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Original article

Molecular modelling and conformational analysis of novel glycoprotein (Gp) IIb/IIIa antagonists. Molecular orbital calculation and the condensed heterocyclic derivatives

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Abstract – A naphthalene compound was chosen as lead compound to develop a new series of fibrinogen receptor antagonists. Eight new compounds with different condensed heterocyclic parts were prepared and their in vitro activities were evaluated. 5-Amidinobenzofuran compound **2**, 6-amidinobenzothiophene **7**, and 5-amidinofuro[2,3-*b*]pyridine **8** were more active than lead compound **1**. Molecular orbital calculation studies suggested the presence of a preferred spatial orientation between the amidine and the carboxylic acid. © 2000 Éditions scientifiques et médicales Elsevier SAS

molecular orbital calculation / condensed heterocycle / conformational analysis / glycoprotein IIb/IIIa antagonist

1. Introduction

In the previous report [1, 2], we described our strategy for the development of naphthalene derivatives of glycoprotein (Gp) IIb/IIIa antagonists. Our studies using molecular modelling techniques suggested that the conformational definition of our compounds rested on three points, which are the amidine, hydrogen-bond donating CONH, and carboxylic acid parts, and that a proper spatial orientation between the amidino group and the carboxylic acid is necessary for binding to the receptor (*figure 1*) [3].

In the structure–function relationship of factor Xa (FXa) inhibitors [4–8], modification of the condensed heterocycles in the amidinoaryl compounds changed their inhibitory activity against FXa and thrombin. Iwanowics and co-workers reported a number of aromatic amidines as inhibitors of thrombin catalytic activity [9]. The amidinium–aspargic acid interaction and the formation of a hydrogen bond between the indole N–H of the condensed heterocycle and serine, together with lipophilic interactions in the pocket, appear to be largely responsible for the binding affinity. A conformational analysis of the amidinoaryl provides a clear and verifiable hypothesis that geometrical parameters and cation-π interactions are very important for molecular recognition. The indole

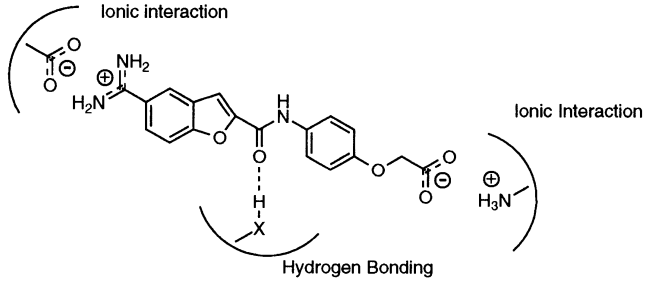

Figure 1. Three point interaction of **2**.

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derivatives, which lead to additional hydrophobic interactions and provide additional π-stacking with receptor regions, have been optimized by Shall and co-workers [10–12]. However we reported that there is not an additional CH-π interaction effect within that region of Gp IIb/IIIa. In our hypothesis, modification of the condensed heterocycle can change the geometrical parameters and provide the compounds with additional interactions.

A condensed heterocyclic compound with a specific structure was evaluated for its Gp IIb/IIIa antagonist activity. In this paper, we report on our approach, in which molecular modelling techniques are used to create condensed heterocyclic compounds from the lead naphthalene compound **1** [1, 2], which is modified from RGD peptides and has very high affinity.

2. Methods

Molecular modelling investigations and analyses were performed with the SYBYL 6.3 [13] running on a Silicon Graphics R4000 workstation. Taking account of the advantages and limitations of the current methodologies for conformational analysis, we considered an approach based on precise molecular orbital calculation of fragment conformations.

2.1. Model building and molecular orbital calculations

The planar structure of the compound **2** was divided into two parts (*figure 2*), the basic part (amidinoaryl moiety) and the acidic part (carboxylic acid moiety). Each part was optimized and evaluated with the basis set of RHF/6-31G* in the Gaussian94 program [14] and Spartan 4 program [15] on Silicon Graphics R8000 workstations. In this case we did not use the zwitterions, that is protonated amidino moiety and deprotonated carboxylate moiety, since the ions were unstable in the vacuum phase. And we could have eight different conformations of the amidino moiety to the rest of the molecule, but we utilized only one conformation, since the contribution of the energies of the rest could be the same or worse. For each of the amidinoaryl-CONH-derivatives, we performed two Gaussian calculations to the *trans*-amide conformations, but not to the *cis*-amide ones. Then we compared the optimized conformers and energies.

3. Results and discussion

Our previous structure–activity and molecular modelling studies of the naphthalene compounds resulted in the observation that the carbonyl group in our compounds occupied the same region as arginine. The molecule needs a hydrogen bond acceptor (amide carbonyl) between the amidinoaryl and carboxylic acid moieties. These studies led to the identification of **1** as lead compound [1, 2]. We started further structure–activity studies utilizing molecular modelling techniques on the amidinoaryl moiety (basic part).

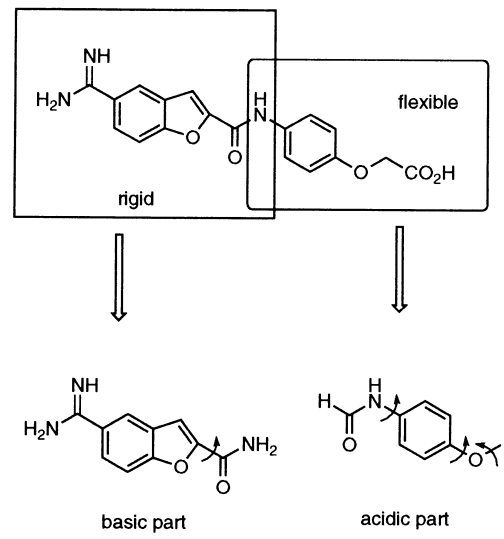


Figure 2. Planar structural formula of compound **2**. This structure was divided into two parts.

Recently, Gp IIb/IIIa antagonists with bicyclic 5-amidinoindoles have been reported by Shall and co-workers [10–12]. They explained that the indole nucleus may provide an additional π-stacking effect to that region of the receptor. In factor Xa inhibitor studies, cation-π interactions [4–8] have been thought to provide a novel mechanism for molecular recognition, and hence compounds having naphthalene and benzofuran rings show up as potent inhibitors. This also means that further conformational restriction may be added to the basic moiety to some extent. Modification of condensed heterocyclic compounds provides a means of varying the flexibility of the amidino group and carboxylic acid. A number of amidino-substituted condensed heterocyclic compounds were therefore designed, modelled and examined for their inhibitory effect against platelet aggregation in order to compare these with the effects of 6-amidinonaphthalene derivatives.

The inhibitory capacities (IC_{50}), shown in *table I*, are the concentrations of antagonists required to achieve 50% inhibition in ADP-induced aggregation of human platelet-rich plasma (PRP) [16]. The 5-amidinobenzofuran **2** and

6-amidinoindole **4** possessed similar activity to the parent compound **1**, and 6-amidinobenzothiophene **7** is approximately 4-fold more potent than **1** in platelet aggregation assays. On the other hand, the 6-amidinobenzofuran **3** and 5-benzothiophene **6** were one order less active than **1** in platelet aggregation assays. Furo[2,3-*b*]pyridine **8** and 3-methylbenzofuran derivative **9** showed similar inhibitory activity to the original benzofuran **2**. The addition of an *N*-methyl group to the indole **5** led to a considerable decrease (> 7 times) in potency. In *figures 3–5*, we indicate the superimposition of the condensed heterocyclic compounds. These potencies suggest that a change in the conformation of the heterocycle nucleus through modification of the ring shape of the basic part may intensify activity. The diminished inhibitory potency suggests that the key binding pharmacophore of inhibitors, which consists of positive and negative charges and hydrogen bonds, may sometimes fail to occupy the corresponding region of the receptor, or that the molecular shape may not be recognized by the receptor, or that the compounds cannot mimic the critical distance between the positive and negative binding sites of the receptor.

