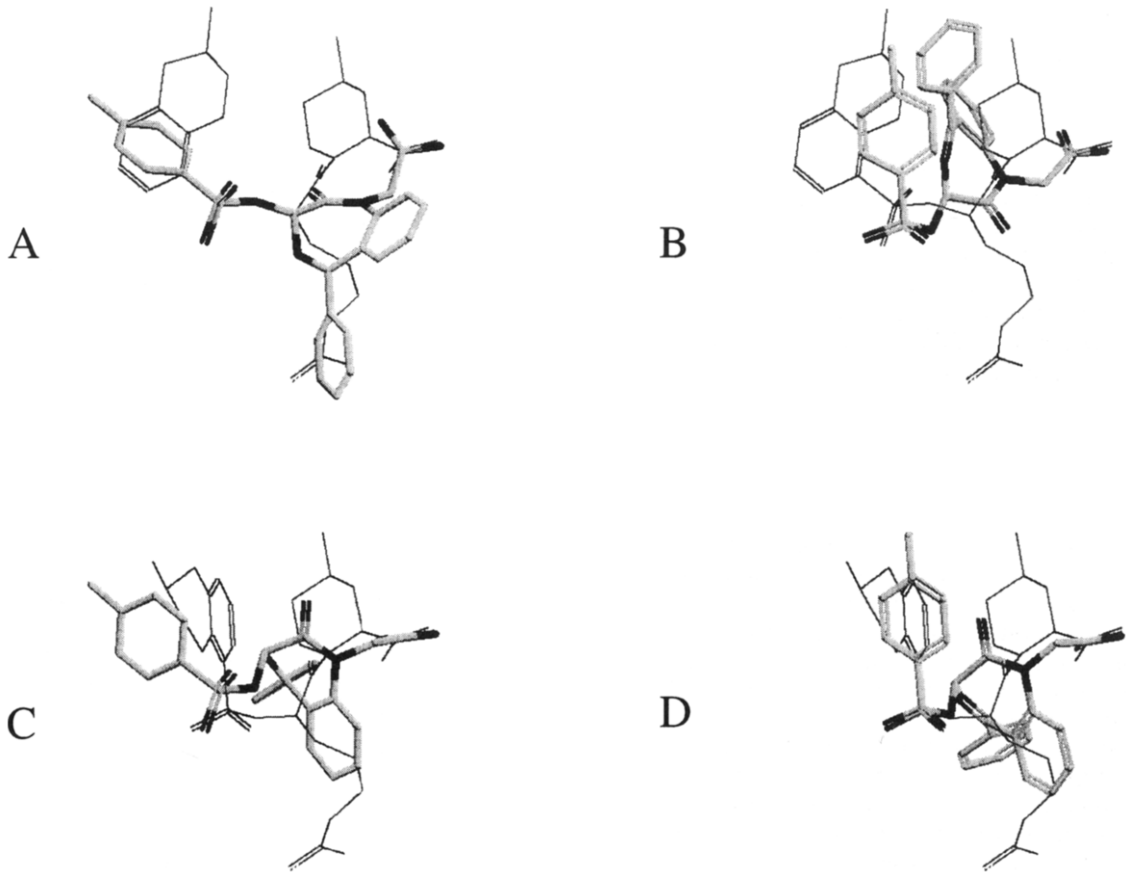
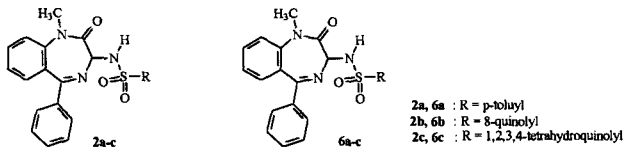


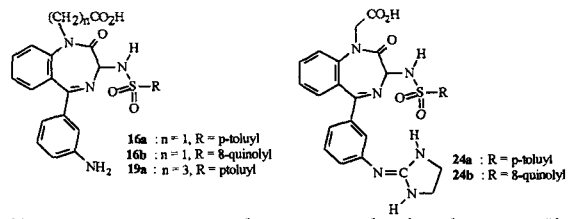
Figure 3. Comparisons of the template **6a** (drawn as thicker sticks with N and O atoms in dark gray) to the models of MD-805 (drawn as thin wires). (A) TempRI with Model I; (B) TempSI with Model I; (C) TempRII with Model II; (D) TempSII with Model II.

2. Chemistry

In order to check the validity of the synthetic strategy approach and to evaluate the pharmacological contribution of each functional group compounds **2a–c** and **6a–c** were synthesized. These molecules are functionalized only in the 1 and 3 positions and contain an unsubstituted 5-phenyl ring:



Next the related analogs which contain the 5-phenyl substituted in the meta-position by either an amino group (**16a–b**) or a guanidino unit (**24a–b**) were prepared. The role of the N^1-substituent was evaluated by preparing **19a**, an analog containing butyric acid as a side chain:



All target compounds were obtained according to *figures 4–8*. Examples in the 3-amino-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepinone series were prepared as outlined in *figure 4*. Sulfonylation of the 3-amino-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepine **1** [29a–b] with suitable arylsulfonyl chlorides, such as tosyl chloride or 8-quinolylsulfonyl chloride, under Seiyaku [30] conditions (method A) led to the targets **2a** and **2b**. Hydrogenolysis (Pd/H_2) of **2b** in MeOH afforded target compound **2c**.

The 3-(sulfonylaryl)-amino-1,4-benzodiazepin-1-acetic acid was prepared from the corresponding 3-amino-1,4-benzodiazepinone **3** [29a–b], a key intermediate in these studies, as illustrated in *figure 5*. Sulfonylation of derivative **3** was achieved according to method A to afford compounds **4a–b**. N^1-Substituents were introduced by alkylation with sodium hydride and the alkyl halide of precursors **4a–b** (Method B) to give **5a–b**. The tert-butyl ester **5a** was released using formic acid at 70 °C to afford **6a**. Saponification of the ester **5b** via 1 N NaOH afforded the carboxylic acid **6b**.

The success associated with the strategy of cyclizing an α-aminoglycine derivative to get the first key intermediate **5** prompted the use of the same method (*figure 6*) to prepare **12**, a second key intermediate. This benzodiazepin-2-one **12**, containing a nitro in the

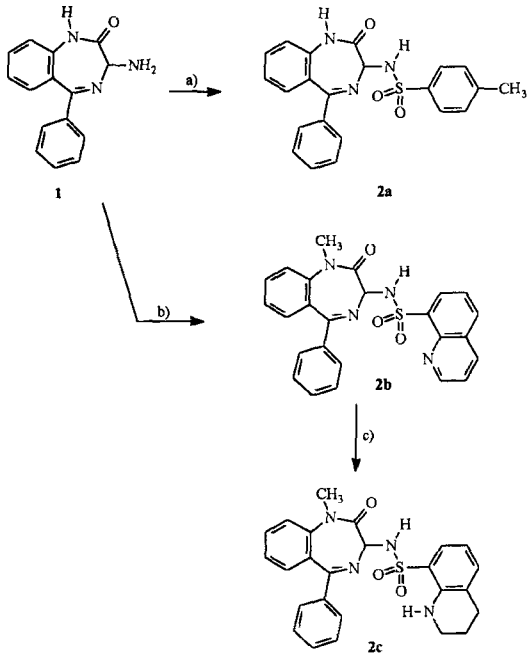


Figure 4. (a) NEt_3, TsCl, $CHCl_3$; (b) NEt_3, 8-quinolyl-SO_2Cl, $CHCl_3$; (c) Pd/C, H_2, MeOH.

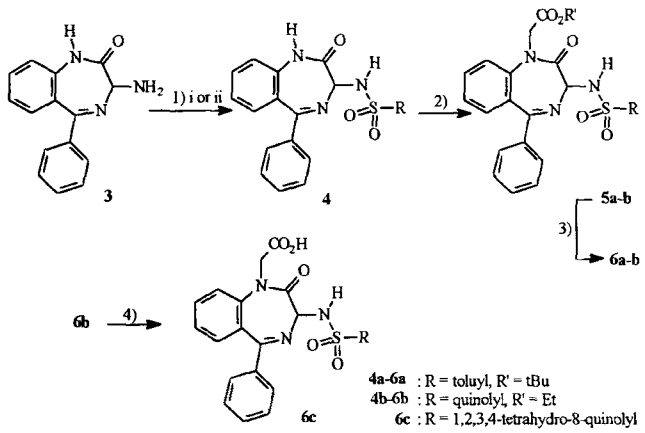


Figure 5. (1) i: NEt_3, *p*-toluyl-SO_2Cl, $CHCl_3$, ii: 8-quinolyl-SO_2Cl; (2) NaH, $BrCH_2CO_2tBu$,DMF to get **5a** or NaH, $BrCH_2CO_2Et$, DMF to obtain **5b**; (3) 1 N NaOH, MeOH for **6b** or HCO_2H, 70 °C for **6a**; (4) Pd/C, H_2, MeOH.

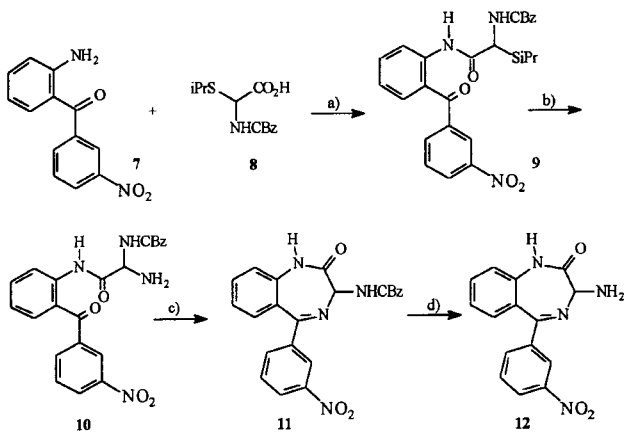


Figure 6. (a) i: BuOCOCl, *N*-Methylmorpholine, CH_2Cl_2, 0 °C, 15 min, 0 –> 23 °C, 12 h; (b) NH_3 (g), $HgCl_2$, THF, 0 –> 23 °C; (c) NH_4OAc, AcOH, 23 °C; (d) HBr (33%), AcOH, HCO_3Na (10%).

meta-position of the 5-phenyl, was obtained by coupling of the 2-amino-3'-nitrobenzophenone **7** [31] with a protected α-aminoglycine synthon **8** [32, 33] to yield the (alkylthio)glycinamide **9**. Displacement of the alkylthio portion with ammonia and $HgCl_2$ afforded the α-amino glycinamide **10** which, when stirred in a NH_4OH/AcOH solution, effected the cyclization to **11**. Removal of the benzyloxycarbonyl (Cbz) protecting group was success fully achieved by hydrolysis with 33% HBr in anhydrous Et_2O.

The preparation of benzodiazepine derivatives **16a** and **16b**, which contain an amino group in the meta-position of the 5-phenyl nucleus, is described in *figure 7*. Improvement in the sulfonylation reaction has been made on intermediate **12**. The arylsulfonyl chlorides previously used were heated with **12** under reflux in pyridine [34] (method C) leading to the isolation of the arylsulfonamides **13a** and **13b**. Specific conditions were investigated in order to increase the likelihood of N¹-alkylation of the 1,4-benzodiazepinones versus the N-alkylation of the sulfonamide portion. Several basic conditions were tested to effect deprotonation of the lactam: $NaHCO_3$, Na_2CO_3, NaH, MeONa and solvent combinations (e.g., THF, DMF) were substantially less effective, affording yields of monoalkylation below 50%. Surprisingly, deprotonation of the lactam **13** by K_2CO_3, followed by treatment with $BrCH_2CO_2tBu$ and heating to 50–70 °C in DMF (method D) selectively yielded the N¹-alkylated 1,4-benzodiazepinones **14a–b**. The selective reduction of the nitro derivatives **14a–b** was accomplished according to the method of Heck et al. [35] using triethylammonium formate/Pd as a catalytic hydrogen transfer agent. Under these conditions compounds **15a–b** were obtained. Tert-butyl esters **15a** and **15b** were hydrolyzed using CF_3CO_2H/AcOH to give carboxylic acids **16a–b**.

The homologous analog of **16a** containing a butyric acid as a side chain, compound **19a**, was prepared as described in *figure 7*. The conditions previously used to alkylate the N-1 position with the ethyl-4-bromobutyrate gave a poor yield (12%) of badly contaminated products. A preliminary bromide-iodide exchange using KI in acetone produced the N¹-alkylated **17a** in 40% yield. Basic hydrolysis of these later esters was achieved via 1 N NaOH solution for 18 h in EtOH. The reduction of the nitro group was carried out by catalytic hydrogenolysis (Pd/C) to yield **19a**. The anilino derivatives **15a** and **15b** were formylated using HCO_2H/Ac_2O according to the conditions of Leclerc et al. [36] to afford the formanilides **20a–b**. Treatment of **20a–b** with $SO_2Cl_2/SOCl_2$ to prepare the corresponding dichloro-imines was unsuccesseful. All attempts to obtain the isocyanides required for the preparation of guanidino derivatives failed. However isocyanide **21a** was formed in good yield by using triphenylphosphine, CCl_4, and triethylamine [37]. Surprisingly, this method was not effective in the preparation of the isocyanide **21b**. This later compound was obtained with a satisfactory yield according to the method of Ugi et al. [38] using diphosgene [perchloro (methylformate)] as a dehydrating agent. The bromination of **21a–b** was carried out by the treatment of their CH_2Cl_2 solution with an equivalent of dioxane dibromide [39]. Treatment of **22a–b** with ethylenediamine gave **23a–b**. The tert-butyl esters **23a–b** were hydrolyzed with either CF_3CO_2H to yield **24a** or with a 33% HBr solution in AcOH to afford **24b**.

