Bioorganic & Medicinal Chemistry Letters 19 (2009) 2997–3001

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Conformational analysis of *N,N*-disubstituted-1,4-diazepane orexin receptor antagonists and implications for receptor binding

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ARTICLE INFO

Article history:
Received 19 March 2009
Revised 8 April 2009
Accepted 9 April 2009
Available online 14 April 2009

Keywords:
Orexin receptor antagonist
Diazepane
Pi-stacking
Macrocyclicization

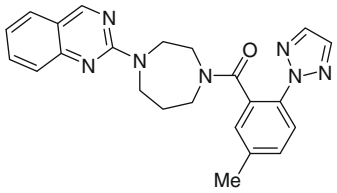
ABSTRACT

NMR spectroscopy, X-ray crystallography, and molecular modeling studies indicate that *N,N*-disubstituted-1,4-diazepane orexin receptor antagonists exist in an unexpected low-energy conformation that is characterized by an intramolecular π-stacking interaction and a twist-boat ring conformation. Synthesis and evaluation of a macrocycle that enforces a similar conformation suggest that this geometry mimics the bioactive conformation.

Antagonism of the orexin (or hypocretin) system has recently been identified as a novel mechanism for the treatment of insomnia.[1] In a recent Communication, we described the discovery of a novel series of HTS-derived dual (OX_1R/OX_2R) orexin receptor antagonists based on a 1,4-diazepane core.[2] This effort culminated in the discovery of **1**, a potent and brain-penetrant compound that demonstrates efficacy for silencing orexin signaling in freely locomoting rats, including the induction of REM and non-REM sleep.

During our synthetic efforts leading to **1**, examination of ^{1}H NMR spectra revealed that compounds in this chemical class exist in multiple conformations in solution, and that several proton resonances appear with unexpected chemical shifts that are indicative of an unusual conformational bias. We therefore undertook an investigation to elucidate the nature and origin of these conformational effects, and to determine if they have ramifications for receptor binding. Herein, we describe the results of our study that suggest an intramolecular π-stacking interaction and the adoption of a twist-boat ring conformation favor an unexpected low-energy conformation in orexin receptor antagonists such as **1**, and that this low-energy structure resembles the bioactive conformation.

As a first step to investigate the conformational preferences of these novel molecules, we performed detailed 600 MHz ^{1}H NMR studies to appraise their three-dimensional structure in solution. Analysis of **1** at room temperature is precluded by broad resonance line widths, but upon cooling the sample to −40 °C in CD_3OD, the resonances narrow significantly to reveal four components in equilibrium (~5:4:1:1) which are likely due to amide and 2-aminoquinazoline rotamers.[3] Though the spectrum is complex with many overlapping signals, proton resonance assignments and NOE correlations of the two major rotamers can be determined.



1 OX_1R K_i = 1.2 nM
OX_2R K_i = 0.6 nM

In the major conformers, **rotamer A** and **rotamer B**, NOE's are observed from the proton at the 4-position of the quinazoline ring to the aromatic methyl group as well as the proton at the 6-position of the phenyl ring, as illustrated in Figure 1A and B. Additionally, the ^{1}H NMR resonances due to the phenyl and methyl protons in **rotamer A**, 6.25 and 1.69 ppm, respectively, are shifted significantly upfield of their expected positions of approximately 7.23 and 2.43 ppm due to the shielding effect of the quinazoline ring.[4] Taken together, this data suggests that the predominant solution conformation is one in which the molecule adopts a horseshoe-

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doi:10.1016/j.bmcl.2009.04.026