3.1. Conformation of amidinoarylcarboxamide

Stable conformations were calculated for amidinoarylcarboxamide **10**–**18** as a surrogate for our compounds (*figures 6–13*). In 5-amidinobenzofuran-2-carboxamide, two major conformers, **11A** and **11B**, existed in relation to the relative orientation of the planes of the amide and benzofuran. These conformations are summarized in *figure 14*. In conformer **11A**, two oxygen atoms are aligned in opposite directions, whereas in conformer **11B**, they face in the same direction. Our calculation RHF/R-31G* [14, 15] indicated that conformer **11A** is more stable than **11B** by 5.14 kcal/mol. The twist angle of the two planes of benzofuran and amide are 0.20 degrees (conformer **11A**) and 16.23 degrees (conformer **11B**). These results suggest that the conformation of **2** is similar to that of **11A**. Molecular modelling studies in which naphthalene compound **1** (conformer **10A**) and **2** (con-

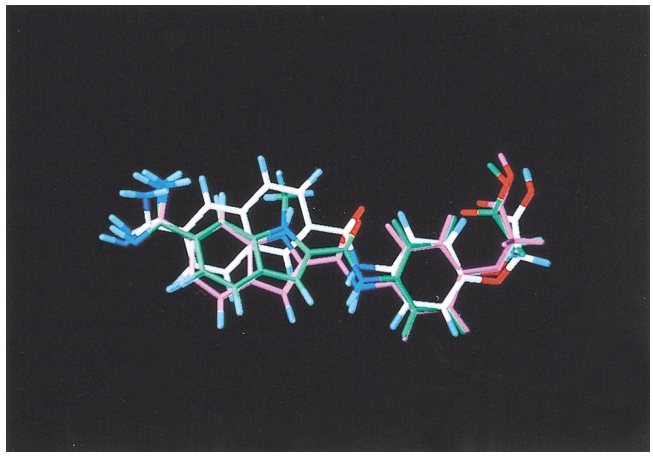


Figure 4. Superimposition of **1** (white), **4** (green) and **5** (magenta).

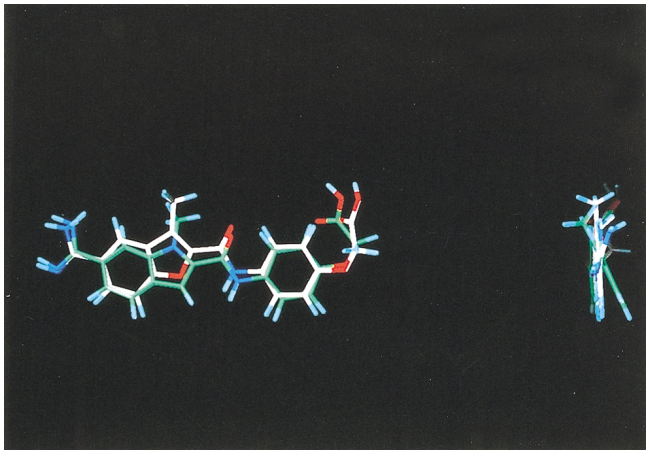


Figure 3. Superimposition of the naphthalene **1** (white) and benzofuran **2** (green). Comparison of the stable conformations of **1** and **2** reveals differing shapes in the amidinoaryl moiety.

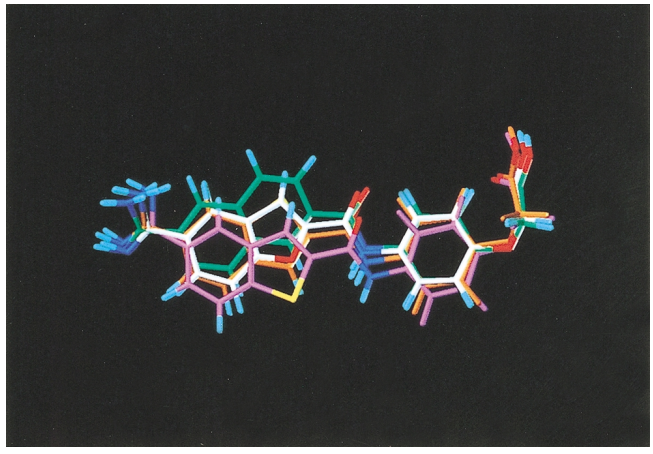
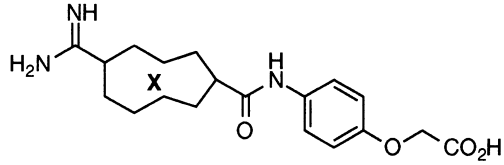
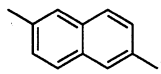
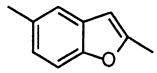
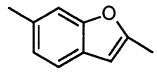
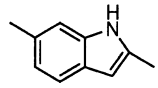
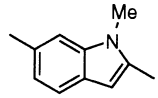
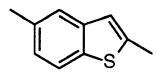
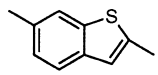
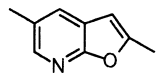
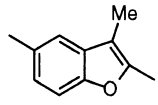


Figure 5. Superimposition of **1** (Green), **2** (white), **6** (magenta) and **7** (orange). The carbonyl oxygen of **2** and **1**, but not that of **6**, is capable of accessing the same space as the carbonyl oxygen of **7.**

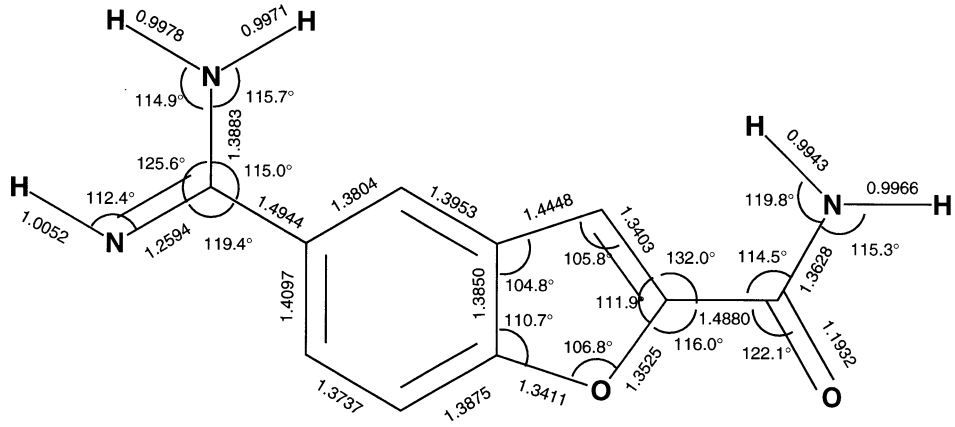
Table I. The condensed heterocyclic derivatives: inhibition of platelet aggregation and conformational parameters of compounds.



Compound number	Structure of X	IC_{50}(μM)[a]	Stable conformation (kcal/mol)[b]	Twist angle[c] (degree)	
				type A[b]	type B[b]
1		0.07 ± 0.02	A 0.15	21.38	21.31
2		0.033 ± 0.009	A 5.14	0.20	16.23
3		0.23 ± 0.03	B 4.64	17.75	0.39
4		0.066 ± 0.012	A 3.77	3.13	15.39
5		0.23 ± 0.02	A 3.66	23.10	28.80
6		0.77 ± 0.033	A 0.53	12.58	15.14
7		0.017 ± 0.002	B 0.21	13.96	0.91
8		0.012 ± 0.002	A 6.32	0.25	18.20
9		0.035 ± 0.002	A 6.76	0.20	26.45
BIBU-52 [18]		0.07 ± 0.006			
Ro 43–5587 [17]		0.085 ± 0.013			

[a] Concentration required to inhibit by 50%. Values are means ± SEM of three experiments. [b] See *figure 14*. [c] Twist angle = angle hetero atom -C-(C=O)-N.