3. Pharmacology; thrombin inhibitor assay: biological results

Conversion of the circulating fibrinogen to fibrin is initiated by a limited proteolysis by thrombin of an arginine residue (Aα16, Bβ14) in each of the two chains of fibrinogen. These proteolytic events result in the release of two peptides, fibrinopeptide A and fibrinopeptide B, from the NH_2-terminal region of the corresponding A and B chains of fibrinogen [40].

The benzodiazepine derivatives listed below (*table I*) were evaluated for their effect on the release of fibrinopeptide A (FPA) from fibrinogen in the presence of α-thrombin. The released FPA is detected by HPLC at 205 nm using a C (18) reverse phase column [41].

Most of the more potent benzodiazepine compounds have a modest and comparable antithrombotic activity (K_i = 42–208 μM). Thus the introduc-

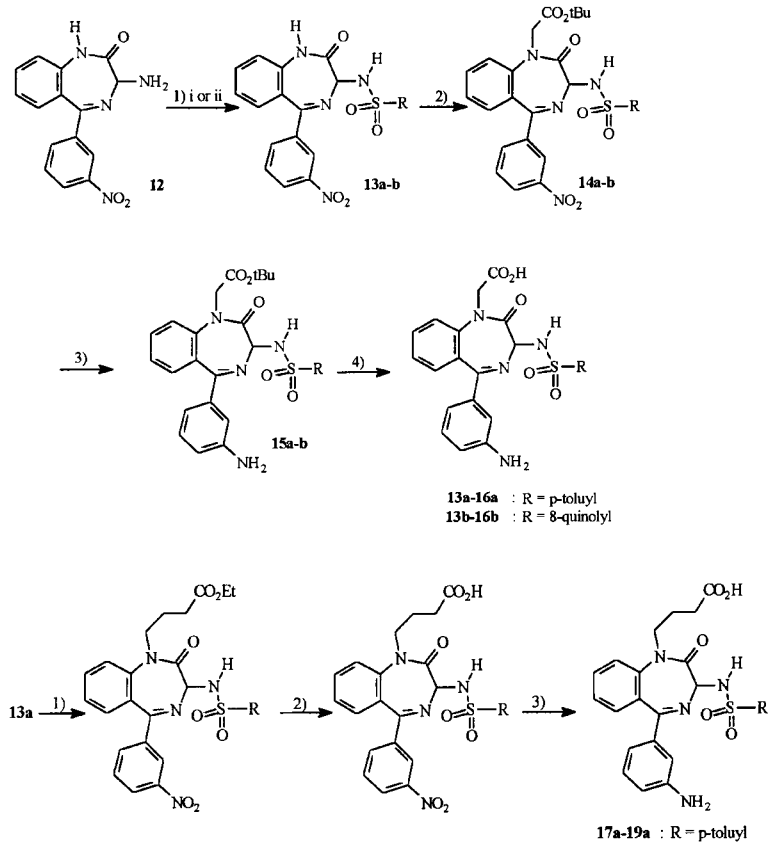


Figure 7. Top: (1) i: NEt_3, *p*-toluyl-SO_2Cl, $CHCl_3$, ii: NEt_3, 8-quinolyl-SO_2Cl; (2) K_2CO_3, $BrCH_2CO_2tBu$, DMF; (3) Pd/C, NEt_3, HCO_2H, CH_3CN; (4) CF_3CO_2H, AcOH. Bottom: (1) KI, $BrCH_2CH_2CH_2CO_2Et$, DMF; (2) 1 N NaOH, EtOH; (3) Pd/C, H_2, MeOH.

tion of a carboxylic acid (**6b**, K_i = 42 mM) increases slightly the inhibitory activity of the compound compared to the N-methyl analog (i.e., **2a**, K_i = 90 μM). Surprisingly, the reduction of the quinoline group (**6b**, K_i = 42 μM) to the 1,2,3,4-tetrahydro-8-quinoline form present in the highly potent inhibitor MD-805 (**6c**, K_i = 208 μM), did not improve the inhibitory activity.

Substitution of the 5-phenyl in the meta-position with an amino group, in order to form interaction with the key Asp199 of the thrombin as described by Banner et al. [2] led to compound **16a** which retained an activity similar (K_i = 82 μM) to that of the more potent benzodiazepine thrombin inhibitors (**6b**, K_i = 42 μM).

The introduction of a longer side chain in the 1 position (compound **19a**, K_i > 500 μM) seems to be not favorable to access to the anion site of the α-thrombin. However, the introduction of a more bulky cyclic guanidino group in the 5-phenyl position (compounds **24a** and **24b**) seems to prevent a good interaction with Asp199 and therefore led to inactive compounds.

The modest activity of these benzodiazepine derivatives to inhibit α-thrombin (K_i = 40–1000 μM) indicated that further pharmacological testing was not warranted. Thus these analogs were not tested for inhibitory activity against other serine proteinases.

4. Follow-up modeling studies and discussion of results

While this work was underway, the crystal structure of thrombin*hirudin (55–65)*MD-805 complex was reported by Banner and Hadvary [2]. A new molecular modeling study of thrombin inhibitors, based on the 3-sulfonylaryl-1,4-benzodiazepine template, was then undertaken.

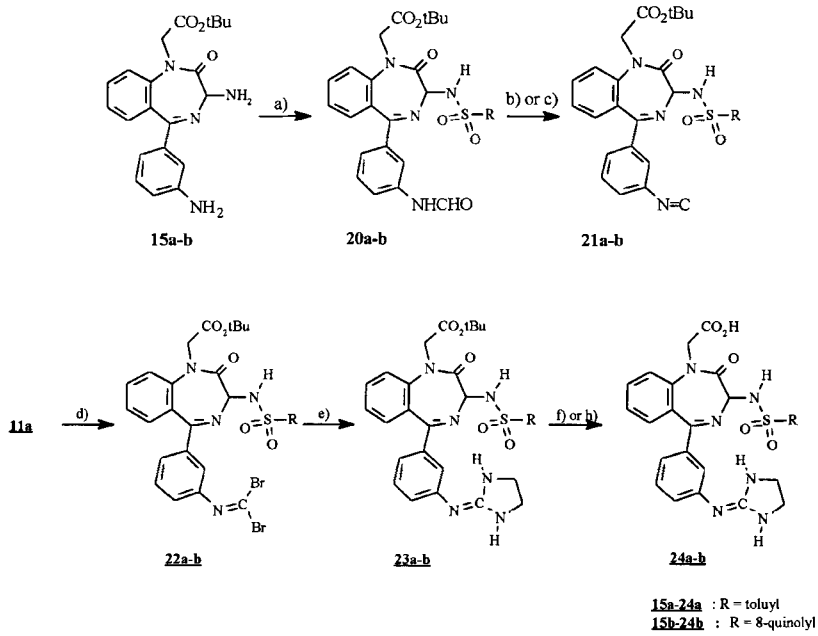
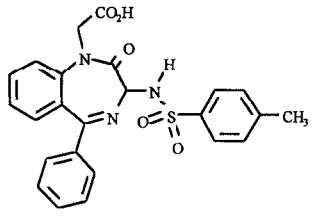


Figure 8. (a) Ac_2O, HCO_2H; (b) CCl_4, PPh_3, $CHCl_3$; NEt_3, $ClCOOCl_3$, CH_2Cl_2; (c) Diphosgen, CH_2Cl_2; (d) Dioxan-bromide complex, CH_2Cl_2; (e) NEt_3, $H_2NCH_2CH_2NH_2$, EtOAc; (f) CF_3CO_2H, $CHCl_3$; (g) 33% HBr, AcOH.

Models of MD-805 in thrombin were built using the crystal coordinates of the thrombin*PPACK complex [42]. The thrombin*MD-805 models were consistent with the description of the experimentally determined structure. In the models the MD-805 Arg amide proton is H-bonded to Gly228 of the protein, the piperidine substituent is in the proximal pocket, the quinoline unit is in the distal pocket, the oxyanion hole is not utilized, and the carboxylic acid of the ligand is near the Lys60F amino group.

Considering the structure of MD-805 and because the number of conformers is considerable, it was interesting to know what represent the conformers selected with respect to the conformational space of this compound. In fact the conformational space available to MD-805 is significant. Comparisons were made using 5 dissimilar conformations of MD-805. These were selected based upon: (a) chair conformation of the substituted piperidine ring, (b) extended conformation of the guanidino side chain, and (c) dissimilarity of the backbone conformations.

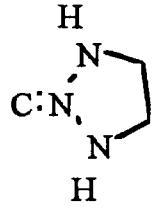
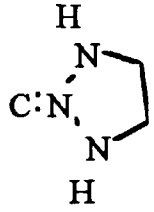
The MD-805 models were then produced, designated Model I and II, differ in the conformation of the quinoline ring. This difference is consistent with Banner's statement that they have no indication of a preferred conformation for that substituent. These models are deficient in that the structure of the Tyr-Pro-Pro-Trp loop, involved in the structure of the proximal and distal pockets, was not modified to accommodate the MD-805 binding:



6a

Models of the benzodiazepine template **6a** were built and flexible fit to the models of bound MD-805. Both stereoisomers of the template were studied using the two MD-805 models resulting in the production of four thrombin 'bound' forms of the template **6a**. These models were named TempRI, TempRII, TempSI, and TempSII depending upon the stereochemistry and MD-805 model used for fitting. *Figure 3* shows a comparison of the MD-805 modeled structures with the template **6a**.

Table I. Antithrombotic activity of the benzodiazepine derivatives.

	R_1	R_2	R_3	K_i (μM)
2a	CH_3	4-CH_3-C_6H_4	H	90
2b	CH_3	8-quinolyl	H	90
2c	CH_3	1,2,3,4-terahydro-8-quinolyl	H	90
6a	CH_2CO_2H	4-CH_3-C_6H_4	H	> 500
6b	CH_2CO_2H	8-quinolyl	H	42
6c	CH_2CO_2H	1,2,3,4-tetrahydro-8-quinolyl	H	208
16a	CH_2CO_2H	4-CH_3-C_6H_4	NH_2	84
16b	CH_2CO_2H	8-quinolyl	NH_2	> 1000
24a	CH_2CO_2H	4-CH_3-C_6H_4		> 1000
24b	CH_2CO_2H	8-quinolyl		N.I.[a]
19a	$(CH_2)_3CO_2H$	4-CH_3-C_6H_4	NH_2	> 500

[a]N.I.: not inhibitory.

Visualizing the four models for **6a** in the context of the PPACK thrombin active site indicated that, in all cases, large portions of the template structure were sterically disallowed because of unfavorable interactions within the active site cavity of the enzyme. For TempRI, the fused aromatic ring of the ligand abuts Glu192 of thrombin. In the model of TempRII the benzodiazepine aromatic ring substituent bumps into Trp60D of thrombin and comes quite close to the arylsulfonamide group. Similarly, the benzodiazepine aromatic substituent of TempSI collides with Trp215 of thrombin, as illustrated in *figure 9*. And for the TempSII model, the benzodiazepine aromatic substituent overlays Gly216 of thrombin.

Alignments of the **6a** template to the thrombin-docked MD-805 models rationalized the low inhibitory activity exhibited by this class. From modeling, it is clear that the position of the fused and/or the substituent aromatic ring (s) induced by the MD-805 overlay would be required to occupy regions of space already filled with amino acids of the protein.

5. Experimental protocols

5.1. Chemistry

Reaction progress was monitored by analytical thin layer chromatography, using the Kieselgel-60 F_{254} plates (Merck, Darmstadt, Germany). Visualization of TLC was done by UV light or iodine vapor. All reactions using nonaqueous reagents were run under a dry nitrogen atmosphere with magnetic stirring. Product isolation involved extraction of the quenched reaction mixture, washing, drying and concentration of the extracts; the solvent and other agents used are indicated in parentheses. Solvents were purified by distillation from the indicated drying agent: dichloro methane, CH_2Cl_2 ($LiAlH_4$); acetonitrile, CH_3CN (P_2O_5); ethyl ether, Et_2O ($LiAlH_4$ or CaH_2); tetrahydrofuran, THF (CaH_2). All analytical samples were homogeneous by TLC. Melting points were determined on a Kofler instrument. Purifications by gravity columns or by flash chromatography were carried out on Merck Silica gel 60 (0.063 – 0.20 μm) using the slurry method of column packing. The ^1H NMR and ^{13}C NMR of all compounds were determined with a Bruker/AC 200 FT spectrometer operating at 200 MHz with tetramethysilane as an internal reference. Chemical shifts (δ, ppm) quoted in the case of multiplets were measured from

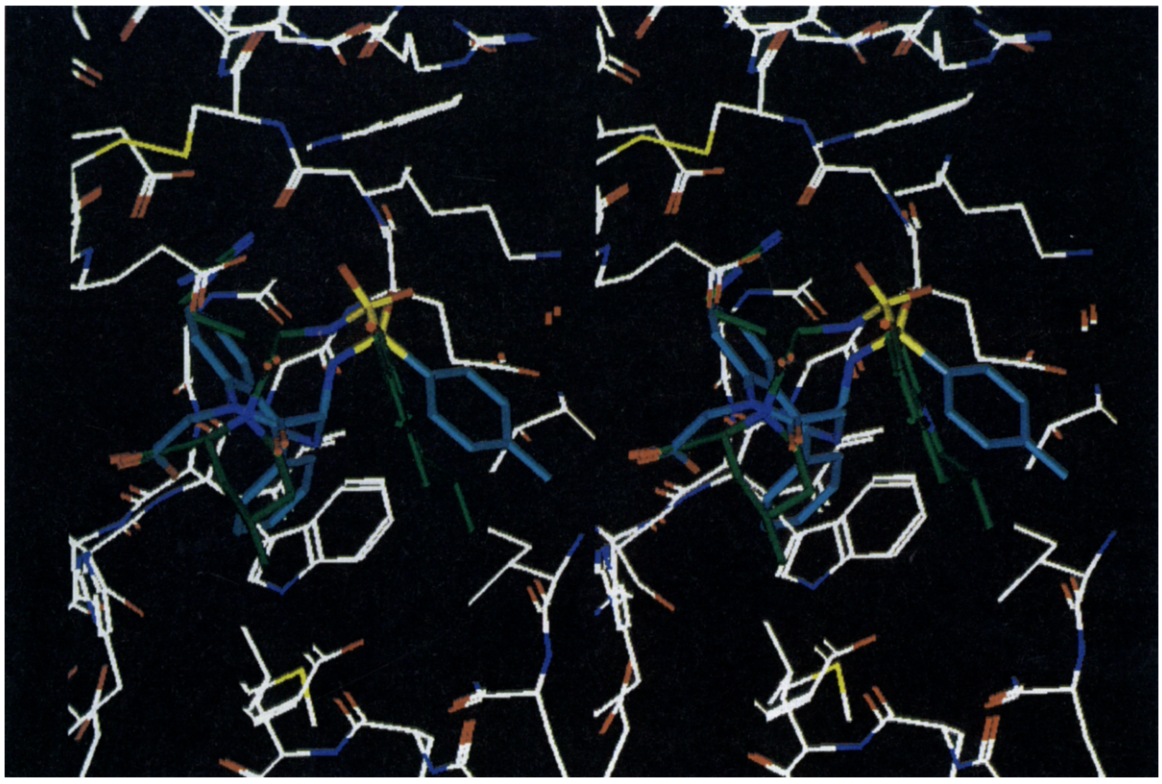


Figure 9. Stereoview of TempSI (light blue) and Model I (green) in the thrombin active site from the PPACK crystal complex.

the approximate center. The mass spectra were obtained with a quadrupole R 10.10C (NERMAG) mass spectrometer in the Fast-Atom-Bombardment (FAB) mode, the electron-impact (EI) or (D/CI). Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ± 0.4% of the theoretical values. 3-Amino-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one **1** was furnished by the Merck Research Laboratories as a para-toluylsulfonic acid salt.