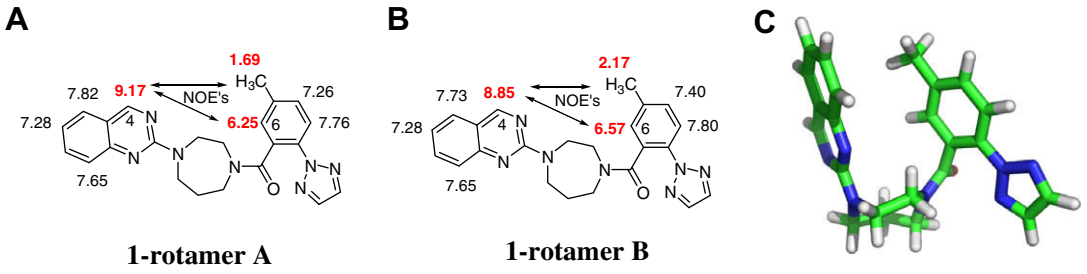
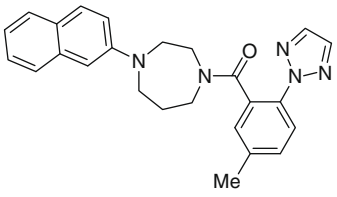


Figure 1. Panel A: ^{1}H NMR data for the most highly populated rotamer of compound **1**, **1-rotamer A**, determined by analysis of **1** at −40 °C in CD_3OD. Key NOE correlations are indicated by black arrows, and proton resonance assignments (in ppm) are illustrated; resonances highlighted in red are most relevant for deducing solution conformation. Panel B: similar data as presented in Panel A, but for the second most populated rotamer, **1-rotamer B** (**Rotamer A** accounts for 45% of the solution and **rotamer B** is 36%). Panel C: a low-energy conformation computed for **1** that is consistent with the NMR data.[5]

or U-shaped structure where the quinazoline and phenyl groups are in close proximity, likely engaged in a π-stacking interaction. Molecular modeling studies predict a low-energy conformation for **1** that is consistent with these observations, as illustrated in Figure 1C.[5]

In **1-rotamer B**, the key proton resonances appear at 6.57 and 2.17 ppm, suggesting a weaker π-stacking interaction than in **rotamer A**. However, in conjunction with the observed NOE's, the NMR data support a U-shaped structure for **rotamer B** as well. In contrast, there are no significant NOE correlations or upfield resonance shifts in either of the minor components that suggest a close spatial relationship between the two aryl groups. Magnetization transfer between the various conformers present in solution prohibits a more detailed analysis of **1**.

We next synthesized the 2-naphthyl analog of **1** to simplify the NMR spectrum and permit a more complete conformational analysis. Compound **2** is a potent dual orexin receptor antagonist, and, more importantly, it exists as a mixture of only two components at room temperature, identified by NOE correlations to be *cis/trans* amide rotamers, in a ratio of approximately 1.4–1. As pictured in Figure 2, the amide rotamers are assigned by NOE's between the proton at C6 of the phenyl ring and the protons on the diazepan ring adjacent to this position, and all key aromatic resonances can be explicitly assigned for both rotamers.



2 OX_1R K_i = 2 nM
OX_2R K_i = 2 nM

In the major component of compound **2**, **rotamer A**, NOE's are observed between the proton at the 6-position of the phenyl group and those at the 1-, 3-, and 4-positions of the naphthyl group (highlighted in red in Fig. 2A). Additionally, a strong NOE between the aromatic methyl protons and the proton at the 5-position of the naphthyl group is observed (highlighted in blue). Furthermore, dramatic upfield shifts of the phenyl and aromatic methyl protons to 5.99 and 1.73 ppm indicate a strong shielding effect due to their proximity to the naphthyl ring. In contrast, no significant NOEs are observed between the aromatic moieties in **rotamer B** (Fig. 2B), and the chemical shifts of the phenyl and methyl protons at 6.87 and 2.27 ppm are closer to their expected isotropic values of approximately 7.23 and 2.43 ppm.[4] This implies **rotamer B** has greater separation between the aromatic moieties, and thus a weaker π-stacking interaction, than in **rotamer A**.