(a) -696.9972937 Hartree
(+5.14 kcal / mol)



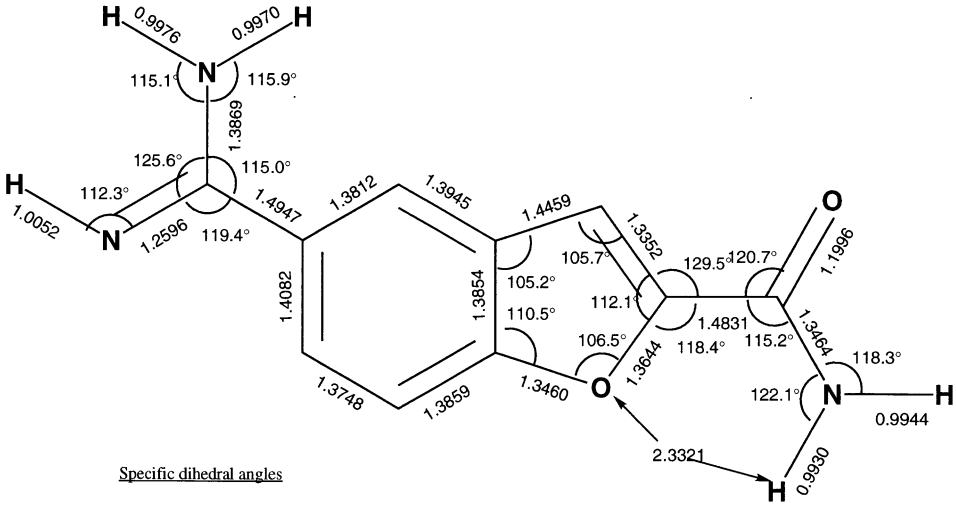
Specific dihedral angles

<c-c-C-N = -24.63 °
<c-c-C=N = 153.27 °
<c-C-N-H = -37.63 °
<c-C-N-H = -170.77 °
<c-C=N-H = 178.48 °

<O-c-CO-N = -163.77 °
<O-c-C=O = 18.46 °
<c-C-N-H = 27.19 °
<c-C-N-H = 173.53 °

(Small 'c' : benzofuran carbon.)

(b) -697.0054805 Hartree



Specific dihedral angles

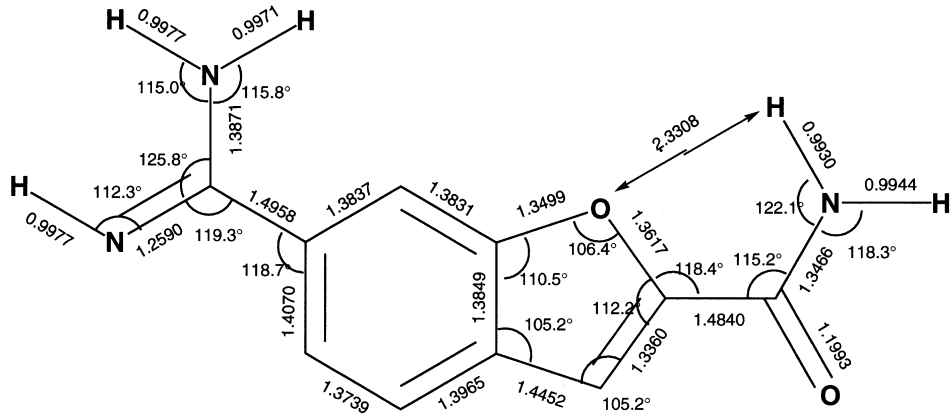
<c-c-C-N = -24.27 °
<c-c-C=N = 153.61 °
<c-C-N-H =-37.08 °
<c-C-N-H = -170.89 °
<c-C=N-H = 178.51 °

<O-c-CO-N = 0.20 °
<O-c-C=O = -179.68 °
<c-C-N-H = 1.42 °
<c-C-N-H = 179.05 °

(Small 'c' : benzofuran carbon.)

Figure 6. 5-Amidinobenzofuran-2-carboxamide **11**.

(a) -697.0047451 Hartree



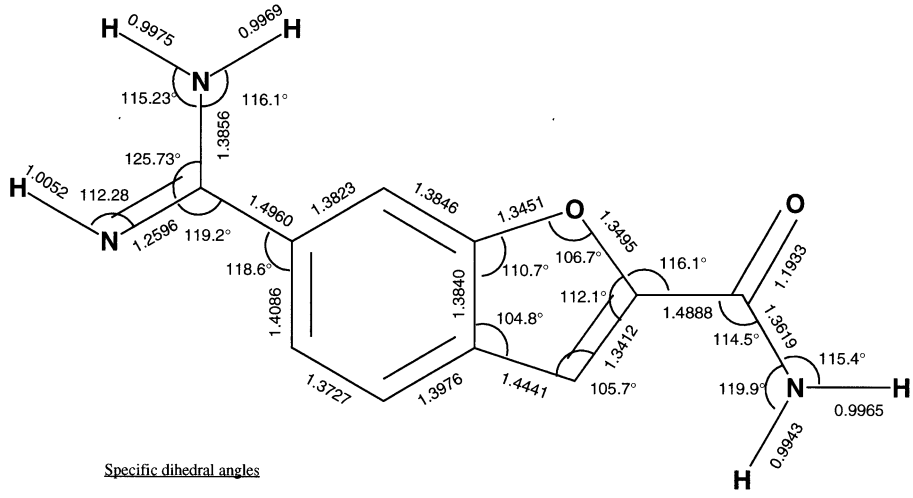
Specific dihedral angles

<c-c-C-N = -25.34 °
<c-c-C=N = 152.47 °
<c-C-N-H = -36.85 °
<c-C-N-H = -170.52 °
<c-C=N-H = 178.70 °

<O-c-CO-N = -0.39 °
<c-CO-N-H = -1.95 °
<c-CO-N-H = -178.69 °
<O-C-C=O = 179.50 °

(Small 'c' : benzofuran carbon.)

(b) -696.997353670 Hartree
(+4.64 kcal / mol)



Specific dihedral angles

<c-c-C-N =24.51 °
<c-c-C=N = -153.31 °
<c-C-N-H = 35.87 °
<c-C-N-H = 170.38 °
<c-C=N-H = -178.77 °

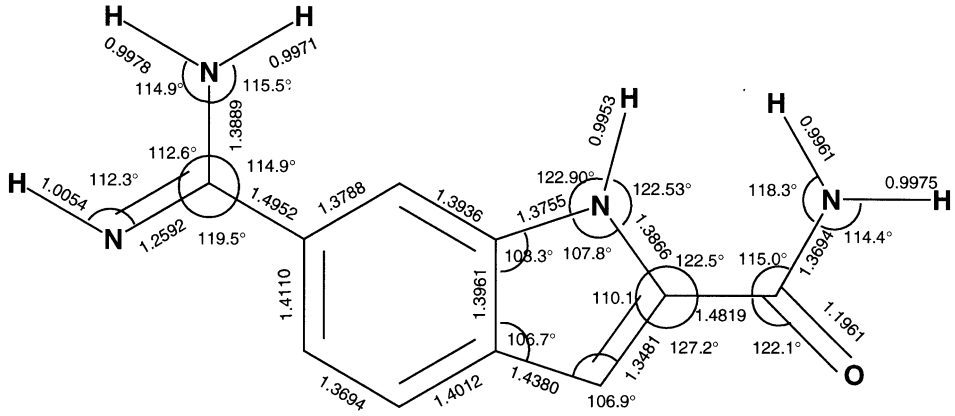
<O-c-CO-N = 162.25 °
<c-CO-N-H =-20.06 °
<c-CO-N-H = -26.82 °
<O-C-C=O = -173.68 °

(Small 'c' : benzofuran carbon.)

Figure 7. 6-Amidinobenzofuran-2-carboxamide **12**.

(a) -677.180453690 Hartree

(+3.77 kcal / mol)



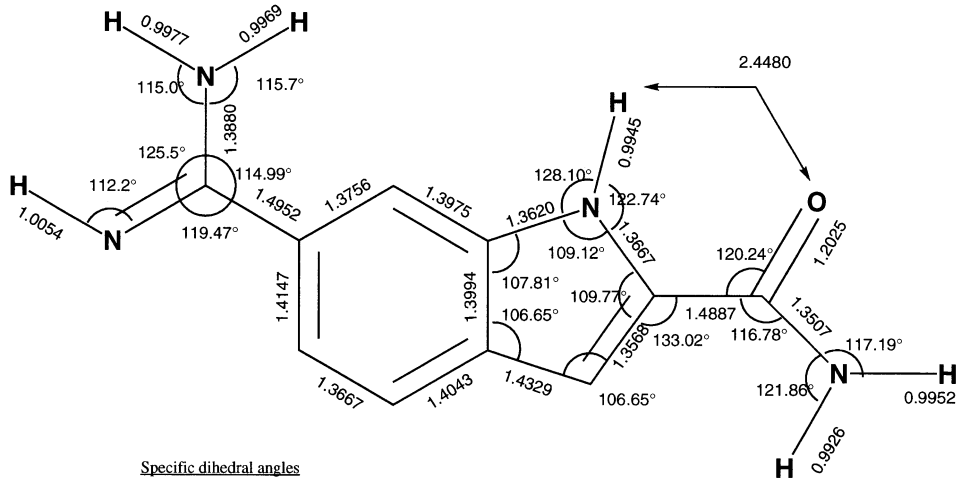
Specific dihedral angles

<c-c-C-N = -26.00 °
<c-c-C=N = 151.86 °
<c-C-N-H = -37.25 °
<c-C-N-H = -170.18 °
<c-C=N-H = 178.69 °

<Naromatic-c-CO-N = 15.39 °
<Naromatic-c-C=O = -163.11 °
<c-C-N-H = 31.14 °
<c-C-N-H = 171.61 °
<c-c-Naromatic-H = -25.47

(Small 'c' : indole carbon.)