5.1.1. Preparation of the compounds ***2a–c***

1,3-Dihydro-1-methyl-5-phenyl-3-[(tosyl)-amino]-2H-1,4-benzodiazepin-2-one ***2a****:* (Method A) Tosyl chloride (1.43 g, 6.32 mmol) and NEt_3 (5.25 mL, 253 mmol) were added to a solution of **1** (0.84 g, 3.16 mmol) in dry $CHCl_3$ (10 mL). The mixture was heated under reflux for 0.5 h. The hot solution was poured in crushed ice and stirred until a precipitation appeared. The solid was filtered off and recrystallized from EtOH to afford 0.92 g (69.5%) of **2** as white crystals: m.p. 235–237 °C; IR (KBr) 3220, 3035, 3000, 2900, 1680, 1600, 1595, 1360, 1140 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.48 (*s*, 3H), 3.34 (*s*, 3H), 4.82 (*s*, 1H), 6.86 (*d*, *J* = 7.3 Hz, 2H), 7, 14–7.50 (*M*, 6H), 7.58–7.75 (*M*, 5H), 9.14 (*s*, 1H); MS (Fab (+), Glycerol), *m/z* 420 (M + 1), 369 (100 %), 358, 351, 333, 308.

1,3-Dihydro-1-methyl-5-phenyl-3-[(8-quinolylsulfonyl)-amino)]-2H-1,4-benzodiazepin-2-one ***2b*** was prepared from **1** (1.2 g, 4.52 mmol) and 8-quinolylsulfonyl chloride (1.23 g, 9.04 mmol) according to method A. It was obtained as a crude solid and recrystallized from EtOH to afford 1.44 g (72%) of **2b** as white crystals: m.p. 258–260 °C; IR (KBr) 3030, 1680, 1605, 1595, 1570, 1490, 1390, 1140, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.36 (s, 3H, $NHCH_3$), 5.29 (*d*, *J* = 7.60 Hz, 1H), 6.62 (*d*, *J* = 7.60 Hz, 2H), 7.06 (*t*, *J* = 7.46 Hz, 1H), 7.70 (*t*, *J* = 8.01 Hz, 1H), 8.15 (*dd*, *J* = 7.08 Hz, *J* = 1.11 Hz, 1H), 8.29 (*dd*, *J* = 8.36 Hz, *J* = 1.46 Hz, 1H), 8.39 (*dd*, *J* = 7.08 Hz, *J* = 1.11 Hz, 1H), 9.11 (*dd*, *J* = 4.15 Hz, *J* = 1.46 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 35.30, 71.97, 121.70, 122.20, 124.53, 125.41, 127.71 (2C), 128.42, 129.06, 129.35 (3C), 130.41 (2C), 131.98, 132.56, 136.45, 137.49, 140.00, 142.80, 151.33, 165.96, 166.23; MS (Fab (+), NBA) *m/z* 457 (M + 1, 100%), 307, 289, 237, 222, 187, 176, 165. Anal. ($C_{25}H_{20}N_4O_3S$) C, H, N.

1,3-Dihydro-1-methyl-5-phenyl-3-[(1,2,3,4-tetrahydro-8-quinolylsulfonyl)-amino]-2H-1,4-benzodiazepin-2-one ***2c***: Pd/C (5%) (0.2 g) was added to a solution of **2b** (2 g, 4.38 mmol) dissolved in MeOH (40 mL) and hydrogenated (1 atm) at room temperature for 12 h. The catalyst was filtered on a Celite pad. The filtrate was concentrated. The oily residue was purified by flash chromatography using silica gel (eluent, EtOAc:hexane, 3:1) to afford 1.3 g (65%) of the title compound **2c**; m.p. 190–192 °C; IR (KBr) 3405, 3250, 2980, 1600, 1590, 1360, 1140, 790, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88 (*m*, *J* = 5.92 Hz, 2H), 2.81 (*t*, *J* = 6.4 Hz, 2H), 3.33 (*t*, *J* = 6.4 Hz, 2H), 3.40 (s, 3H), 5.06 (*d*, *J* = 8.8 Hz, 1H), 5.52–6.70 (m, 2H), 7.08–7.65 (M, 8H); ^{13}C NMR ($CDCl_3$) δ 20.68, 27.79, 35.36, 41.62, 70.55, 114.93, 121.70, 123.40, 124.58, 127.53, 127.96 (3C), 128.66, 129.76 (2C), 130.27, 130.69, 132.00, 133.83, 137.56, 142.75, 143.37, 166.40, 166.51.MS (Fab (+), NBA) *m/z* 457 (M + 1, 100%), 307, 289, 264, 249, 237, 222, 187, 176, 165. Anal. ($C_{25}H_{20}N_4O_3S$) C, H, N.

5.1.2. Preparation of the compounds ***6a–c***

1,3-Dihydro-5-phenyl-3-[(tosyl)-amino]-2H-1,4-benzodiaze pin-2-one ***4a*** was prepared according to method A from **3** (0.8 g, 3.26 mmol), tosyl chloride (0.62 g, 3.26 mmol) and anhydrous NEt_3 (3.66 mL, 26 mmol). The solid obtained was recrystallized from EtOH to afford 0.92 g (66%) of **4a**: IR (KBr) 3250, 3075, 2975, 2925, 1700, 1600, 1570, 1480, 1450, 1380, 1340, 1170, 1150, 1100, 1025, 920, 820, 770, 700, 680 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.44 (s, 3H), 4.76 (s, 1H), 6.78 (*d*, *J* = 7.34 Hz, 2H), 7.13–7.34 (*m*, 6H), 7.40 (*d*, *J* = 7.74 Hz, 2H), 7.62 (*dd*, *J* = 7.61 Hz, *J* = 2.10 Hz, 1H), 7.73 (*d*, *J* = 7.74 Hz, 2H), 9.14 (*s*, 1H), 10.69 (*s*, 1H); ^{13}C NMR (Me_2SO-d_6) δ 21.01, 71.51, 121.56, 123.43 125.91, 126.77 (2C), 127.92 (2C), 129.32 (4C), 130.33, 130.5, 132.29, 137.66, 138.78, 139.92, 142.50, 165.96, 166.48; MS (D/CI, NH_3 + isobutane) *m/z* 406 (M + 1), 250, 223, 207, 189. Anal. ($C_{22}H_{19}N_3O_3S$) C, H, N.

1,3-Dihydro-5-phenyl-3-[(8-quinolylsulfonyl)-amino]-2H-1,4-benzodiazepin-2-one ***4b*** was prepared from **3** following method A and obtained as a white solid (80%): m.p. 152–154 °C; IR (KBr) 3600–3200, 2950, 1680, 1600, 1560, 1360, 1140, 840, 800, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.25 (*d*, *J* = 9.75 Hz, 1H), 6.65 (*d*, *J* = 4.5 Hz, 2H), 7.18 (*t*, *J* = 7.5 Hz, 2H), 7.23 (*d*, *J* = 4.26 Hz, 2H), 7.20–7.42 (*m*, 2H), 7.58–7.70 (*m*, 1H), 7.49 (*dd*, *J* = 18.37 Hz, *J* = 4.20 Hz, 1H), 7.82 (*t*, *J* = 7.75 Hz, 1H), 8.35 (*d*, *J* = 2.84 Hz, 1H), 8.39 (*dd*, *J* = 5 Hz, *J* = 1.34 Hz, 1H), 8.45 (*d*, *J* = 9.75 Hz, 1H), 8.58 (*dd*, *J* = 8.37 Hz, *J* = 1.58 Hz, 1H), 8.93 (*dd*, *J* = 4.20 Hz, *J* = 1.58 Hz, 1H), 10.94 (*s*, 1H); ^{13}C NMR (Me_2SO-d_6) d 72.06, 121.61, 122.49, 123.44, 125.78, 125.98, 127.93 (2C), 128.66, 128.99 (2C), 129.31, 130.40, 132.28, 133.43, 137.14, 137.71, 138.58, 139.04, 142.64, 151.08, 165.77, 166.61; MS (Fab (+), NBA) *m/z* 444 (M + 1), 307, 289, 251, 235, 166.

2,3-Dihydro-2-oxo-5-phenyl-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***5a***: (Method B) To a solution of the sulfonamide **4a** (1.2 g, 2.96 mmol) in dry DMF (15 mL) was added NaH (0.18 g, 3.55 mmol) at 0 °C under a N_2 atmosphere. The reaction mixture was allowed to stir for 1 h at 0 °C and treated with $BrCH_2CO_2tBu$ (0.86 g, 4.44 mmol). The temperature was raised to 20 °C and the solution allowed to stir an additional hour. The solvent was evaporated and the oily residue taken up with CH_2Cl_2 (50 mL). This solution was washed with H_2O and brine (2 x 25 mL). The organic portion was dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. The oil obtained crystallized in anhydrous Et_2O (15 mL). The crude solid was purifed by recrystallization from EtOH to afford 0.90 g (58%) of **5a** as colorless crystals: IR (KBr) 3075, 2985, 2925, 1740, 1680, 1605, 1580, 1440, 1380, 1340, 1250, 1160, 1110, 1010, 820, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (*s*, 9H), 2.48 (*s*, 3H), 4.36 (*d*, J_{gem} = 17.1 Hz, 1H), 4.46 (*d*, J_{gem}, 1H), 5.23 (*d*, *J* = 8.85 Hz, 1H), 6.47 (*d*, *J* = 8.86 Hz, 1H), 7.09–7.18 (*dt*, *J* = 7.09 Hz, *J* = 1.52 Hz, 1H), 7.20–7.47 (*m*, 8H), 7.59 (*dd*, *J* = 6.13 Hz, *J* = 2.77 Hz, 1H), 7.81 (*dt*, *J* = 8.33 Hz, *J* = 1.78 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.52, 27.84, 50.73, 70.5, 82.57, 121.85, 125.22, 127.22 (2C), 127.96 (2C), 129.23, 129.54 (2C), 129.7 (2C), 130.38, 130.68, 132.25, 137.59, 139.34, 141.57, 143.09, 166.15, 166.68, 166.98;MS (D/CI, NH_3 + isobutane) *m/z* 520 (M^+ + 1, 100%), 464, 351, 281, 246, 207, 189, 171, 139, 110, 83. Anal. ($C_{18}H_{19}N_3O_5S$) C, H, N.

2,3-Dihydro-2-oxo-5-phenyl-3-[(8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-ethyl-methylcarboxylate ***5b*** was prepared according to method B from **4b** (1.2 g, 2.70 mmol) to afford 1.32 g (93%) of **5b**: IR (KBr) 3210, 2820, 1740, 1670, 1595, 1560, 1340, 1140, 800, 700 cm^{-1}; MS (CI, NH_3 + isobutane) *m/z* 529 (M^+), 422, 338, 321, 309, 295, 209, 194, 130 (100%).

2,3-Dihydro-2-oxo-5-phenyl-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-acetic acid ***6a***: The ester **5a** (0.35 g, 0.67 mmol) dissolved in formic acid (3 mL) was heated at 70 °C and allowed to stir under a N_2 atmosphere for 15–20 min. The excess formic acid was removed under reduced pressure and the residue taken up in $CHCl_3$ (10 mL). The organic phase was washed with brine (2 x 5mL), dried ($MgSO_4$) and concentrated under a reduced pressure to dryness. The solid was recrystallized from MeOH to afford 0.24 g (77%) of the attempted acid **6a**: R_f 0.21 (40:10 EtOAc/MeOH); m.p. 210–212 °C; IR (KBr) 3600–2200, 1700, 1680, 1600, 1560, 1500, 1460, 1380, 1340, 1230, 1160, 1130, 960, 830, 800, 700, 690 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 244 (*s*, 3H), 4.59 (*d*, J_{gem} = 17.11 Hz, 1H), 4.64 (*d*, J_{gem}, 1H), 4.88 (*d*, *J* = 10.1 Hz, 1H), 6.83 (*d*, *J* = 7.26 Hz, 2H), 7.09–7.50 (*m*, 7H), 7.52–7.75 (*m*, 4H), 9.19 (*d*, *J* = 10.1 Hz, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.99, 49.12, 71.07, 122.55, 125.12, 126.78 (2C), 127.64 (2C), 128.62, 129.31 (2C), 130.51, 132.32, 137.33, 141.53, 142.57, 165.46, 166.25, 169.7; MS (D/CI, NH_3 + isobutane) *m/z* 464 (M^+ + 1, 100%), 420, 246, 237, 207, 183, 174, 157, 139, 110, 91. Anal. ($C_{24}H_{21}N_3O_5S$) C, H, N.