A computational analysis of **2** was performed, and a Boltzmann average was calculated over all low-energy conformations within 5 kcal/mol of the minima using the MMFFs forcefield.[5] The lowest energy conformer has a structure consistent with the NMR data obtained for **rotamer A**, and is pictured in Figure 3. The aromatic groups are engaged in a π-stacking interaction with a geometry that is in between that of well defined edge-to-face (or T-shaped) and parallel-displaced[6] orientations. The centroid separation distance is 5.2 Å, versus calculated distances in the optimized benzene dimer of 4.96 Å for edge-to-face or 3.4–3.6 Å for parallel-displaced interactions.[7] A structure consistent with **rotamer B**, though populated to a lesser degree, is also identified among the low-energy conformations and has a centroid separation distance of 7.3 Å.[8]

To further our understanding of the conformational preferences of compounds in this chemical class, we determined the structure of **1** by X-ray crystallography.[9] As pictured in Figure 4, the solid state structure of **1** is very similar to the predominant low- energy conformations determined by ^{1}H NMR analysis and molecular modeling studies on **1** and **2**, wherein the central seven-membered

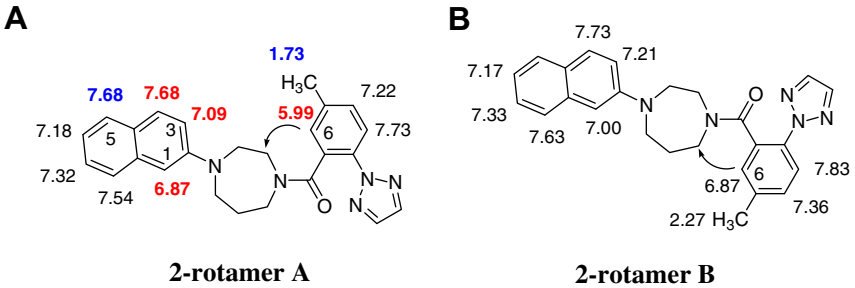


Figure 2. Panel A: ^{1}H NMR data for the major rotamer of compound **2**, **2-rotamer A**, determined by analysis of **2** at 25 °C in CD_3OD. The NOE used to assign the amide rotamer is indicated by a black arrow, and the NOE's used to establish the U-shaped conformation are illustrated by the proton resonance assignments (in ppm) highlighted in red and blue. Panel B: similar data as presented in Panel A, but for the minor rotamer, **2-rotamer B**. **Rotamer A** accounts for 58% of the solution and **rotamer B** is 42%.

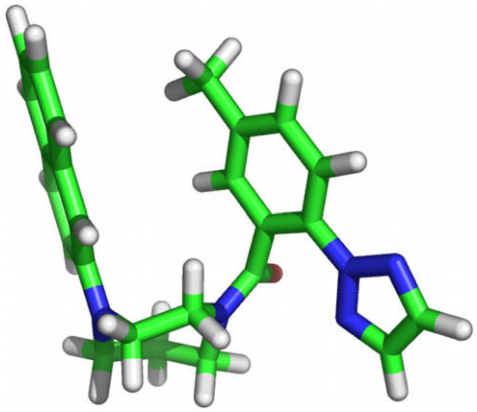
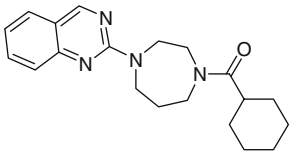


Figure 3. Predominant, low-energy structure of **2** using the MMFFs forcefield in chloroform at a constant dielectric constant of 4. The structure is consistent with the ^{1}H NMR for **2-rotamer A**, and has a centroid separation distance of 5.2 Å.