(b) -677.186465860 Hartree



Specific dihedral angles

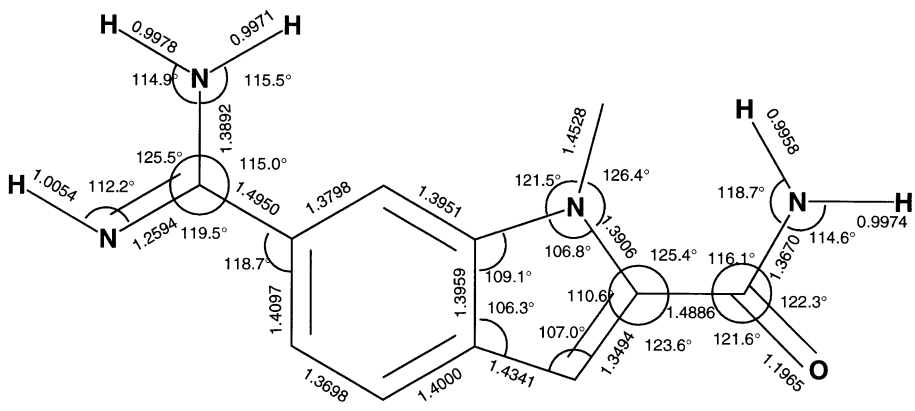
<c-c-C-N = -25.31 °
<c-c-C=N = 152.58 °
<c-C-N-H = -36.65 °
<c-C-N-H = -170.10 °
<c-C=N-H = 178.76 °

<Naromatic-c-CO-N = 176.87 °
<Naromatic-c-C=O = -3.96 °
<c-C-N-H = -13.77 °
<c-C-N-H = 173.69 °
<c-c-Naromatic-H = -2.20 °

(Small 'c' : indole carbon.)

Figure 8. 6-Amidinoindole-2-carboxamide **13**.

(a) -716.205880180 Hartree
(+3.66 kcal / mol)



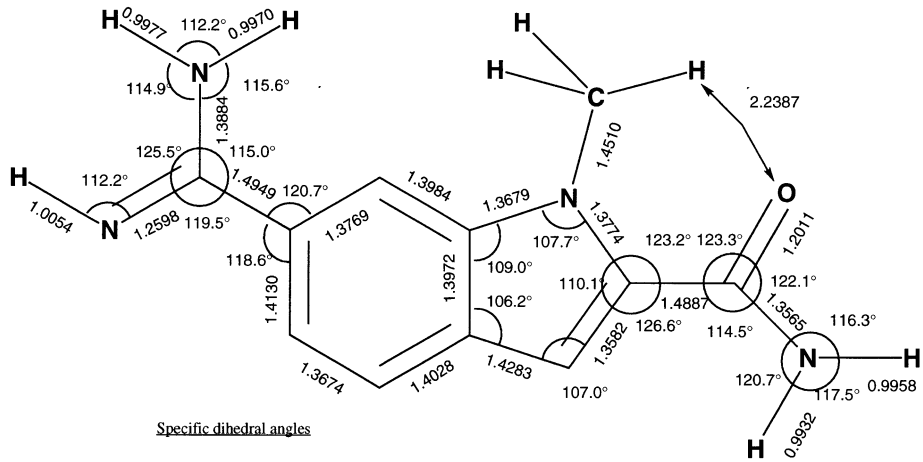
Specific dihedral angles

<c-c-C-N = -25.95 °
<c-c-C=N = 151.94 °
<c-C-N-H = -37.17 °
<c-C-N-H = -170.07 °
<c-C=N-H = 178.64 °

<Naromatic-c-C-N = 28.80 °
<Naromatic-c-C=O = -152.48 °
<c-CO-N-H = 25.06 °
<c-CO-N-H = 167.65 °
<c-Naromatic-C-H = 98.10 °
<c-Naromatic-C-H = -141.66 °
<c-Naromatic-C-H = -22.20 °

(Small 'c' : indole carbon.)

(b) -716.2117152 Hartree



Specific dihedral angles

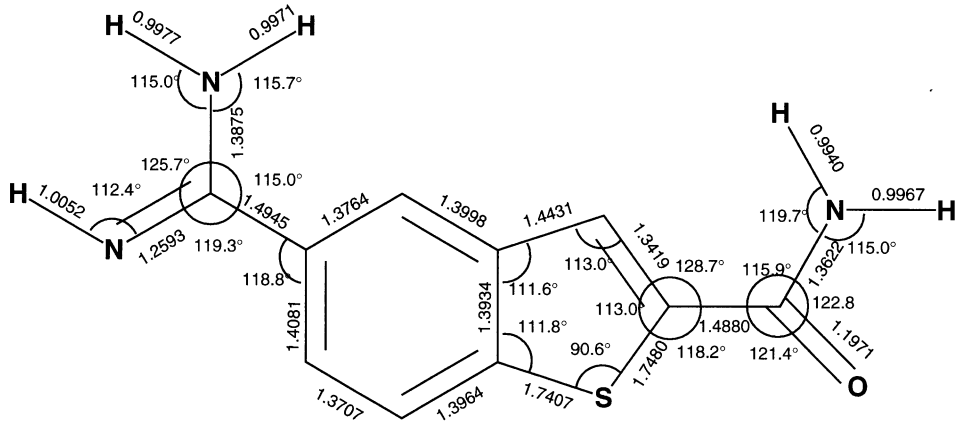
<c-c-C-N = -25.85 °
<c-c-C=N = 152.05 °
<c-C-N-H = -36.66 °
<c-C-N-H = -169.91 °
<c-C=N-H = 178.67 °

<Naromatic-c-C-N = -156.90 °
<Naromatic-c-C=O =25.25 °
<c-C-N-H = 22.02 °
<c-C-N-H = 175.47 °
<c-Naromatic-C-H = -4.69 °
<c-Naromatic-C-H =116.22 °
<c-Naromatic-C-H = -123.92 °

(Small 'c' : indole carbon.)

Figure 9. 6-Amidino-3-methylindole-2-carboxamide **14**.

(a) -1019.662614200 Hartree
(+0.53 kcal / mol)



Specific dihedral angles

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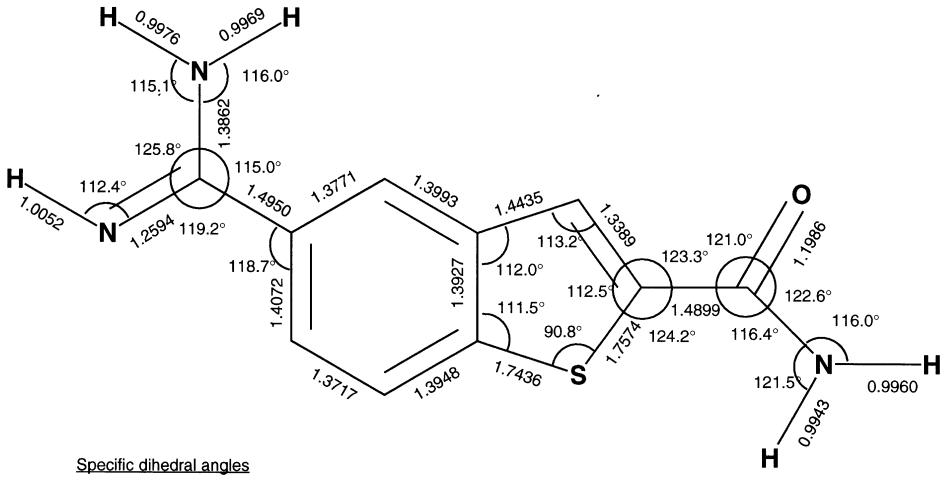
<c-c-C=N = 152.98 °
<c-C-N-H = -37.40 °
<c-C-N-H = -170.67 °
<c-C=N-H = 178.61 °

<S-c-C-N = 164.86 °
<S-c-C=O = -16.44 °
<c-C-N-H = -26.74 °
<c-C-N-H = -172.07 °

(Small 'c' : benzothiophen carbon.)