2,3-Dihydro-2-oxo-5-phenyl-3-[(8-quinolylsulfonyl)-amino]-1H-l,4-benzodiazepin-1-acetic acid ***6b***: To a solution of **5b** (4 g, 7.5 mmol) in MeOH (40 mL) was added a 1 M NaOH solution (7.6 mL, 7.6 mmol). The reaction mixture was allowed to stir for 24 h at room temperature, then acidified with a solution of 1 M HCl (7.8 mL) and evaporated to dryness in vacuo. The residue was taken up with CH_2Cl_2 (50 mL) and washed with H_2O (3 x 10 mL). The organic portion was dried ($MgSO_4$), filtered and evaporated in vacuo. The solid was washed with hot EtOAc to give 2.4 g (64%) of the title acid **6b**: R_f 0.47 (80:20:2 EtOAc/MeOH/AcOH); m.p. 222–226 °C; IR (KBr) 3600–2900, 1700, 1670, 1600, 1560, 1360, 1140, 800, 700 cm^{-1} ; ^1H NMR (Me_2SO-d_6) δ 4, 15 (*d*, J_{gem} = 17 Hz, 1H), 4.35 (*d*, J_{gem}, 1H), 5.22 (*d*, *J* = 9.28 Hz, 1H), 6.55 (*d*, *J* = 7.40 Hz, 1H), 7.09–7.20 (*m*, 4H), 7.25–7.40 (*m*, 3H), 7.60–7.69 (*m*, 3H), 7.79 (*t*, *J* = 7.64 Hz, 1H), 7.32 (*d*, *J* = 6.15 Hz, 1H), 7.36 (*d*, *J* = 8.44 Hz, 1H), 7.59 (*d*, *J* = 6.92 Hz, 1H), 8.98 (*dd*, *J* = 4.2 Hz, *J* = 1.5 Hz, 1H); ^{13}C NMR (Me_2SO-d_6) δ 50.12, 71.63, 122.56 (2C), 124.75, 125.25, 125.60, 127.75 (2C), 128.73, 129.03 (2C), 129.42, 130.29, 132.16, 133.48, 137.15, 137.46, 139.05, 141.91, 142.72, 151.28, 165.20, 166.18, 170.51; MS (D/CI, NH_3 + isobutane) *m/z* 501 (M^+ + 1), 282, 246, 194, 162, 130 (100%), 83.

2,3-Dihydro-2-oxo-5-phenyl-3-[(1,2,3,4-tetrahydro-8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-acetic acid ***6c***: To a solution of **6b** (1 g, 2 mmol) in MeOH (30 mL) was added Pd/C (5%) (0.25 g) and the suspended mixture was hydrogenated (1 atm) at room temperature for 15 h. The catalyst was filtered on a Celite pad and the filtrate concentrated under a reduced pressure. The solid obtained was recrystallized from MeOH to afford 0.65 g (65%) of compound **6c** as colorless crystals: m.p. 222–224 °C; IR (KBr) 3300–2900, 1700, 1690, 1600, 1570, 1330, 1130, 1020, 700 cm^{-1}; ^1H NMR (CD_3COCD_3) δ 1.80–1.86 (*m*, 2H), 2.78–2.86 (*m*, 2H), 3.29–3.38 (*m*, 2H), 4.61 (*d*, J_{gem} = 17.7 Hz, 1H), 4.76 (*d*, J_{gem}, 1H), 5.06 (dd, *J* = 10.1 Hz, 1H), 6.49 (*t*, *J* = 7.39 Hz, 1H), 7.11–7.69 (*m*, 3H); ^{13}C NMR (CD_3COCD_3) δ 21.48, 42.16, 49.57, 71.57, 115.01, 123.06, 123.40, 123.98, 125.92, 128.25, 128.55 (2C), 130.54, 130.68 (2C), 131.19, 132.96, 134.34, 139.02, 142.83, 144.20, 166.77, 169.80; SM (D/CI, NH_3 + isobutane) *m/z* 505 (M^+ + 1, 100%), 443, 427, 377, 246, 213, 134.

5.1.3. Preparation of the compounds ***16a*** *and* ***16b***

2-Amino-3'nitrobenzophenone ***7*** was obtained according the procedure described by Detar and al. [31]: m.p. 88–90 °C. Anal. ($C_{13}H_{10}N_2O_3$) C, H, N.

α-Isopropylthio-Nα-(benzoyloxycarbonyl)glycine ***8*** was prepared following the procedure described by Ben-Ishai [33].

2-[N-(α-(Isopropylthio)-Nα-(benzyloxycarbonyl)-glycinyl)-amino]-3'-nitrobenzophenone ***9***: α-(Isopropylthio)-$N_α$-(benzyloxycarbonyl)glycine **8** (0.54 g, 37.2 mmol) was dissolved in dry CH_2Cl_2 (400 mL), cooled to 0 °C, and treated with N-methylmorpholine (4.1 mL, 37.2 mmol) followed by isobutyl chloroformate (4.8 mL, 37.2 mmol) under a N_2 atmosphere. The resulting reaction mixture was stirred at 0 °C for 15 min more and then was heated to reflux. The refluxing reaction mixture was treated dropwise over 20 min with a solution of 2-amino-3'-nitrobenzophenone **7** (7.63 g, 33.54 mmol) in dry CH_2Cl_2 (75 mL). After the addition was complete, the reaction mixture was heated under reflux for 20 min and then allowed to stir at room temperature overnight. The reaction mixture was washed in succession with 10% citric acid solution (2 x 200 mL), saturated $NaHCO_3$ solution (2 x 100 mL), and brine. The dried ($MgSO_4$) organic phase was concentrated to give 15.6 g (92%) as white crystals: R_f (EtOAc:cyclohexane, 1:1); IR (KBr) 3400, 3300, 3270, 3225, 1720, 1700, 1640, 1600, 1580, 1540, 1500, 1450, 1350, 1320, 1300, 1260, 1220, 1160, 1040, 975, 910, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (*d*, *J* = 6.70 Hz, 3H), 1.44 (*d*, *J* = 6.70 Hz, 3H), 3.28 (*m*, 1H), 5.16 (*s*, 2H), 5.61 (*d*, *J* = 8.12 Hz, 1H) , 5.96 (*d*, *J* = 7.72 Hz, 1H, NH), 7.16 (*t*, *J* = 7.47 Hz, 1H) , 7.26 (*s*, 1H), 7.35 (*m*, 1H), 7.51 (*d*, *J* = 7.88 Hz, 1H), 7.60–7.74 (*m*, 2H), 8.02 (*d*, *J* = 7.72 Hz, 1H), 8.45 (*d*, *J* = 8.17 Hz, 1H), 8.55 (*s*, 1H), 8.62 (*d*, *J* = 8.36 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 23.02, 23.69, 35.35, 57.65, 66.91, 121.37, 122.38, 122.76, 124.12, 126.32, 127.67 (2C), 127.79, 128.11 (2C), 129.29, 132.85, 134.72, 134.96, 135.73, 139.37, 147.64, 155.04, 167.01, 196.12; MS (EI) *m/z* 433, 388, 371, 269, 238, 223, 191, 146, 108, 91 (100%). Anal. ($C_{26}H_{25}N_3O_6S$) C, H, N.

2-[N-(α-(Amino-Nα-(benzyloxycarbonyl)glycinyl)amino]-3'-nitrobenzophenone ***10***: The crude (isopropylthio) glycinamide **9** (15 g, 33.5 mmol) was dissolved in dry THF (200 mL). This solution was cooled to 0 °C and saturated with ammonia. $HgCl_2$ (10.02 g, 36.9 mmol) was then added in one portion to the stirred mixture, while a continuous stream of ammonia gas was bubbled into the reaction mixture. After 3 h, the suspended solids were removed, and the solvent was rotary evaporated to give crude 17 g of **10** as an oil which was used immediately without further purification (Note: all operations should be confined to a well-ventilated hood): R_f 0.23 (EtOAc:cyclohexane, 1:1); IR (KBr) 3400, 3300, 3270, 3225, 2975, 2925, 1720, 1700, 1640, 1600, 1580, 1540, 1500, 1450, 1350, 1320, 1300, 1260, 1220, 1160, 1040, 975, 910, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (*d*, *J* = 6.7 Hz, 3H), 1.44 (*d*, *J* = 6.7 Hz, 3H), 3.28 (*heptuplet*, 1H), 5.16 (*s*, 2H), 5.61 (*d*, *J* = 8.12 Hz, 1H), 5.96 (*d*, *J* = 7.72 Hz, 1H), 7.16 (*t*, *J* = 7.47 Hz, 1H), 7.26 (*s*, 1H), 7.35 (*m*, 1H), 7.51 (*d*, *J* = 7.88 Hz, 1H), 7.60–7.14 (*m*, 2H), 8.02 (*d*, *J* = 7.72 Hz, 1H), 8.45 (*dm*, *J* = 8.17 Hz, 1H), 8.55 (*s*, 1H), 8.62 (*d*, *J* = 8.36 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 23.02, 23.69, 35.35, 57.65, 66.91, 121.37, 122.38, 122.76, 124.12, 126.32, 127.67 (2C), 127.79, 128.11 (2C), 129.29, 132.85, 134.96, 135.73, 139.37, 139.57, 147.64, 155.04, 167.01, 196.12; MS (EI) *m/z* 433, 388, 371, 269, 238, 223, 191, 146, 108, 91. Anal. ($C_{26}H_{25}N_3O_6S$) C, H, N.

1,3-Dihydro-5-(3-nitrophenyl)-3-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one ***11***: Crude α-amino-glycinamide **10** (16.5 g, 36.85 mmol) was dissolved in glacial AcOH (300 mL) and was treated with NH_4OAc (14.33 g, 186 mmol). The resulting mixture was then protected from moisture and stirred at room temperature overnight. The heterogeneous reaction mixture was concentrated under reduced pressure to remove the AcOH, and the residue was partitioned between EtOAc (200 mL) and 1 N NaOH solution (40 mL). The mixture was stirred for approximately 1 h. The solids were collected and washed with EtOAc to afford 10.1 g (64%) of analytically pure **11**: m.p. 166–168 °C; R_f 0.42 (EtOAc:cyclohexane, 1:1); IR (KBr) 3370, 1695, 1595, 1575, 1520, 1480, 1440, 1410, 1360, 1030, 920, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.18 (s, 2H), 5.38 (*d, J* = 8.18 Hz, 1H), 6.73 (*d, J* = 8.18 Hz, 1H), 7.18–7.46 (*M*, 8H), 7.51–7.62 (*M*, 2H), 7.30 (*m, J* = 7.80 Hz, 1H), 8.30 (*m, J* = 8.20 Hz, 1H), 8.37 (*s*, 1H), 9.85 (*s*, 1H); ^{13}C NMR ($CDCl_3$) δ 67.16, 69.08, 121.92 (2C), 124.55 (2C), 121.15, 126.30, 128.13 (2C), 128.47 (2C), 129.30, 130.47, 132.79, 135.54, 136.06, 137.62, 139.91, 148.05, 155.73, 165.59, 168.49; MS (D/CI, NH_3 + isobutane) *m/z* 431 (M^+ + 1), 398, 369, 323, 307, 295, 279, 268, 252, 221, 205, 91 (100%). Anal. ($C_{23}H_{18}N_4O_5$) C, H, N.

3-Amino-1,3-dihydro-5-(3-nitrophenyl)-2H-1,4-benzodiazepin-2-one ***12***: The benzodiazepinone **11** (20 g, 46.8 mmol) was treated with 33% HBr (180 mL) in AcOH at 0 °C under a N_2 atmosphere. The solution was allowed to stir at 0 °C for 10 min and then the temperature was raised to 25 °C. A large excess of anhydrous Et_2O was added after 30 min to the solution until the HBr salt precipitated. The solid was filtered, washed with anhydrous Et_2O, and dissolved with a small portion of H_2O and treated with 10% $NaHCO_3$ to reach a pH 7.5–8. The free precipitated amine was filtered and dried over P_2O_5. The amine was recrystallized from MeOH to afford 13.5 g (97%) of 3-amino benzodiazepinone **12**: IR (KBr) 3400, 3050, 1680, 1600, 1595, 1560, 1520, 1480, 1340, 1020, 760, 680 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.28 (*s*, 1H, $CHNH_2$), 7.15–7.38 (*m*, 4H), 7.55–7.88 (*M*, 4H), 8.33 (*d, J* = 7.21 Hz, 2H, NH_2), 10.77 (*s*, 1H, NHCO); ^{13}C NMR (Me_2SO-d_6) δ 70.73, 121.44, 123.11, 123.46, 124.73, 125.29, 129.86 (2C), 130.00, 132.13, 135.54, 139.07, 139.94, 147.72; MS (D/CI, NH_3 + isobutane) *m/z* 296 (M^+), 280 (100%), 268, 252, 221, 206, 193, 178, 166, 146, 119.