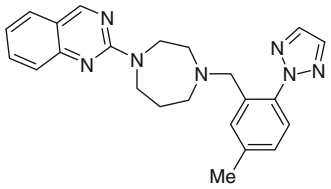
ring adopts a twist-boat conformation[10] and the aryl moieties are in close proximity with a centroid separation distance of 5.1 Å. Within the crystal lattice there are also π−π stacking interactions in symmetry-related instances of the quinazoline rings which presumably assist in stabilizing the low-energy conformer in the solid-state.[11]

To better understand the driving force for enforcing the U-shaped conformation, we performed studies to investigate the energetic contribution of the π-stacking interaction. Along with hydrogen-bonding, π-stacking is a critical non-covalent interaction that helps to control macromolecular structure, including the helical nature of DNA,[12] the secondary and tertiary structure of proteins,[13] crystal packing of aromatic molecules,[14] and architectural organization in supramolecular chemistry.[15] In drug discovery, there has been much attention given to *inter*molecular π-stacking between a pharmaceutical agent and its biological target.[16] However, much less focus has been placed on *intra*molecular π-stacking interactions that control the conformational preferences of drug molecules.[17]

Quantum mechanical studies have reproduced experimental binding energies for the benzene dimer with relatively high accuracy depending on the level of theory employed.[18] Therefore, we chose to quantitate the π-stacking ability of the quinazoline and phenyl rings in compound **1** beginning with the crystal structure coordinates and optimizing to a local minima using the MP2/aug-cc-pVDZ level of theory.[19] Using the Counterpoise keyword in Gaussian, this interaction is calculated to be −7.5 kcal/mol. Relative to the parallel-displaced benzene dimer at the same level of theory (−4.2 kcal/mol), the quinazoline–phenyl dimer interaction is calculated to be stronger.[20] When we employ the crystal coordinates for only the quinazoline and phenyl rings and perform a single point calculation (thus, keeping the coordinates fixed at the crystal structure solid state conformation), the two-body binding interaction is calculated to be −3.5 kcal/mol, less than an optimized benzene dimer interaction, but consistent with an energetically favorable π-stacking interaction.



3 OX_1R K_i = 9,800 nM
OX_2R K_i = 550 nM



4 OX_1R K_i = 930 nM
OX_2R K_i = 240 nM

To investigate if the π-stacking interaction is the primary driving force for adopting the U-shaped structure, we synthesized cyclohexyl amide **3** that can not form a π-stack, as well as tertiary amine **4** which lacks the amide carbonyl. As expected from previous SAR, both **3** and **4** display significantly reduced binding affinity for the orexin receptors.[2a] NMR analysis indicates that compound **3** exists as a mixture of four components in solution in approximately a 1:1:1:1 ratio, but in one or more conformations, weak to moderate NOE's are evident between the cyclohexyl and quinazoline rings. This suggests a U-shaped structure is among the low-energy conformations present in solution. In contrast, the ^{1}H NMR spectrum of **4** consists of a single conformation at room temperature, with no evidence of π-stacking as indicated by a lack of NOE correlations or shielding effects on the chemical shift of key protons. Taken together, this suggests that π-stacking is neither required, nor solely sufficient, to favor the U-shaped structure as a low-energy conformation.

There are no pertinent examples of 1,4-diazepane structures in the Cambridge Structural Database that contain an exocyclic amide carbonyl attached to a ring nitrogen. However, there are several non-amide containing structures, and in each case, the central ring exists as a chair or twist-chair.[21] In contrast, the diazepane ring of **1** exists in a twist-boat conformation, as illustrated in Figure 4.

It is not clear why an exocyclic amide might trigger a change in ring conformation from chair to boat, but the increased π-stacking potential of **1** and **2** relative to **4**, and the ability of **3** to display the cyclohexyl group in proximity to the quinazoline ring, is consistent with this supposition. Accordingly, in the boat conformation, the ring nitrogen substituents occupy flagpole positions and are therefore in close proximity, readily available to engage in a π-stacking interaction. However, if the ground state is a chair conformation, an energetic penalty must be paid to change the ring conformation in order to bring the aryl moieties into proximity. The NMR data for compound **4** suggest that in this case, the energetic penalty of adopting a boat conformation is not overcome by a favorable π-stacking interaction.