(b) -1019.663462600 Hartree



Specific dihedral angles

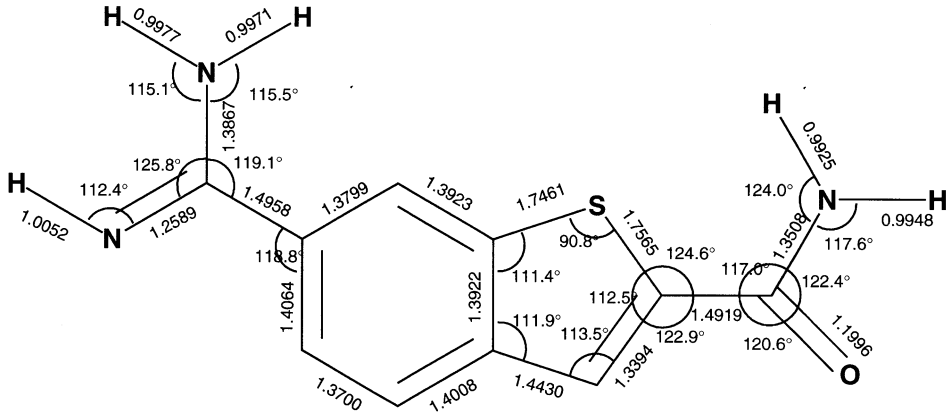
<c-c-C-N = -24.17 °
<c-c-C=N = 153.67 °
<c-C-N-H = -36.79 °
<c-C-N-H = -170.79 °
<c-C=N-H = 178.67 °

<S-c-C-N = 12.58 °
<S-c-C=O = -166.57 °
<c-C-N-H = -20.49 °
<c-C-N-H = 172.98 °

(Small 'c' : benzothiophen carbon.)

Figure 10. 5-Amidinobenzo[*b*]thiophen-2-carboxamide **15**.

(a) -1019.6625612 Hartree



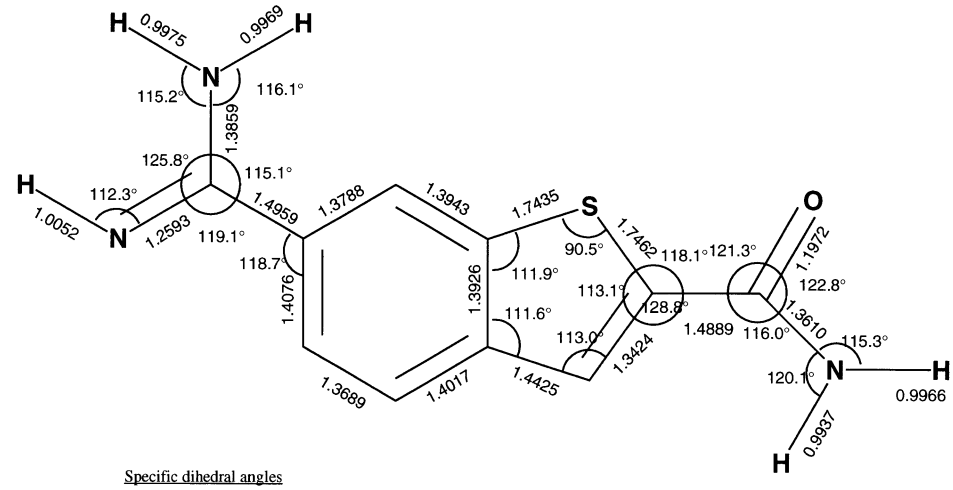
Specific dihedral angles

<c-c-C-N = -25.34 °
<c-c-C=N = 152.47 °
<c-C-N-H = -37.00 °
<c-C-N-H = -170.78 °
<c-C=N-H = 178.76 °

<S-c-C-N = 0.91 °
<S-c-C=O = -178.97 °
<c-C-N-H = 2.50 °
<c-C-N-H = 179.04 °

(Small 'c' : benzothiophen carbon.)

(b) -1019.6622187 Hartree
(+0.21 kcal / mol)



Specific dihedral angles

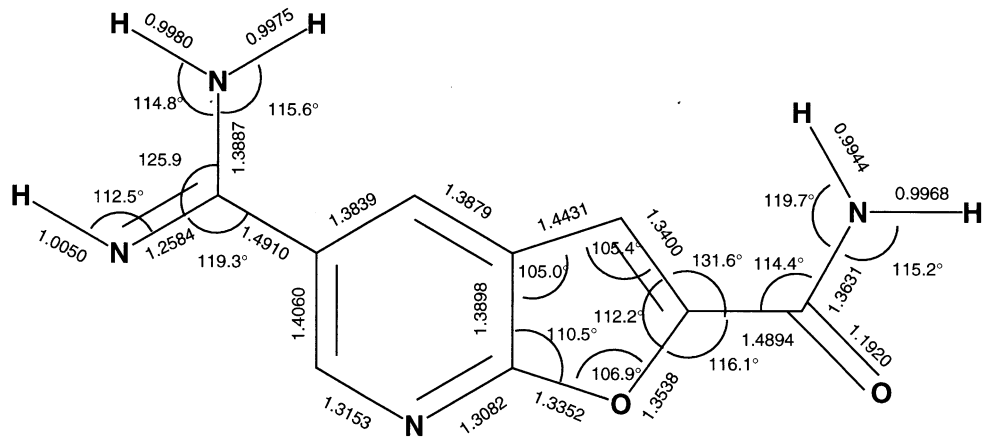
<c-c-C-N = -24.46 °
<c-c-C=N = 153.38 °
<c-C-N-H = -36.43 °
<c-C-N-H = -170.74 °
<c-C=N-H = 178.82 °

<S-c-C-N =166.04 °
<S-c-C=O = -15.15 °
<c-C-N-H = -25.45 °
<c-C-N-H = -172.33 °

(Small 'c' : benzothiophen carbon.)

Figure 11. 6-Amidinobenzo[*b*]thiophen-2-carboxamide **16**.

(a) -712.992796940 Hartree
(+6.32 kcal / mol)



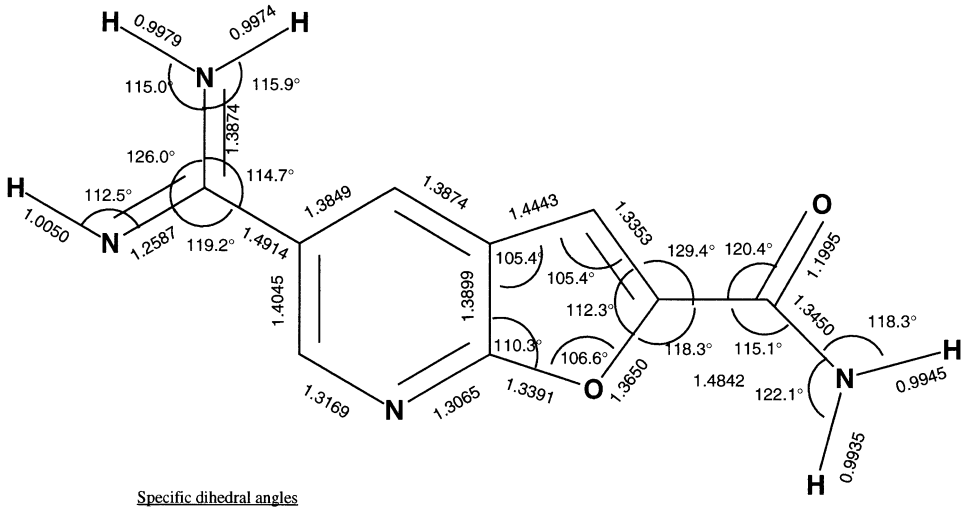
Specific dihedral angles

<c-c-C-N = -25.58 °
<c-c-C=N = 152.15 °
<c-C-N-H = -39.29 °
<c-C-N-H = -172.03 °
<c-C=N-H = 178.15 °

<O-c-CO-N = -161.80 °
<O-c-CO = 20.69 °
<c-C-N-H = 28.23 °
<c-C-N-H = 173.95 °

(Small 'c' : furo[2, 3-b]pyridine carbon.)