1,3-Dihydro-2-oxo-5-(3-nitrophenyl)-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-2-one ***13a*** was prepared from **12**, using method C, and obtained as a white solid (96%): m.p. 250–252 °C; IR (KBr) 3275, 1680, 1595, 1520, 1340, 1300, 1160, 1140, 1090, 1020, 920, 810, 760, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.98 (*s*, 3 H), 4.85 (*s*, 2 H), 7.18–7.48 (*M*, 6 H), 7.50–7.80 (*M*, 5 H), 8.28 (*d, J* = 7.95 Hz, 1H), 9.25 (*s*, 1 H), 11.01 (*s*, lH); ^{13}C NMR (Me_2SO-d_6) δ 21.0, 71.7, 121.6, 123.16, 123.73, 124.99, 125.16, 126.63 (2C), 129.44 (2C), 129.77, 130.05, 132.79, 135.59, 138.99 (2C), 139.65, 142.14, 147.68, 164.26, 166.18; MS (CI, NH_3 + isobutane) *m/z* 451 (M + 1), 335, 313, 295, 268, 189, 174 (100%), 156, 139. Anal. ($C_{22}H_{18}N_4O_5S$) C, H, N.

1,3-Dihydro-5-(3-nitrophenyl)-3-[(8-quinolylsulfonyl)-amino]-2H-1,4-benzodiazepin-2-one ***13b***: (Method C) To a solution of amino-benzodiazepinone **12** (3 g, 10 mmol) dissolved in anhydrous pyridine (30 mL) was added the 8-quinolylsulfonyl chloride (3.40 g, 15 mmol) by portions at 0 °C. The reaction mixture was allowed to stir for 15 min at 0 °C, then heated under reflux for 1.5 h. The solvent was evaporated under reduced pressure and the oily residue was poured in a 50% H_2O/EtOH mixture to precipitate the sulfonamide. The solid was filtered and recrystallized from hot EtOH to give 4.12 g (84%) of the pure sulfonamide as white crystals: m.p. 288–289 °C; IR (KBr) 3250, 1700, 1620, 1520, 1340, 1320, 1170, 1130, 1020, 920, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.47 (*s*, 1H), 7.21 (*t, J* = 7.88 Hz, 1H), 7.26–7.40 (m, 3H), 7.53 (*t, J* = 8.04 Hz, 1H), 7.61–7.76 (m, 3H), 760 (*t, J* = 7.67 Hz, 1H), 8.25 (*dd, J* = 7.75 Hz, *J* = 1.95 Hz, 1H), 8.35 (*dd, J* = 13.49 Hz, *J* = 1.23 Hz, 1H), 8.39 (*dd, J* = 12.52 Hz, *J* = 1.23 Hz, 1H), 8.54 (*dd, J* = 8.43 Hz, *J* = 1.61 Hz, 1H), 8.74 (*s*, 1H), 8.84 (*dd, J* = 4.22 Hz, *J* = 1.61 Hz, 1H), 11.01 (*d*, 1H); ^{13}C NMR (Me_2SO-d_6) δ 72.40, 121.81, 122.45, 123.25, 133.68, 125.07, 125.21, 125.76, 28.62, 129.59, 129.76, 130.24, 132.79, 133.62, 135.25, 137.20, 138.96 (2C), 139.20, 142.52, 147.53, 150.95, 164.09, 166.63; MS (D/CI, NH_3 + isobutane) *m/z* 488 (M^+ + 1), 335, 295, 194, 171, 153, 130, 100 (100%), 93, 77. Anal. ($C_{24}H_{17}N_5O_5S$) C, H, N.

2,3-Dihydro-5-(3-nitrophenyl)-2-oxo-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***14a***: (Method D) To a solution of the sulfonamide **13a** (3.90 g, 6.43 mmol) dissolved in DMF (50 mL) was added by portion solid K_2CO_3 (0.58 g, 6.43 mmol) at 0 °C under a N_2 atmosphere. The reaction mixture was stirred at 0 °C for 30 min. The tert-butyl-acetyl bromide was then added in a dropwise manner to the above solution at 0 °C. The temperature was raised to 25 °C and the reaction mixture was allowed to stir for 2 h. The solvent was evaporated and the oily residue dissolved in $CHCl_3$ (30 mL). The organic solution was washed with H_2O (3 x 15 mL) and brine (2 x 30 mL). The organic portion was dried ($MgSO_4$), filtered and concentrated under a reduced pressure to dryness. The solid obtained was triturated with anhydrous Et_2O (50 mL) and recrystallized from a mixture $CHCl_3$/EtOH (1:3) to afford 3.2 g (88%) of the ester **14a** as colorless crystals: m.p. 173–175 °C; IR (KBr) 3350, 3075, 2975, 2925, 2875, 1750, 1690, 1595, 1565, 1535, 1495, 1450, 1435, 1410, 1370, 1340, 1320, 1240, 1160, 1100, 1010, 940, 520, 780, 700, 660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (*s*, tBu, 9H), 2.49 (*s*, $PhCH_3$, 3H), 4.36 (*d,* J_{gem} = 17.20 Hz, 1H), 4.59 (*d,* J_{gem}, 1H), 5.30 (*d, J* = 8.64 Hz, 1H), 6.50 (*d, J* = 8.64 Hz, 1H), 7.25–7.54 (*M*, 7H), 7.66 (*ddd, J* = 8.45 Hz, *J* = 6.90 Hz, *J* = 1.55 Hz, 1H), 7.83 (*d, J* = 8.28 Hz, 2H), 8.10 (*t, J* = 1.85 Hz, 1H), 8.28 (*ddd, J* = 7.47 Hz, *J* = 1.85 Hz, *J* = 1.75 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.54, 27.82 (3C), 50.37, 70.58, 82.72, 122.5, 124.16, 125.17, 125.76, 126.99 (2C), 128.60, 129.06, 129.53, 129.64 (2C), 132.83, 135.48, 139.02, 139.34, 141.48, 143.62, 148.06, 165.13, 165.70, 166.48; MS (FAB (+), NBA) *m/z* 565 (M^+ + 1), 509, 338, 310 (100%), 252, 205, 190, 154, 136, 107. Anal. ($C_{28}H_{28}N_4O_7S$) C, H, N.

2,3-Dihydro-5-(3-nitrophenyl)-2-oxo-3-[(8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***14b*** was prepared using method D from **13b** (4 g, 8.20 mmol), K_2CO_3 (1.1 g, 8.20 mmol) and tert-butylacetyl bromide (1.12 mL, 8.2 mmol) to afford 4.5 g (97%) of **14b** as white crystals: m.p. 234–236 °C; IR (KBr) 3225, 3075, 2975, 1730, 1705, 1600, 1560, 1540, 1430, 1360, 1340, 1230, 1140, 790, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (*s*, 9H, tBu), 4.35 (*d,* J_{gem} = 17.2 Hz, 1H), 4.56 (*d,* J_{gem}, 1H), 5.47 (*d, J* = 9.75 Hz, 1H), 7.10–7.40 (*M*, 5H), 7.48–7.62 (*M*, 3H), 7.12–7.73 (*t, J* = 7.54 Hz, 1H), 8.16 (*m*, 2H), 8.25–8.36 (*m*, 2H), 8.41 (*d, J* = 7.23 Hz, 1H), 9.11 (*dd, J* = 4.21 Hz, *J* = 1.66 Hz, 1H); ^{13}C NMR (Me_2SO-d_6) δ 27.45, 49.92, 71.93, 81.55, 122.47, 123.02, 123.24, 125.17, 125.52, 125.74, 127.92, 128.62, 129.37, 129.58, 129.60, 132.92, 133.68, 135.04, 137.21, 138.88, 141.48, 142.51, 147.47, 151.07, 164.37, 165.76, 167.21; MS (Fab (+), NBA) *m/z* 602 (M + 1), 546, 409, 353, 338, 310, 264, 192, 154, 129, 107 (100%).

2,3-Dihydro-5-(3-aminophenyl)-2-oxo-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***15a***: Compound **14a** (4 g, 7.08 mmol) and 5% Pd/C (0.75 g, 0.7 mmol) were suspended in CH_3CN (50 mL) and then anhydrous NEt_3 (4.5 mL, 31.8 mmol) dissolved in CH_3CN (10 mL) was added in a dropwise manner. The mixture was heated to boiling and 97% formic acid (1.15 mL, 30.5 mmol) was added over a period of a few minutes. After the addition, the mixture was quickly heated to boiling for 5–10 min and then cooled. CH_2Cl_2 was added and the catalyst was removed by filtration. The solvent and excess of NEt_3 were removed under reduced pressure and **15a** was purified by flash chromatography using silica gel (eluent, EtOAc:cyclohexane, 2:1) to give 3.2 g (85%) as a white solid: m.p. 158–160 °C; IR (KBr) 3450, 3375, 3050, 2975, 2925, 2875, 1740, 1690, 1595, 1490, 1450, 1370, 1340, 1240, 1160, 1100, 1020, 950, 820, 710, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (*s*, 9H), 2.46 (*s*, 3H), 4.36 (*d*, J_{gem} = 17.20 Hz, 1H), 4.59 (*d*, J_{gem}, 1H), 5.20 (*d*, *J* = 8.3 Hz, 1H), 6.45 (*d*, *J* = 8.3 Hz, 2H, NH), 6.53 (*d*, *J* = 7.65 Hz, 1H), 6.63 (*s*, 1H), 6.78 (*d*, *J* = 7.37 Hz, 1H), 7.10 (*dd*, *J* = 7.86 Hz, *J* = 7.32 Hz, 1H), 7.20–7.42 (*m*, 4H), 7.58 (*ddd*, *J* = 8.45 Hz, *J* = 6.75 Hz, *J* = 2.00 Hz, 1H), 7.80 (*d*, *J* = 6.50 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.5, 27.9, 50.8, 70.5, 82.6, 116.1, 117.6, 120.8, 121.7, 125.10, 127.20, 128.60, 129.10, 129.5 (2C), 130.56, 132.20, 138.60, 139.30, 141.50, 142.90, 145.60, 166.25, 166.7O, 167.10. Anal. ($C_{28}H_{30}N_4O_5S$) C, H, N.

2,3-Dihydro-5-(3-amino)-2-oxo-3-[(8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***15b*** was prepared from **14b**, according the procedure given for **15a**, to afford 0.6 g (84%) of **15b** as colorless crystals: m.p. 238–240 °C; IR (KBr) 3450–3375, 2975, 1740, 1695, 1600, 1595, 1495, 1455, 1340, 1230, 1160, 1140, 840, 800, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (*s*, 9H), 4.25 (*d*, J_{gem} = 17.15 Hz, 1H), 4.43 (*d*, J_{gem}, 1H), 5.40 (*s*, 1H), 6.00 (*t*, *J* = 1.91 Hz, 1H), 6.13 (*d*, *J* = 7.75 Hz, 1H), 6.60 (*dd*, *J* = 7.64 Hz, *J* = 1.64 Hz, 1H), 6.88 (*t*, *J* = 7.80 Hz, 1H), 7.21–7.31 (*M*, 4H), 7.50–7.56 (*M*, 2H), 7.70 (*dd*, *J* = 1.40 Hz, 1H), 8.12 (*dd*, *J* = 8.20 Hz, *J* = 1.34 Hz, 1H), 8.28 (*dd*, *J* = 8.39 Hz, *J* = 1.69 Hz, 1H), 8.39 (*dd*, *J* = 7.26 Hz, *J* = 1.34 Hz, 1H), 9.07 (*dd*, *J* = 4.26 Hz, *J* = 1.69 Hz, 1H); ^{13}C NMR (Me_2SO-d_6) δ 27.45, 50.10, 71.63, 81.37, 114.51, 115.65, 116.63, 122.12, 122.40, 124.68, 125.70, 128.19, 128.59, 128.76, 129.27, 129.66, 132.11, 133.35, 137.04, 138.69, 141.23, 142.56, 148.19, 150.99, 166.18, 166.41, 167.07; MS (D/CI, NH_3 + isobutane) *m/z* 572 (M^+ + 1), 100%, 516, 379, 323, 280, 154, 136.

2,3-Dihydro-2-oxo-5-[(3-aminophenyl)-amino]-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-acetic acid ***16a***: To a solution of **15a** (0.4 g, 0.66 mmol) in AcOH (5 mL) was added an excess of anhydrous trifluoroacetic acid. The reaction mixture was heated under reflux for 3 h and the solvents distilled off. The oily residue was taken up in $CHCl_3$ (10 mL) and washed with H_2O (2 x 5 mL) and brine (2 x 5 mL). The organic portion was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The solid was purified by flash chromatography using silica gel (eluent, EtOAc:cyclohexane 60:40) to afford 0.22 g (70%) of the amino acid **16a** as white crystals: m.p. > 260 °C; IR (KBr) 3700–2800, 1680, 1600, 1495, 1460, 1440, 1380, 1340, 1310, 1160, 1150, 1000, 1030, 720 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.41 (*s*, 3H), 3.91 (*d*, J_{gem} = 16.62 Hz, 1H), 4.68 (*d*, J_{gem}, 1H), 4.82 (*d*, *J* = 5.92 Hz, 1H), 5.05 (*s*, 2H, NH_2), 5.95 (*d*, *J* = 7.74 Hz, 1H), 6.24 (*s*, 1H), 6.58 (*d*, *J* = 7.84 Hz, 1H), 6.88 (*t*, *J* = 7.61 Hz, 1H), 7.08–7.30 (*M*, 2H), 7.39 (*d*, *J* = 8.07 Hz, 2H), 7.50–7.60 (*M*, 2H), 7.52–7.78 (*M*, 3H), 9.02 (*s*, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.99, 51.92, 71.36, 115.88, 117.10, 122.23, 124.14, 126.71 (2C), 128.29, 129.33 (2C), 131.84, 138.34, 139.85, 142.43, 142.79, 148.10, 165.49, 166.54, 172.90; MS (FAB (–), NBA) *m/z* 477 (M–1, 100%), 459, 385, 352, 340, 320.