In summary, the data generated by NMR spectroscopy, X-ray crystallography, and molecular modeling suggest that the U-shaped structure is a low-energy conformation of orexin receptor antagonists **1** and **2**. Although this provides an interesting perspective on a novel class of biologically active compounds, a key unan-

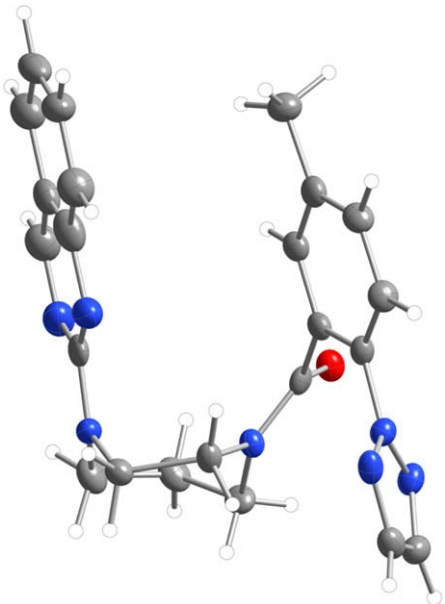


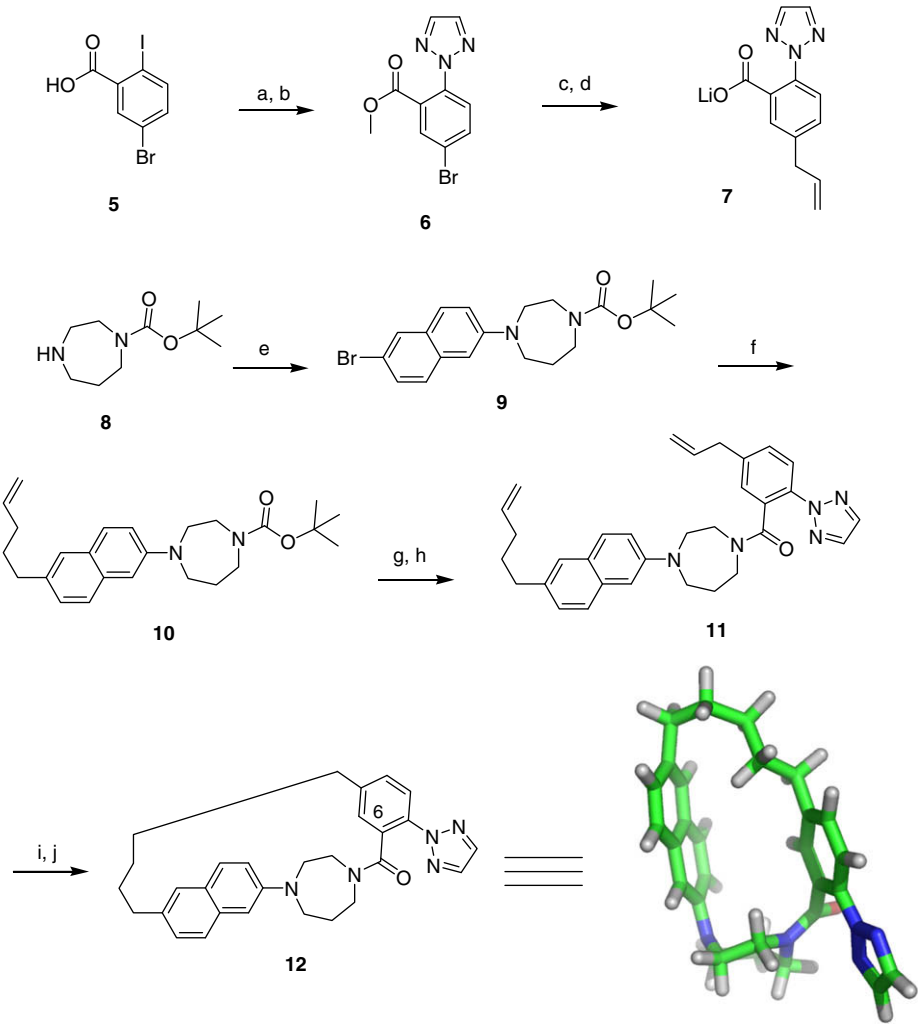
Figure 4. X-ray crystal structure of **1** illustrating the twist-boat conformation of the central diazepan ring, as well as the overall U-shaped structure present in the solid state.