(b) -713.002866870 Hartree



Specific dihedral angles

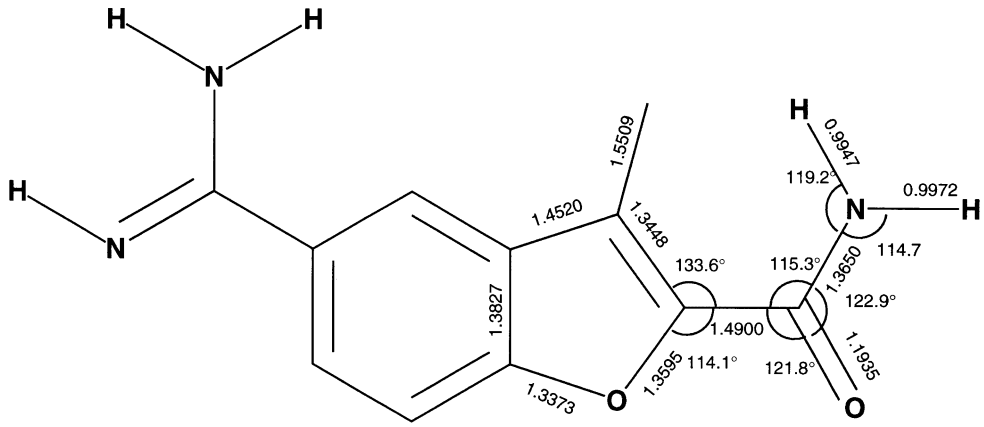
<c-c-C-N = -25.31 °
<c-c-C=N = 152.41 °
<c-C-N-H = -38.68 °
<c-C-N-H = -172.04 °
<c-C=N-H = 178.16 °

<O-c-CO-N =0.25 °
<O-c-CO =-179.65 °
<c-C-N-H = 1.03 °
<c-C-N-H = 179.30 °

(Small 'c' : furo[2, 3-b]pyridine carbon.)

Figure 12. 5-Amidinofuro[2,3-b]pyridine-2-carboxamide **17**.

(a) -736.0349835 Hartree
(+6.76 kcal / mol)



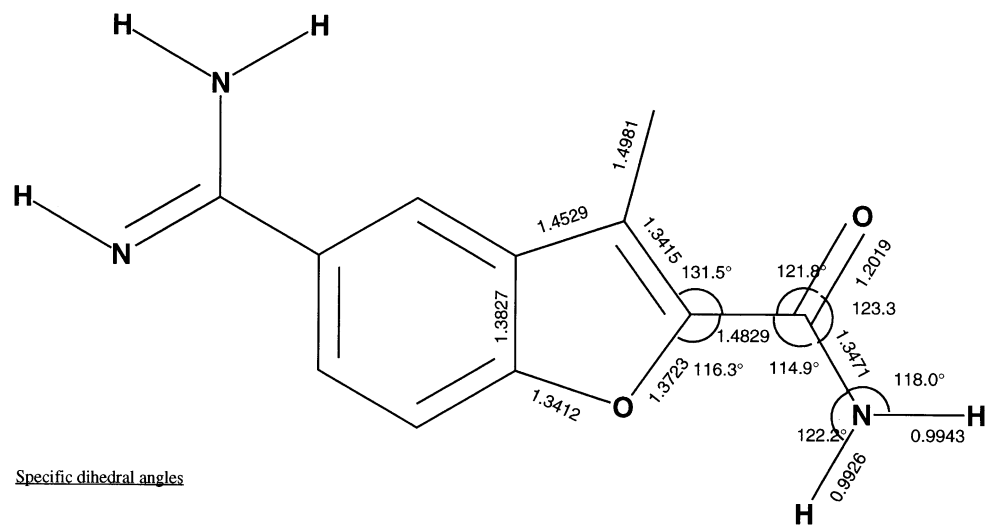
Specific dihedral angles

<c-c-C-N : -24.54 °
<c-c-C=N : 153.36 °
<c-C-N-H : -37.73 °
<c-C-N-H : -170.73 °
<c-C=N-H : 178.45 °

<O-c-C-N : -153.55 °
<O-c-CO : 28.01 °
<c-C-N-H : 28.67 °
<c-C-N-H : 172.33 °

(Small 'c' : benzofuran carbon.)

(b) -736.0457562 Hartree



Specific dihedral angles

<c-c-C-N : -24.19 °
<c-c-C=N : 153.70 °
<c-C-N-H : -37.23 °
<c-C-N-H : -170.78 °
<c-C=N-H : 178.46 °

<O-c-C-N : 0.20 °
<O-c-CO : -179.68 °
<c-C-N-H : 1.36 °
<c-C-N-H : 179.06 °

(Small 'c' : benzofuran carbon.)

Figure 13. 5-Amidino-3-methylbenzofuran-2-carboxamide **18**.

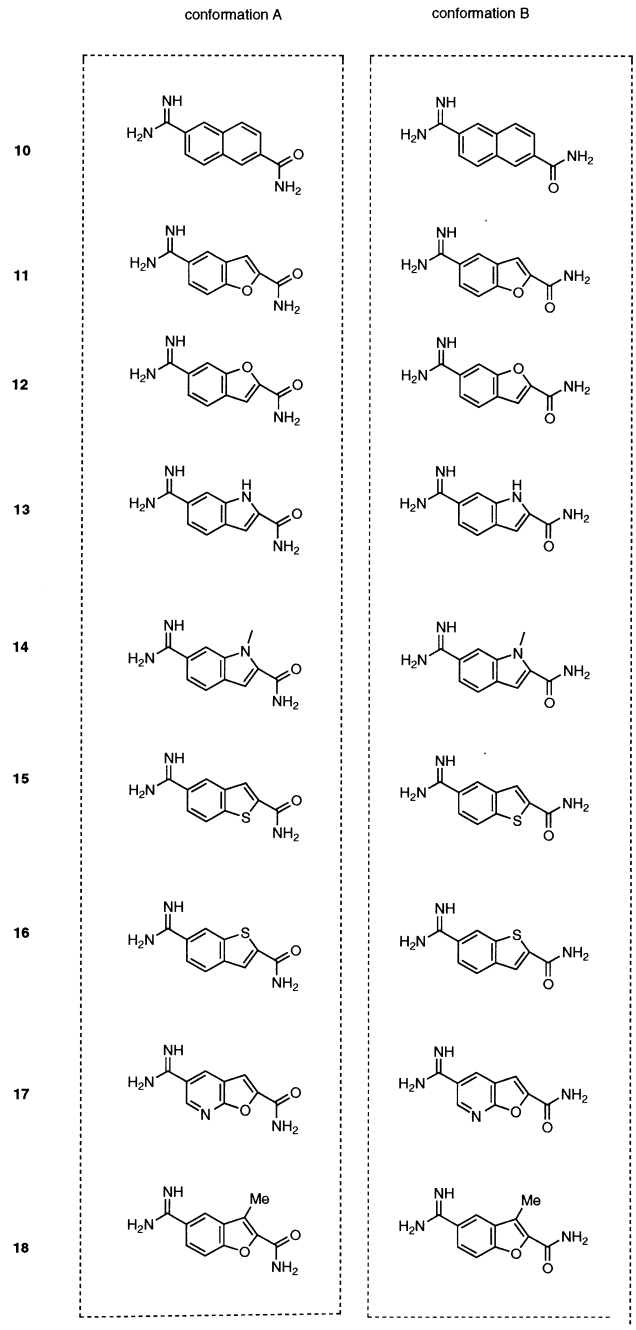


Figure 14. Conformation of amidinoarylcarboxamide.

former **11A**) showed good carbonyl oxygen overlap indicated that these compounds may bind to the receptor in a similar mode (*figure 3*).