2,3-Dihydro-2-oxo-5-(3-aminophenyl)-3-[(8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-acetic acid ***16b***: To a solution of **15b** (0.5 g, 0.87 mmol) in dry $CHCl_3$ (20 mL) was added an excess of trifluoroacetic acid (2 mL). The reaction mixture was heated under reflux for 3 h. The solvents were evaporated and the oily residue was purified by flash chromatography using silica gel (eluent, $CHCl_3$/MeOH/AcOH, 84:15:1) to afford 0.3 g (68%) of the amino acid **16b** as white crystals: m.p. 238–240 °C; IR (KBr) 3600–2800, 1600, 1595, 1380, 1210, 1170, 1140, 1020, 840, 770, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.51 (*d*, J_{gem} = 17.7 Hz, 1H), 4.7 (*d*, J_{gem}, 1H), 5.55 (*s*, 1H), 6.96 (*dt*, *J* = 7 Hz, *J* = 1.65 Hz, 1H), 7.24–7.50 (*m*, 5H), 7.55–7.90 (*m*, 4H), 8.40 (*dd*, *J* = 8.31 Hz, *J* = 1.34 Hz, 1H), 8.48 (*dd*, *J* = 1.34 Hz, *J* = 7.35 Hz, 1H), 8.75 (*dd*, *J* = 9.66 Hz, *J* = 1.64 Hz, 1H), 9.01 (*dd*, *J* = 4.51 Hz, *J* = 1.64 Hz, 1H); ^{13}C NMR (CD_3OD) δ 50.06, 72.91, 123.84, 123.95, 125.38, 126.34, 126.88, 127.87, 130.09, 130.81, 130.96, 131.96 (3C), 132.01, 133.01, 134.22, 134.22, 135.37, 138.10, 140.85, 141.56, 141.82, 143.08, 167.45, 167.78, 171.09; MS (D/CI, NH_3 + isobutane) *m/z* 516 (M^+ + 1), 442, 367, 338, 321, 289, 279, 261, 209 (100%).

5.1.4. Preparation of the compound ***19a***

2,3-Dihydro-2-oxo-5-(3-nitrophenyl)-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-ethyl-propylcarboxylate ***17a***: To a solution of **13a** (2.5 g, 5.54 mmol) in dry DMF was added K_2CO_3 under a N_2 atmosphere. The reaction mixture was allowed to stir at 0 °C for 0.5 h and then ethyl-4-iodobutanoate (1.6 g, 6.64 mmol) was added. The temperature was raised to 25 °C for 1 h and heated to 70 °C for 3 h. The solvent was evaporated and the oily residue taken up with $CHCl_3$ (50 mL). The organic solution was washed with H_2O (2 x 20 mL), brine (2 x 20 mL), dried ($MgSO_4$) and filtered. The solvent was evaporated under reduced pressure. The oily residue was purified by flash chromatography using silica gel (eluent, EtOAc/cyclohexane, 1:1) to give a pale white solid. The solid was recrystallized from EtOH to afford 1.25 g (40%) of the ester **17a** as colorless crystals: m.p. 173–175 °C; IR (KBr) 3250, 2975, 2925, 2875, 1730, 1680, 1600, 1520, 1430, 1440, 1380, 1350, 1170, 1160, 1100, 820, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (*t*, *J* = 7.11 Hz, 3H), 1.60–2.32 (*m*, 4H), 2.48 (*s*, $PhCH_3$, 3H), 3.64–3.90 (*m*, 1H), 3.90–4.12 (*dq*, *J* = 7.11 Hz, *J* = 2 Hz, 2H), 4.15–4.40 (*m*, 2H), 5.19 (*d*, *J* = 8.57 Hz, 1H), 6.61 (*d*, *J* = 8.57 Hz, 1H), 7.22–7.59 (*m*, 7H), 6.62–7.63 (*m*, 1H), 7.81 (*d*, *J* = 8.28 Hz, 2H), 8.07 (*t*, *J* = 1.08 Hz, 1H), 8.22–8.33 (*m*, 1H); ^{13}C NMR (Me_2SO-d_6) δ 13.83, 20.96, 22.49, 29.96, 45.74, 59.73, 71.60, 122.84, 123.50, 125.38, 125.59, 126.67 (2C), 128.8, 147.79, 163.99, 165.40, 171.78; MS (FAB (+), NBA) *m/z* 565 (M + 1, 100%), 549, 491, 409, 394, 366, 307.

2,3-Dihydro-2-oxo-5-(3-nitrophenyl)-3-[(tosyl)amino]-1H-1,4-benzodiazepin-1-butyric acid ***18a***: To a solution of **17a** (1.2 g, 2.12 mmol) in MeOH was added a 1 M NaOH solution (2.12 mL, 2.12 mmol) at room temperature. The reaction mixture was allowed to stir for 24 h and the solvent was evaporated off. The oily residue was taken up with $CHCl_3$ (50 mL) and acidified with a 1 M HCl solution. The organic portion was separated and extracted with a satured $NaHCO_3$ solution (2 x 25 mL). The basic solution was acidified with a 1 M HCl solution and extracted with $CHCl_3$ (3 x 20 mL). The combined organic portion was dried ($MgSO_4$), filtered and evaporated to

give 0.63 g (53%) of pure **18a** as yellowish crystals: m.p. 208–210 °C; R_f 0.25 (CH_2Cl_2:MeOH, 96:4); IR (KBr) 3450, 3375, 3600–2400, 1710, 1670, 1600, 1595, 1520, 1440, 1380, 1160, 1100, 1040, 960, 820, 720 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.32–1.70 (m, 2H, $CH_2H_2CH_2$), 1.75–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.37 (s, 3H, $PhCH_3$), 3.77 (*dt, J* = 17.10 Hz, *J* = 6.90 Hz, 1H), 4.21 (*dt, J* = 17.70 Hz, *J* = 6.9 Hz, 1H), 7.26–7.49 (*m*, 4H), 7.55–7.73 (*M*, 4H), 7.74–7.85 (*m*, 3H), 8.31 (*d, J* = 8.11 Hz, 1H), 9.24 (*s*, 1H, $NHSO_2$); ^{13}C NMR (Me_2SO-d_6) δ 20.95, 22.61, 30.22, 45.60, 71.59, 122.62, 123.43, 125.35, 125.53, 126.63 (2C), 128.04, 129.15, 129.37 (2C), 129.95, 132.92, 135.32, 138.26, 139.43, 141.24, 142.79, 147.77, 163.99, 165.32, 173.49; MS (D/CI, NH_3 + isobutane) *m/z* (M^+ + 1), 408, 391, 381, 354, 296, 277, 189, 174 (100%), 156, 139, 104.

2,3-Dihydro-2-oxo-5-(3-aminophenyl)-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-butyric acid ***19a***: A solution of **18a** (0.2 g, 0.5 mmol) in MeOH (25 mL) was, after the addition of Pd/C (5%, 50 mg), hydrogenated at 1 atm of H_2 for 12 h. The reaction mixture was filtered, the residue was washed with hot MeOH (ca. 10 mL), and the filtrate evaporated and dried under high vacuum. The residue was purified by flash chromography using silica gel (eluent, $CHCl_3$/MeOH/AcOH, 84:15:1.5) to give a pale white solid. This later was recrystallized from MeOH to give 0.13 g (52%) of **19a** as white crystals: m.p. 218–220 °C; IR (KBr) 3450, 3375, 3600–2400, 1710, 1680, 1600, 1595, 1500, 1460, 1380, 1340, 1160, 1100, 1040, 960, 840, 720 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.30–1.68 (*m*, 2H), 1.95 (*t, J* = 7.03 Hz, 1H), 2.40 (*s*, 3H), 3.72 (*dt,* J_{gem} = 17.10 Hz, *J* = 7.00 Hz, 1H), 4.18 (*dt,* J_{gem}, *J* = 7.00 Hz, 1H), 4.74 (*d, J* = 9.00 Hz, 1H), 5.07 (*s*, 2H), 6.0 (*d, J* = 7.70 Hz, 1H), 6.27 (*s*, 1H), 6.61 (*d, J* = 7.70 Hz, 1H), 6.91 (*t, J* = 7.71 Hz, 1H), 7.18 (*d, J* = 7.74 Hz, 1H), 7.32 (*m*, 1H), 7.38 (*d, J* = 7.90 Hz, 1H), 7.64 (*d, J* = 7.90 Hz), 1H, 7.66 (m, 2H), 9.09 (*d, J* = 9.90 Hz, 1H); ^{13}C NMR (Me_2SO-d_2) δ 20.95, 22.59, 39.12, 45.48, 71.27, 114.43, 116.07, 116.90, 122.79, 125.04, 126.58 (2C), 128.44, 129.31 (2C), 129.53, 129.65, 132.11, 137.93, 139.55, 140.79, 142.47, 148.28, 165.74, 166.38; MS (D/CI, NH_3 + isobutane) *m/z* 507 (M^+ + 1), 409, 378, 363, 324, 296, 189, 169, 102 (100%).

5.1.5. Preparation of the compounds ***24a*** *and* ***24b***

2,3-Dihydro-5-(3-formylaminophenyl)-2-oxo-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***20a***: The acetic-formic anhydride was prepared by heating Ac_2O (14 mL, 30.3 mmol) with HCO_2H (21.5 mL, 20.2 mmol) at 50 °C for 15 min and cooled immediately to 0 °C. To this solution was added the anilino derivative **15a** (1.44 g, 2.02 mmol). The mixture was heated to 50 °C for 30 min, evaporated to dryness, and recrystallized from a mixture (EtOAc/$CHCl_3$, 1:1) to afford 0.85 g (77%) of formamide: m.p. 208–210 °C; R_f 0.26 (EtOAc/cyclohexane, 1:1); IR (KBr) 3200, 2995, 2875, 1740, 1700, 1690, 1595, 1450, 1370, 1340, 1240, 1160, 1100, 1030, 820, 790, 710, 690 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.29 (*s*, 9H), 2.39 (*s*, 3H), 4.51 (*s*, 2H), 4.88 (*d, J* = 7.10 Hz, 1H), 6.55 (*d, J* = 7.10 Hz, 1H), 7.18–7.60 (*m*, 8H), 7.65–7.77 (*m*, 3H), 8.22 (*s*, 1H), 9.19 (*s*, 1H), 10.22 (*s*, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.29, 27.51, 50.36, 71.14, 81.46, 118.2, 120.44, 121.50, 122.40, 124.49, 125.14, 126.57 (2C), 129.5, 132.46, 137.6, 139.54, 141.53, 142.51, 159.6, 165.79, 167.23. Anal. ($C_{29}H_{30}N_4O_6S$) C, H, N.

2,3-Dihydro-5-(3-formylaminophenyl)-2-oxo-3-[(8-quinolyl-sulfonyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***20b*** was prepared following the exact procedure given for **20a** from **15a** using Ac_2O and HCO_2H; R_{dt} 99%: m.p. 168–170 °C; IR (KBr) 3300, 3320, 2990, 2800, 1740, 1695, 1670, 1620, 1595, 1560, 1500, 1440, 1430, 1370, 1340, 1290, 1240, 1030, 920, 850, 840, 800, 760 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.21 (*s*, 9H), 4.48 (*d, J* = 9.38 Hz, 1H), 6.41 (*d, J* = 7.90 Hz, 1H), 7.17 (*t, J* = 7.95 Hz, 1H), 7.22–7.42 (*m*, 1H), 7.45 (*s*, 1H), 7.60–7.85 (*m*, 4H), 8.20 (*d, J* = 1.58 Hz, 1H), 8.28–8.40 (*m*, 4H), 8.55 (*dd, J* = 8.17 Hz, *J* = 1.66 Hz, 1H), 8.67 (*d, J* = 9.38 Hz, 1H), 8.86 (*dd, J* = 4.27 Hz, *J* = 1.66 Hz, 1H), 10.17 (*s*, 1H, NHCHO); ^{13}C NMR (Me_2SO-d_6) δ 27.83 (3C), 50.20, 71.78, 82.58, 122.09, 122.28, 125.31, 125.43, 125.99, 127.82, 128.33, 128.75, 128.84 (2C), 129.76, 130.71, 132.51, 133.54 (2C), 136.86, 139.09, 139.55, 141.66, 143.50, 151.47, 164.68, 165.48, 166.61; MS (Fab (+), NBA) *m/z* (M^+ + 1), 544, 460, 443, 424, 407, 351, 356, 324, 308 (100%).

2,3-Dihydro-2-oxo-5-(3-phenylisocyanide)-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***21a***: To a solution of mono substituted formamide **20a** (0.85 g, 1.55 mmol) in anhydrous $CHCl_3$ (50 mL) was successively added CCl_4 (0.15 mL, 1.55 mmol), NEt_3 (0.22 mL, 1.55 mmol) and triphenylphosphine (20% excess) (0.53 g, 2.0 mmol). The mixture was heated to 60 °C under a N_2 atmosphere for 2.5 h. The solvent was distilled off at slighty reduced pressure and the residue was extracted with $CHCl_3$ (3 x 50 mL). The combined organic portions were washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness in vacuo. The oily residue was purified by flash chromatography using silica gel (elued, EtOAc/cyclohexane, 1:1) to afford 0.7 g (85%) of **21a** as white crystals: m.p. 160–162 °C; IR (KBr) 3225, 2975, 2700, 1740, 1670, 1610, 1580, 1430, 1230, 1160, 990, 810, 790, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (*s*, 9H, tBu), 2.52 (*s*, 3H, $PhCH_3$), 4.36 (*d,* J_{gem} = 17 Hz, 1H), 4.55 (*d,* J_{gem}, 1H), 5.26 (*d, J* = 8.70 Hz, 1H), 6.51 (*d, J* = 8.70 Hz, 1H, NH), 7.15–7.45 (*M*, 10H, Harom), 7.63 (*ddd, J* = 8.36 Hz, *J* = 6.87 Hz, *J* = 8.70 Hz, 1H), 7.81 (*d, J* = 8.36 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.72, 27.65 (3C), 30.43, 70.54, 82.71 (N=C), 122.36, 125.60, 127, 10 (2C), 128.2, 128.6, 129.09, 129.6 (2C), 129.74 (2C), 130.68, 132.70, 139.23, 139.31, 141.54, 143.65, 165.02, 165.22, 165.74, 166.49; MS (Fab (+), NBA *m/z* 545 (M^+), 489, 333, 318, 306, 290 (100%), 260, 246, 232. Anal. ($C_{29}H_{29}N_4O_5S$) C, H, N.

2,3-Dihydro-5-(3-phenylisocyanide)-2-oxo-3-[(8-quinolyl-sulfonyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***21b***: To a solution of formamide **20b** (2.56 g, 4.26 mmol) in dry CH_2Cl_2 (25 mL) was added anhydrous NEt_3 (1.25 mL, 8.9 mmol) at –30 °C under a N_2 atmosphere. Diphosgene (0.3 mL, 2.35 mmol) dissolved in dry CH_2Cl_2 (5 mL) was added in a dropwise manner to the above solution and was allowed to stir at 0 °C over one hour. The temperature was then raised to 20 °C and the solution allowed to stir for an additional 30 min. The solution was extracted with H_2O (20 mL) and 7.5% $NaHCO_3$ solution (2 x 10 mL). The organic portion was separated, dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. The residue obtained was purified by flash chromatography using silica gel (eluent, EtOAc/cyclohexane, 2:1) to afford 1.26 g (51%) of **21b** as white crystals: m.p. decomposed at *T* > 210 °C; IR (KBr) 3250, 3075, 2995, 2975, 2145, 1740, 1700, 1600, 1595, 1570, 1500, 1440, 1410, 1380, 1340, 1320, 1230, 1160, 950, 910, 800, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (*s*, 9H), 4.35 (*d,* J_{gem} = 17.10 Hz, 1H), 4.49 (*d,* J_{gem}, 1H), 5.38 (*d, J* = 10.04 Hz, 1H), 6.30 (*t, J* = 1.55 Hz, 1H), 6.91 (*dt, J* = 7.70 Hz, *J* = 1.40 Hz, 1H), 7.10–7.35 (*m*, 4H), 7.50–7.65 (*m*, 2H), 7.76 (*dd, J* = 7.45 Hz, *J* = 7.39 Hz, 1H), 8.25 (*d, J* = 10.04 Hz, 1H), 8.27 (*dd, J* = 8.21 Hz, *J* = 1.34 Hz, 1H), 8.35 (*t,*

J = 1.46 Hz, 1H), 8.39 (*d*, J = 1.38 Hz, 1H), 9.11 (*dd*, J = 4.25 Hz, J = 1.69 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 27.63 (2C), 50.21, 71.60, 82.6, 122.11, 122.30, 125.33, 125.45, 126.00, 127.84, 128.34, 128.88 (2C), 129.76, 130.73, 132.54 (2C), 133.58, 136.88, 139.11, 139.57, 141.67, 143.53, 151.59, 164.7, 165.5, 166.63; MS (FAB (+), NBA) *m/z* 582 (M + 1), 526, 461, 399, 391, 374, 333, 318, 317, 290 (100%), 260, 246, 232, 219, 207.

2,3-Dihydro-2-oxo-5-[(phenyl-3-(dibromoisocyanide)]-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***22a***: To a solution of **21a** (0.35 g, 0.64 mmol) dissolved in dry CH_2Cl_2 (20 mL) was added bromide dioxane complex (0.19 g, 7.7 mmol) dissolved in dry CH_2Cl_2 (3 mL) at 0 °C under a N_2 atmosphere. The reaction mixture was allowed to stir for 15 min and the solvent was evaporated. The oily residue obtained crystallized from dry Et_2O to give 0.45 g (99%) of **22a** as white crystals: ^{1}H NMR ($CDCl_3$) δ 1.38 (*s*, tBu, 3H), 2.51 (*s*, $PhCH_3$, 3H), 4.42 (*s*, CH_2CO_2R, 2H), 5.26 (*s*, J = 8.70 Hz, 1H), 6.48 (*d*, J = 8.70 Hz, 1H), 6.77 (*t*, J = 1.75 Hz, 1H), 7.00 (*d*, J = 8.14 Hz, 1H), 7.04 (*d*, J = 7.88 Hz, 1H), 7.22–7.40 (*M*, 7H); ^{13}C NMR ($CDCl_3$) δ 21.68, 27.90, 59.75, 70.50, 82.71, 121.92, 122.10, 125.43, 127.22, 127.62, 128.95, 129.58, 130.33, 132.61, 138.71, 139.28, 141.64, 143.41, 147.13, 165.94, 166.31, 166.60; MS (D/CI, NH_3 + isobutane) *m/z* 705 (M^+), 628, 545 (10%), 507, 467, 401, 386, 360, 346, 336, 306.

2,3-Dihydro-2-oxo-5-[(phenyl-3-(dibromoisocyanide)]-3-[(8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***22b*** was prepared according the procedure given for **22a** from **21a** using bromide dioxane complex to afford **22b**: R_{dt} 90%: m.p. 198–200 °C; IR (KBr) 3350, 3050, 2990, 2875, 1740, 1690, 1600, 1595, 1550, 1490, 1360, 1330, 1230, 1160, 1140, 810, 790, 700, 690 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34 (*s*, 9H, tBu), 4.39 (*s*, 2H, CH_2CO), 5.37 (*s*, 1H, CH), 5.94 (*t*, J = 1.64 Hz, 1H), 6.67 (*d*, J = 7.80 Hz, 1H), 6.81 (*dd*, J = 7.80 Hz, J = 1.14 Hz, 1H), 7.12 (*t*, J = 7.84 Hz, 1H), 7.20–7.35 (*m*, 4H), 7.48–7.64 (*m*, 2H), 7.73 (*dd*, J = 7.63 Hz, J = 7.83 Hz, 1H), 8.25 (*dd*, J = 3.77 Hz, J = 1.05 Hz, 1H), 8.39 (*m*, 1H), 9.11 (*dd*, J = 4.16 Hz, J = 1.42 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 27.84, 50.56, 71.76, 82.56, 94, 119.78, 121.02, 121.82, 122.83, 125.10, 125.49, 127.54, 128.50, 128.63, 128.84, 128.98, 130.24, 132.29, 132.94, 136.85, 138.74, 139.61, 141.66, 143.34, 147.11, 151.31, 165.62, 165.71, 166.74; MS (Fab (+), NBA) *m/z* 742 (M + 1), 686, 582, 478, 450, 290 (100%), 284, 245, 232.

2,3-Dihydro-2-oxo-5-[phenylimino-3-(imidazolidine)]-3-[(tosyl)-amino)]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***23a***: To a solution of **22a** dissolved in dry EtOAc (10 mL) was added simultaneously NEt_3 (0.45 mL, 3.2 mmol) dissolved in dry EtOAc (2 mL) and ethylene diamine (0.046 mL, 0.70 mmol) dissolved in EtOAc (2 mL) at 0 °C under a N_2 atmosphere. The reaction mixture was allowed to stir an additional hour and then the solvent was evaporated to dryness in vacuo. The residue was dissolved in $CHCl_3$ (30 mL) and washed with H_2O (2 x 15 mL) and with brine (2 x 10 mL). The combined organic portions were dried ($MgSO_4$), filtered and evaporated to dryness in vacuo to afford 0.3 g (77%) of **22a** as white crystals: IR (KBr) 3550, 2995, 2975, 1740, 1700, 1660, 1600, 1570, 1490, 1460, 1440, 1380, 1285, 1240, 1160, 1100, 820, 720 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.33 (*s*, tBu, 9H), 2.41 (*s*, $PhCH_3$, 3H), 3.27 (*s*, 4H), 4.50 (*s*, 2H), 4.87 (*s*, 1H), 6.32 (*d*, J = 7.10 Hz, 1H), 6.61 (*s*, 1H), 6.95–7.12 (*m*, 2H), 7.25–7.52 (*m*, 6H), 7.68 (*d*, J = 8.05 Hz, 1H); ^{13}C NMR (Me_2SO-d_6) δ 21.01 ($PhCH_3$), 27.57 (3C), 42.54 ($NHCH_2CH_2NH$), 50.23, 71.13, 81.46, 120.96, 122.02, 123.72, 124.15, 124.80, 126.63 (2C), 128.07, 128.59, 129.26 (2C), 129.92, 132.18, 137.62, 139.65, 141.45, 142.58, 157.68, 165.68, 166.59, 167.34.

2,3-Dihydro-2-oxo-5-[phenylimino-3-(imidazolidine)]-3-[(8-quinolylsulfonyl)-amino)]-1H-1,4-benzodiazepin-1-tert-butyl-acetate ***23b*** was prepared following the procedure given for **23a** utilizing **22b** in place of **22a**: R_{dt} 51%: IR (KBr) 3400, 2995, 2985, 2875, 1740, 1690, 1660, 1600, 1570, 1500, 1430, 1370, 1340, 1270, 1160, 1150, 1030, 960, 910; ^{1}H NMR ($CDCl_3$) δ 1.35 (*s*, 9H, tBu), 3.44 (*s*, 4H, CH_2CH_2), 4.28 (*d*, J_{gem} = 17.10 Hz, 1H), 4.41 (*d*, J_{gem}, 1H), 5.37 (*s*, 1H, CH), 6.23 (*dt*, J = 7.01 Hz, J = 1.73 Hz, 1H), 6.44 (*t*, J = 1.73 Hz, 1H), 6.85–7.01 (*m*, 2H), 7.14–7.32 (*M*, 3H), 7.40–7.58 (*M*, 2H), 7.66 (*dd*, J = 7.40 Hz, J = 7.37 Hz, 1H), 8.09 (*dd*, J = 8.35 Hz, J = 1.35 Hz, 1H), 8.24 (*dd*, J = 8.38 Hz, J = 1.70 Hz, 1H), 8.35 (*dd*, J = 7.27 Hz, J = 1.35 Hz, 1H), 9.03 (*dd*, J = 4.26 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 27.65 (2C), 42.38 (2C), 50.55, 71.69, 82.44, 121.48, 122.01, 123.55, 124.19, 125.05, 125.42, 128.56, 128.85, 129.10, 130.60, 132.00, 132.96, 136.60, 138.71, 139.40, 141.58, 143.53, 148.62, 151.22, 157.68, 166.08, 166.69 (2C).

2,3-Dihydro-2-oxo-5-[(phenylimino-3)-imidazolidine]-3-[(tosyl)-amino]-1H-1,4-benzodiazepin-1-acetic acid ***24a***: To a solution of **23a** (0.4 g, 7.32 mmol) in AcOH (5 mL) was added an excess of trifluoroacetic acid (1 mL). The reaction mixture was heated to 50 °C for 5 h. The solvents were evaporated off and the solid residue was recrystallized several times from MeOH to give 0.25 g (64%) of **24a** as white crystals: m.p. 228–230 °C; IR (KBr) 3600–2600, 1660, 1600, 1340, 1140, 1100 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.41 (*s*, 3H), 3.63 (*s*, 4H), 4.50 (*d*, J_{gem} = 17 Hz, 1H), 4.61 (*d*, J_{gem}, 1H), 4.91 (*d*, J = 9.98 Hz, 1H), 6.76 (*d*, J = 6.16 Hz, 1H), 5.87 (*s*, 1H), 7.20–7.45 (*m*, 6H), 7.55–7.75 (*m*, 4H), 8.44 (*s*, 2H), 9.23 (*d*, J = 9.98 Hz, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.90, 42.64, 49.09, 71.09, 122.59, 124.88, 125.14, 128.68, 128.97, 129.27, 132.55, 135.54, 138.64, 139.75, 141.53, 142.53, 158.17, 165.34, 165.49, 169.49, 169.70 ; MS (Fab (+), NBA) *m/z* 547 (M + 1), 489, 460, 399, 391.

2,3-Dihydro-2-oxo-5-[(phenylimino-3)-imidazolidine]-3-[(8-quinolylsulfonyl)-amino]-1H-1,4-benzodiazepin-1-acetic acid ***24b***: To a solution of **23b** (0.3 g, 0.47 mmol) dissolved in acetic acid (5 mL) was added a 33% HBr solution (2.5 mL) at 0 °C under a N_2 atmosphere. The reaction mixture was allowed to stir at 0 °C for 30 min and the solvent was evaporated to dryness in vacuo. The oily residue was purified by flash chromatography using silica gel (eluent, EtOAc/MeOH/AcOH, 60:40:1) to afford 0.17 g (63%) of **24b** as white crystals: T > 260 °C; IR (KBr) 3600–2800 (COOH), 1670, 1640, 1600, 1520, 1460, 1440, 1360, 1100, 1160, 1080, 1040, 960, 810, 680 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 3.68 (*s*, $NHCH_2C_2NH$, 4H), 4.51 (*d*, J_{gem} = 17.60 Hz, 1H), 4.65 (*d*, J_{gem}, 1H), 5.60 (*d*, CHNH, J = 9.68 Hz, 1H), 6.66 (*t*, J = 2.30 Hz, 2H), 7.2–7.42 (*m*, 4H), 7.62–7.89 (*m*, 4H), 8.25 (*s*, 1H), 8.33 (*d*, J = 1.42 Hz, 1H), 8.36 (*d*, J = 2.30 Hz, 1H), 8.53 (*d*, J = 9.68 Hz, 1H), 8.57 (*dd*, J = 8.61 Hz, J = 1.5 Hz, 1H), 8.94 (*dd*, J = 4.17 Hz, J = 1.54 Hz, 1H), 10.32 (*s*, 1H, COOH); ^{13}C NMR (Me_2SO-d_6) δ 42.61 (2C), 48.75, 71.66, 122.50, 122.76, 124.85, 125.16, 125.65, 126.44, 127.59, 128.56 (2C), 129.19 (2C), 129.48, 132.5 (2C), 133.42, 135.36, 137.10, 138.96, 141.30, 142.51, 151.15, 158.08, 165.42, 169.53; MS (D/CI, NH_3 + isobutane) *m/z* 584 (M^+ + 1), 512, 460, 443, 424, 399, 329 (100%), 322, 310.