For naphthalene compound **1**, the energy barrier to rotation around the amide bond was less than 1.0 kcal/mol. This amide can exist in the two conformers **10A** and **10B** due to the low barrier. The binding conformation of naphthalene compounds to the receptor is thus indicated to correspond to conformer **10A**. Comparison of the conformation of **1** with the stable conformer **2** indicated that naphthalene **1** has a twisted conformation (ca. 20 degrees) between the naphthalene ring and the amide plane (*figure 3*). The difference in potency of **1** and **2** may be attributable to the twist angle of aryl and amide plants.

The calculation for 6-amidinobenzofuran-2-carboxamide **12** indicates that the conformer **12B** is more stable than **12A** by 4.64 kcal/mol and again, the twist angle between the benzofuran and amide planes is 0.39 degrees (conformer **12B**) and 17.75 degrees (conformer **12A**). These results indicate that the binding conformation of **3** to the receptor is conformation **12B**. We showed that amide N–H in 6-amidinobenzofurancarboxamide occupies a different spatial region from that in 5-amidinobenzofurancarboxamide. The diminished inhibitory potency of **3** compared with **2** suggests that the key binding pharmacophore of inhibitors, which consists of positive and negative charges and hydrogen bonds, may sometimes fail to occupy the corresponding region of the receptor, or that the molecular shape of **3** may not be recognized by the receptor, or that the compounds cannot mimic the critical distance between the positive and negative binding sites of the receptor.

In 6-amidinoindole-2-carboxamide **13**, the conformer **13A** is more stable than **13B** by 3.77 kcal/mol and the twist angle of the planes is 3.13 degrees (conformer **13A**) and 13.39 degrees (conformer **13B**). These results indicate the bound conformation of **4** to be conformer **13A**. In the addition of an N-methyl group to the indole **4**, the conformation **14A** is more stable than **14B** by 3.66 kcal/mol. But the twist angle of the planes is 23.10 degrees (conformer **14A**) and 28.80 degrees (conformer **14B**) (*figure 4*). The diminished inhibitory potency of **5** compared to **9** and **4** indicates that, for potent activity, it is necessary for the aromatic rings and the amide group to share the same plane.

In 5-amidinobenzothiophene-2-carboxamide **15**, the C–S bond length of the benzothiophene is greater than the C–O or C–N bond distance of benzofuran and indole. The benzothiophene ring of **6** may occupy a different spatial region than in **1** and **2** (*figure 5*).

In 6-amidinobenzothiophene-2-carboxamide **16**, the conformer **16B** is more stable than **16A**, but the barriers to rotation are less than 1.0 kcal/mol. This amide can exist in two conformations due to the low barriers. The twist angle of the benzothiophene and amide planes is

0.91 degrees (conformer **16B**) and 13.95 degrees (conformer **16A**). Overlay of **2** onto **7** (conformer **16A**) shows that the carbonyl oxygen in **2** occupies the same space as in **7**. As the bound conformation of **7** is thereby indicated to correspond to the slightly unfavourable conformer **16A**, **7** may prove a potent inhibitor (*figure 5*).

In 5-amidinofuro[*b*]pyridine-2-carboxamide **17**, the conformer **17A** is more stable than **17B** by 6.3 kcal/mol and the twist angle of the planes is 0.25 degrees (conformer **17A**) and 18.20 degrees (conformer **17B**). These results indicate the bound conformation of **8** to be conformer **17A**. A further attempt to enhance the potency of the benzofuran of **2** through introduction of another hydrogen bond acceptor was successful.

The introduction of a methyl group (3-position) to the 5-amidinobenzofuran-2-carboxamide **18** led to the energetically less favourable conformation **18B** (6.76 kcal/mol), in which the twist angle of the benzofuran and amide planes is 0.20 degrees (conformer **18A**) and 26.45 degrees (conformer **18B**). These results indicate that the bound conformation of **9** to the receptor is conformation **18A**. From these results, we assume that **2** and **9** affect similar conditions (stable conformation and twist angle) and steric bulkiness is tolerated at the 3-position.

Our studies of the condensed heterocycle series suggested that a certain spatial orientation between the amidino group and the carboxylic acid may be essential in order to bind to the receptor. These compounds establish the importance of the conformational requirements of amidine, the hydrogen-bond donating CONH and carboxylic acid in condensed heterocyclic derivatives. The distance between the cationic moiety and the negative moiety is nearly identical (ca. 15 Å) in our potent inhibitors. Derivatives **2**, **4**, **5**, **8** and **9** bind with a higher affinity than **3**, **5** and **6**, suggesting the presence of a preferred spatial orientation between the aryl and amide planes.

4. Conclusion

In this paper, we reported on the design and evaluation of novel fibrinogen receptor antagonists. Our modifications improved in vitro potency over previously reported naphthalene compounds. Conformational studies in which molecular modelling techniques were used on our compounds established the importance of geometrical parameters (position, length and angle) and interactions with the receptor.

5. Experimental protocols

5.1. Chemistry

Reagents were purchased from commercial suppliers and used without further purification. Reaction solvents were distilled from an appropriate drying agent before use. Melting points were measured on a Yanaco micromelting point apparatus and are uncorrected. IR and NMR spectra, which were in agreement with the structures cited, were recorded on a Shimadzu IR-420 instrument for IR and a Brucker AM-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) and a Brucker AC-200 spectrometer (200 MHz for ^{1}H-NMR and 50 MHz for ^{13}C-NMR) for NMR using TMS as an internal standard. Chemical shifts are reported in parts per million (ppm), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). EI-MS and SI-MS were taken on a Hitachi M-2000 mass spectrometer, respectively. Compounds **1–8** [1, 2] were prepared according to the methods described in the literature.

5.1.1. Spectroscopic data

5.1.1.1. 4-(6-Amidino-2-naphthalenecarboxamido)phenoxyacetic acid **1**

IR (KBr) cm^{-1}: 3 300, 1 640, 1 510. ^{1}H-NMR (DMSO-d_6) δ 4 (H s), 6.95 (2 H, d, *J* = 9.0 Hz) 7 (H d, *J* = 9.0 Hz) 7 (H dd, *J* = 8.5, 1.5 Hz) 8 (H dd, *J* = 81.5, 8.5 Hz), 8.24 (1 H, d, *J* = 8.7 Hz), 8.32 (1 H, d, *J* = 8.7 Hz), 8.54 (1 H, s), 8.68 (1 H, s), 9.08 (2 H, brs), 9.49 (2 H, brs), 10.45 (1 H, s), 12.6–13.4 (1 H, brs). Anal. calcd. for $C_{20}H_{17}N_3O_4$ plus $0.5C_2HO_2F_3$ and $1.1H_2O$: C, 44.40; H, 3.67; N, 7.40. Found: C, 44.08; H, 3.53; N, 7.73.

5.1.1.2. 4-(5-Amidinobenzofuran-2-carboxamido)phenoxyacetic acid **7a**

M.p.: > 250 °C; IR (KBr) 1 740, 1 690, 1 610, 1 540, 1 505 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.57 (s, 1 H), 9.38 (bs, 2 H), 9.11 (bs, 2 H), 8.34 (d, *J* = 1.7 Hz, 1 H), 7.97 (d, *J* = 8.8 Hz, 1 H), 7.91 (s, 1 H), 7.89 (dd, *J* = 1.7, 8.8 Hz, 1 H), 7.72–7.69 (m, 2 H), 6.97–6.93 (m, 2 H), 4.67 (s, 2 H); ^{13}C-NMR (DMSO-d_6): δ 170.1, 165.6, 156.6, 155.7, 154.4, 150.7, 131.4, 127.3, 126.7, 124.0, 123.9, 122.1, 114.4, 112.5, 64.62, 10.2; Anal. ($C_{18}H_{15}N_3O_5$ plus HCl and H_2O) C, H, N; HPLC t_{RA} 6.1 min, t_{RB} 11.5 min.