5.2. Biochemistry assay: thrombin inhibition

Materials; buffer: 0.04 M sodium phosphate/0.084 M sodium chloride/0.1 polyethylene glycol 6-8000 (PEG) pH 7.4.

Fibrinogen (FGN): 0.30–0.40 nM in buffer (E_{280} = 1.51 a. u./mg/mL, Mol. Wt = 340K) [43].

α-Thrombin: 2.0–5.0 nM in buffer (E_{280} = 1.83 a.u./mg/mL, mol. wt. = 36.5 K) [44]. Thrombin was diluted in PEG-treated tubes where the tubes were filled with 1% PEG 20 000 overnight, drained, dried and used without rinsing with water.

Compound to be tested (I): The compound to be tested was dissolved in either water, buffer or, as a last resort, DMSO at a concentration of 1–5 nM.

Experimental: To 0.9 mL of 0.30–0.40 μM fibrinogen ([FGN] < Km), was added 10 μL of either solvent (the control) or 10 μL of solvent containing the compound of interest. After warming to 37 °C, 0.1 mL of buffer (the zero time control) or 0.1 mL of 2.0–5.0 nM α-thrombin was added with mixing. After a specified time 0.1 mL of 3 M perchloric acid was added with mixing along with another 0.3 mL of H_2O. After complete mixing of the 1.41 mL acid-quenched sample, the contents were centrifuged for 5 min at 10 000 RPM and 1.0 mL of the supernatent was injected onto the HPLC. In runs where the maximum amount of releasable FPA was desired, 0.1 mL of 25–50 nM thrombin was added for 30 minutes [45].

HPLC: The FPA was detected at 205 nm from HPLC, using a C (18) reverse phase column and a gradient of 100% 0.08 M phosphate buffer, pH 3.1, to 40% acetonitrile/phosphate buffer, pH 3.1 [41, 43, 45].

Determination of K_i: K_i values depicted in *table I* are determined from:

$$(k_{cat}/K_M)_0 / (k_{cat}/K_M)_i = 1 + [I]/K_i$$

where $(k_{cat}/K_M)_0$ and $(k_{cat}/K_{MM})_i$ are the specificity constants for release of FPA in the absence and presence of inhibitor, respectively. The specificity constants are conveniently evaluated [45] from first-order plots of thrombin-catalyzed release of FPA from fibrinogen under condidtions where the concentration of fibrinogen is much less than the Michaelis–Menten constant, K_M (K_M = 3.6 μM in fibrinogen; 7.2 μM in FPA [45]).

6. Molecular modeling investigations

All small molecule ligands were constructed with the Advanced Modeling Facility (AMF) [46]. All energy minimizations were performed using the MM2X force field within the OPTIMOL program [47]. The minimization algorithm used in OPTIMOL, is a variable Metric optimizer based upon the BFGS (Broyden Fletcher Goldfarb Shanno) method. The criteria for RMS gradient convergence is 10^{-3} Kcal/mol A [47].

6.1. Initial comparisons of the benzodiazepine template to MD-805 structure

Various conformations of the structures were produced via the conformational searching and energy minimization routines of OPTIMOL using the MM2X force field. The ligands were superimposed using the COMPARE facility within AMF. Any compared structures that were more than 7 kcal higher in energy than the minima were discarded.

6.2. Follow-up comparisons of the benzodiazepine template to MD-805 structure

Models of MD-805 in thrombin were produced using the build facilities of AMF and the crystal coordinates of the thrombin*PPACK complex [42]. The

Table II. MM2X energies (Kcal/mol) for thrombin*MD 805 models.

	Model I			Model II		
	Total energy (kcal/mol)	Ligand energy (kcal/mol)	Ligand/active site energy (kcal/mol)	Total energy (kcal/mol)	Ligand energy (kcal/mol)	Ligand/active site energy (kcal/mol)
Total E	–194.34	–31.29	–163.06	–197.97	–28.13	–169.85
Bond	2.08	2.08		2.25	2.25	
Angle	10.16	10.16		10.25	10.25	
Stretch-bend	0.51	0.51		0.53	0.53	
Torsion	10.48	14.48		16.30	16.30	
Net VanderWaals	–29.16	12.08	–41.24	–34.45	11.04	–45.49
Net electro static	–192.41	–70.59	–121.81	–192.85	–68.50	–124.35

MD-805 structures were energy minimized within the context of a rigid representation of the thrombin active site. The energetic information is given in *table II*. The MD-805 models produced, designated Model I and II, differ in the conformation of the quinoline ring.

Subsequent comparison of these MD-805 models with the actual crystal structure indicates that they both differ slightly from the crystallographic form of MD-805. These subtle differences, however, do not alter the results and conclusions drawn from the use of these modes of MD-805 in comparison with the **6a** scaffold:

The template building facility within AMF was used to construct the *R*- and *S*-stereoisomers of the benzodiazepine template **6a**. Molecular comparison tools in Quanta [48] were used to flexibly fit benzodiazepine template **6a** to the rigid MD-805 conformation. Each stereoisomer of the template was fit to MD-805 Models I and II using the carboxylic acid carbon, the sulfonamide N and S atoms, and the sulfonamide aryl substituent as match points. This resulted in four thrombin 'bound' forms of the template **6a** named TempRI, TempRII, TempSI, and TempSII depending upon the stereochemistry and model used for fitting. The resulting benzodiazepine templates were then energy minimized in the active site. The results were visualized with Quanta to assess the 'bound' forms of the template **6a**.

Furthermore comparing to the assumption of Kikumoto [18] we can say that in addition to a guanadinium binding site, there is a hydrophobic pocket on thrombin which binds the substituted piperidine ring. Our hope was to bring a three-dimensional component to that type of model, e.g., by successfully employing a more constrained benzodiazepine scafford one would gather data on the geometrical disposition between the guanidino and the hydrophobic binding sites. Unfortunately, given our limited success at identifying potent inhibitors of thrombin, this was not achieved.

Acknowledgements

D. Dumas and G. Leclerc acknowledge the Merck Research Laboratories for their financial support. We wish to express our sincere gratitude to D.W. Banner (Pharmaceutical Research Department, Hoffmann–La Roche, Switzerland) and M.J. Rabiet (INSERM–CENG, Grenoble) for their helpful discussions and suggestions in the preparation of this work. We also thank Mr. F. Thomasson (UJF, Chimie Pharmacie, Grenoble) for his contribution to the ^{1}H and ^{13}C NMR spectra and Mr Bosso (CERMAV, UJF Grenoble) for mass spectral analysis.

References

[1] Okamoto S., Hijikata A., Kikumoto R., Tonomura S., Hara H., Ninomiya K., Maruyama A., Sugano M., Tamao Y., Biochem. Biophys. Res. Commun. 101 (1981) 440.
[2] Banner D.W., Hadvary P.J., Biol. Chem. 266 (1991) 20085.
[3] Powers J.C., Kam C.-M., in: Berliner L.J. (Ed.), Thrombin: Structure and Function, Plenum Press, New York, 1992.
[4] Venturini C.M., Kaplan J.E., Semin. Thromb. Hemostas. 18 (1992) 275.
[5] Stasek J.-E., Garcia J.G.N., Semin. Thromb. Hemostas. 18 (1992) 117.
[6] Toma K., Suzuki K., J. Mol. Graphics 7 (1989) 146.
[7] Maraganore J.M., Thromb. Haemostas. 70 (1993) 208.
[8] Hirsh J.N., Engl. J. Med. 324 (1991) 1865.
[9] Markwardt F., Hauptmann J., Nowak G., Kleben Ch., Walsmann P., Thromb. Haemostas. 47 (1982) 226.
[10] Bichler J., Fichtl B., Siebeck M., Fritz H., Arzeim. Forsch. Drug Res. 38 (1988) 704.
[11] Markwardt F., Semin. Thromb. Hemostas. 17 (1991) 79.
[12] Johnson P.H., Sze P., Winant R., Payne P.W., Lazar J.B., Semin. Thromb. Hemostas. 15 (1989) 302.
[13] Grütter M.G., Priestle J.P., Rahuel J., Grossenbacher H., Bode W., Hofsteenge J., Stone S.R., EMBO J. 9 (1990) 2361.
[14] Bajusz S., Barabas E., Tolnay P., Szell E., Bagdy D., Int. J. Pept. Protein. Res. 123 (1978) 217.
[15] Kettner C., Mersinger L., Knabb R., J. Biol. Chem. 265 (1990) 18289.
[16] Kettner C., Mersinger L., Siefring G., J. Cell. Biochem. Suppl. 12B (1988) 292.
[17] Kettner C.A., Shenvi A.B., J. Biol. Chem. 259 (1984) 15106.
[18] Kikumoto R., Tamao Y., Tezuka T., Tonomura S., Hara H., Ninomiya K., Hijikata A., Okamoto S., Biochemistry 23 (1984) 85.
[19] Stürzebecher J., Markwardt F., Voigt B., Wagner G., Walsmann P., Thromb. Res. 29 (1983) 635.
[20] Stürzebecher J., Walsmann P., Voigt B., Wagner G., Thromb. Res. 36 (1984) 457.
[21] Powers J.C., Harper J.W., Inhibitors of serine protease, in: Barett A.J., Salvesen G. (Eds.), Proteinase Inhibitors, Elsevier, Amsterdam, 1986, pp. 55–152.
[22] Kam C.-M., Fujikawa K., Powers J.C., Biochemistry 27 (1988) 2547.
[23] Evan B.E., Rittle K.E., Bock M.G., DiPardo R.M., Freidinger R.M., Whitter W.L, Gould N.P., Lundell G.F., Homnick C.F., Veber D.F., Anderson P.S., Chang R.S.L., Lotti V.J., Cerino D.J., Chen T.B., Kling P.J., Kunkel K.A., Springer J.P., Hirsfield J., J. Med. Chem. 30 (1987) 1229.
[24] Evan B.E., Rittle K.E., Bock M.G., Dipardo R.M., Freidinger R.M., Whitter W.L., Lundell G.F., Veber D.F., Anderson P.S., Chang R.S.L., Lotti V.J., Cerino D.J., Chen T.B., Kling P.J., Kunkel K.A., Springer J.P., Hirsfield J., J. Med. Chem. 31 (1988) 2235.
[25] Okada M., Takahashi T., Yokota M., Kawasaki T., Nagaoka S., Yamanouchi Pharmaceutical Co. Ltd, JP 02,193,971 (CA: 114 81626 f).
[26] Wakselman M., Mazaleyrat J.P., Reboud M., EP 0357 510, CNRS (CA: 113, 132827 m).
[27] Han W.T., Bristol-Meyers Squibb. Co, US Patent No. 5, O37, 819 (CA: 115, 232060 r).
[28] Stürbecher J., Stürbecher U., Vieweg H., Wagner G., Hauptmann J., Markward F., Thrombosis Res. 54 (1989) 245.
[29] (a) Bock M.G., DiPardo R.M., Evans B.E., Rittle K.E., Veber D.F., Freindinger R.M., Tetrahedron Lett. 28 (1987) 939; (b) Bock M.G., DiPardo R.M., Evans B.E., Rittle K.E., Veber D.F., Freindinger R.M., Hirschfield J., Spinger J.P., J. Org. Chem. 52 (1987) 3232.
[30] Daiichi Seiyaku Co., Ltd (Japan), Drug of the future 7 (1982) 810.
[31] Detar D.F., Relyea D.I., J. Am. Chem. Soc. 76 (1954) 1680.
[32] Bock M.G., DiPardo R.M., Freindinger R.M., J. Org. Chem. 51 (1986) 3718.
[33] Zolher U., Ben-Ishai D., Tetrahedron 31 (1975) 863.
[34] Lamattina J.L., Suleske X., Hebeisen P., Kende A.S., Org. Synth. Coll. VII (1990) 149.
[35] Terpko M.O., Heck R.F., J. Org. Chem. 45 (1980) 4992.
[36] Rouot B., Leclerc G., Wermuth C.G., Miesch F., Schwartz J., J. Med. Chem. 19 (1976) 1049.

[37] Appel R., Kleinstück R., Zeihn K.D., Angew. Chem. Int. Edit. 10 (1971) 132.
[38] Skorna G., Ugi I., Angew. Chem. Internat. Ed. Engl. 16 (1977) 259.
[39] Bellimoria J.D., Maclagan N.F., J. Chem. Soc. (1954) 3257.
[40] Higgins D.L., Shafer J.A., CRC Crit. Rev. Clin. Lab. Sci. 26 (1988) 1–41.
[41] Ng A.S., Lewis S.D., Shafer J.A., Meth. Enzym. 222 (1993) 341.
[42] Bode W., Mayr I., Baumann U., Huber R., Stone S.R., Hofsteenge J., Emb. J. 8 (1989) 3467.
[43] Lewis S.D., Shafer J.A., Thromb. Res. 35 (1984) 111.
[44] Fenton II J.W., Fasco M.J., Stockrow A.B., Aronson D.L., Young A.M., Finlayson J.S., J. Biol. Chem. 252 (1977) 3587.
[45] Higgins D.L., Lewis S.D., Shafer J.A., J. Biol. Chem. 258 (1983) 9276.
[46] AMF – The Advanced Modeling Facility, Molecular Systems Department, Merck Research Laboratories, a division of Merck & Co., Inc., version 1.0.
[47] Holloway M.K. et al., J. Med. Chem. 38 (1995) 305.
[48] QUANTA software © 1986–1996, Molecular Simulations, Inc., Waltham, MA.