5.1.1.3. 4-(6-Amidinobenzofuran-2-carboxamido)phenoxyacetic acid **7b**

M.p.: > 250 °C; IR (KBr) 3 700–2 800, 1 730, 1 690, 1 520, 1 505 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.63 (bs, 1 H), 9.45 (bs, 2 H), 9.32 (bs, 2 H), 8.20 (s, 1 H), 8.06 (d,

J = 8.3 Hz, 1 H), 7.89 (d, *J* = 1.6 Hz, 1 H), 7.79 (dd, *J* = 1.6, 8.3 Hz, 1 H), 7.72–7.69 (m, 2 H), 6.97–6.93 (m, 2 H), 4.67 (s, 2 H); ^{13}C-NMR (DMSO-d_6): δ 170.1, 165.4, 155.7, 154.4, 153.3, 151.8, 131.7, 131.4, 126.2, 123.4, 123.3, 122.1, 114.5, 112.2, 109.9, 64.63; Anal. ($C_{18}H_{15}N_3O_5$ plus 2.2CF_3CO_2H and 1.8H_2O) C, H, N; HPLC t_{RA} 6.2 min, t_{RB} 11.5 min.

5.1.1.4. 4-(6-Amidino-1H-indole-2-carboxamido)phenoxyacetic acid **7c**

M.p.: 205–245 °C (decomp.); IR (KBr) 3 700–3 100, 1 660, 1 520, 1 400 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.33 (bs, 1 H), 9.27 (bs, 2 H), 8.82 (bs, 2 H), 7.95 (s, 1 H), 7.91 (d, *J* = 8.4 Hz, 1 H), 7.70 (d, 2 H), 7.51 (s, 1 H), 7.45 (dd, *J* = 1.3, 8.4 Hz, 1 H), 6.95 (d, 2 H), 4.67 (s, 2 H); ^{13}C-NMR (DMSO-d_6): δ 170.1, 166.4, 166.3, 158.7, 154.2, 135.4, 135.4, 132.0, 130.8, 122.7, 121.8, 118.9, 114.5, 113.2, 103.5, 64.69; Anal. ($C_{18}H_{16}N_4O_4$ plus 2HI and 1.5H_2O) C, H, N; HPLC t_{RA} 7.9 min, t_{RB} 15.2 min.

5.1.1.5. 4-(6-Amidino-1-methylindole-2-carboxamido)phenoxyacetic acid **7d**

M.p.: > 250 °C; IR (KBr) 3 700–2 800, 1 640, 1 505, 1 390 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.28 (bs, 2 H), 8.90 (bs, 2 H), 8.19 (s, 1 H), 7.92 (d, *J* = 8.4 Hz, 1 H), 7.67 (d, 2 H), 7.54 (dd, *J* = 1.5, 8.4 Hz, 1 H), 7.37 (s, 1 H), 6.94 (d, 2 H), 4.64 (s, 2 H), 4.09 (s, 3 H); ^{13}C-NMR (DMSO-d_6): δ 170.1, 165.8, 159.4, 155.0, 137.0, 135.9, 132.0, 129.3, 122.3, 122.0, 121.8, 119.1, 114.4, 111.9, 105.1, 64.70, 31.72; HPLC t_{RA} 9.7 min, t_{RB} 17.5 min.

5.1.1.6. 4-(5-Amidinobenzo[b]thiophen-2-carboxamido)phenoxyacetic acid **7e**

M.p.: > 210 °C (deccomp.).; IR (KBr) 3 700–2 700, 1 680, 1 640, 1 510 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.58 (bs, 1 H), 9.42 (bs, 2 H), 9.26 (bs, 2 H), 8.42 (m, 2 H), 8.32 (d, *J* = 8.6 Hz, 1 H), 7.84 (dd, *J* = 1.8, 8.6 Hz, 1 H), 7.68–7.65 (m, 2 H), 6.97–6.93 (m, 2 H), 4.67 (s, 2 H); ^{13}C-NMR (DMSO-d_6): δ 170.0, 165.6, 159.3, 154.3, 144.7, 142.8, 138.8, 131.7, 125.6, 125.3, 125.2, 124.7, 123.6, 122.0, 114.5, 64.70; Anal. ($C_{18}H_{17}N_3O_4S$ plus HI and 0.5CF_3CO_2H) H, N, C: calcd., 41.17; found, 42.52; HPLC t_{RA} 9.7 min, t_{RB} 17.1 min.

5.1.1.7. 4-(6-Amidinobenzo[b]thiophen-2-carboxamido)phenoxyacetic acid **7f**

M.p.: > 250 °C; IR (KBr) 3 700–2 800, 1 740, 1 680, 1 635, 1 500 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.59 (bs, 1 H), 9.41 (bs, 2 H), 9.21 (bs, 2 H), 8.58 (s, 1 H), 8.44 (s, 1 H), 8.23 (d, *J* = 8.5 Hz, 1H), 7.81 (dd, *J* = 1.5, 8.5 Hz, 1 H), 7.67 (d, 2 H), 6.95 (d, 2 H), 4.67 (s, 2 H); ^{13}C-NMR (DMSO-d_6): δ 170.1, 165.6, 159.3, 154.4, 144.5, 142.6, 139.8, 131.7, 125.8, 125.6, 125.0, 124.1, 123.6, 122.0, 114.5, 64.80; Anal. ($C_{18}H_{17}N_3O_4S$ plus HI and 0.7CF_3CO_2H) C, H, N; HPLC t_{RA} 8.5 min, t_{RB} 15.9 min.

5.1.1.8. 4-(5-Amidinofuro[2,3-b]pyridine-benzofuran-2-carboxamido)phenoxyacetic acid **7g**

M.p.: > 250 °C (decomp.).; IR (KBr) 3 350, 1 660, 1 600, 1 500 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.69 (bs, 1 H), 9.53 (bs, 2 H), 9.38 (bs, 2 H), 8.87 (d, *J* = 2.1 Hz,1 H), 8.75 (d, *J* = 2.1 Hz, 1 H), 7.92 (s, 1 H), 7.75–7.67 (m, 2 H), 6.97–6.90 (m, 2 H), 4.67 (s, 2 H); ^{13}C-NMR (DMSO-d_6): δ 174.2, 164.10, 162.8, 155.4, 154.6, 149.9, 146.5, 133.4, 129.2, 122.3, 122.0, 119.1, 114.5, 1.9.7, 64.67; Anal. ($C_{17}H_{14}N_4O_5$ plus 1.4CF_3CO_2H) C, H, N: calcd., 10.90; found, 11.48; HPLC t_{RA} 4.4 min, t_{RB} 7.4 min.

5.1.1.9. 4-(5-Amidino-3-methylbenzofuran-2-carboxamido)phenoxyacetatic acid **7h**

M.p.: 220–228 °C (decomp.); IR (KBr) 3 300, 3 100, 1 730, 1 670, 1 610, 1 535, 1 510 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.42 (s, 1 H), 9.38 (bs, 2 H), 9.14 (bs, 2 H), 8.34 (d, *J* = 1.8 Hz, 1 H), 7.92 (dd, *J* = 1.8, 8.6 Hz, 1 H), 7.90 (d, *J* = 8.6 Hz, 1 H), 7.73–7.70 (m, 2 H), 6.94–6.91 (m, 2 H), 4.67 (s, 2 H), 2.64 (s, 3 H); ^{13}C-NMR (DMSO-d_6): δ 170.1, 165.5, 157.1, 155.1, 154.3, 144.7, 131.5, 129.4, 127.0, 123.5, 122.4, 122.2, 121.8, 114.4, 112.3, 64.62, 8.72; Anal. ($C_{19}H_{17}N_3O_5$ plus HI and 0.8CF_3CO_2H) C, H, N; HPLC t_{RA} 9.4 min, t_{RB} 19.3 min.

5.2. Biology

5.2.1. Determination of suppressive activity on ADP induced aggregation of human platelets [16]

Platelet rich plasma was prepared from the blood taken from healthy humans by centrifugation in the presence of 0.38% sodium citrate, and used for the determination. Two minutes after the test compounds were added to the above-mentioned platelet rich plasma, ADP (adenosin-5′-diphosphate, 1–5 μM) was added, at a concentration where primary aggregation alone was observed. The suppression of ADP aggregation by the compounds was evaluated. The percent suppression was determined by varying the concentration of the compounds and the concentration of the compound at which the aggregation was suppressed by 50% (IC_{50}) was calculated, which was taken as the activity of the compound.

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