swered question for the rational design of next generation orexin receptor antagonists is whether the bioactive conformation is similar to the U-shaped structure. If so, rational design of antagonists with geometric constraints to enforce this conformation may lead to increased potency and improved pharmaceutical properties.[22] To test our hypothesis that the bioactive conformation of diazepane orexin receptor antagonists is similar to the low-energy conformation observed spectroscopically for **1** and **2**, we designed and synthesized a macrocyclic analog that is locked in a U-shaped geometry, as illustrated in Scheme 1.

Preparation of the macrocycle begins with a copper-catalyzed C–N cross-coupling between 5-bromo-2-iodobenzoic acid (**5**) and 1,2,3-triazole.[23] After esterification, the resulting aryl bromide (**6**) is allylated under standard Stille conditions and the ester hydrolyzed to provide lithium salt **7**. In a parallel sequence, the coupling partner is prepared beginning with a cross-coupling of BOC-homopiperazine (**8**) with 2,6-dibromonaphthalene to provide **9**. A Negishi coupling with pentenylzinc bromide yields olefin **10**. Following deprotection, this intermediate is coupled with **7** to provide ring-closing metathesis (RCM) precursor **11**. Treatment of **11** with the Zhan-1B catalyst, followed by hydrogenation of the resulting alkene, provides macrocycle **12** in low yield.[24]

COSY, HMBC, and NOE correlations, as well as HRMS, confirm the structure of **12**. In DMSO-d_6 solution at 75 °C, **12** exists as two major conformers in a 1:1 ratio, with two minor components making up less than 4% of the mixture. NOE correlations are difficult to identify and assign due to rapid magnetization transfer, but the C6 phenyl protons of the major conformers are observed at 5.00 and 5.49 ppm, supporting the fact that **12** exists in a conformation similar to the low-energy conformations of **1** and **2** in which the aromatic groups are in close proximity.

In the orexin receptor binding assays, the RCM precursor **11** displays activity against OX_1R and OX_2R of 63 nM and 120 nM, respectively, whereas the corresponding values for macrocycle **12** are 51 nM and 42 nM. Thus, closing the macrocycle and enforcing the U-shaped conformation is potency neutral on OX_1R and provides a slight potency increase on OX_2R. The observation that **12** does not obtain the levels of potency observed for **1** and **2** may be a result of the macrocycle geometry not perfectly mirroring the bioactive conformation, or simply that the saturated carbon linker is not fully accommodated by the receptor. Evidence in support of the latter conclusion is provided by the observation that **11** lost significant potency relative to its simplified analog **2**. Nevertheless, the fact that the geometrically constrained macrocycle **12** binds to both receptors with significant levels of potency supports our hypothesis that the U-shaped conformation determined for **1** and **2** mimics the bioactive conformation.



Scheme 1. Synthesis of the macrocycle. Reagents and conditions: (a) 1*H*-1,2,3-triazole, CuI, K_3PO_4, DMF, 60 °C; (b) HCl(g), MeOH (36% for two steps); (c) tetraallyltin, tetrakis(triphenylphosphine) Pd(0), LiCl, DMF, 125 °C (83%); (d) LiOH, THF/MeOH/H_2O, 45 °C (quant.); (e) 2,6-dibromonaphthalene, NaO^{t}Bu, BINAP, $Pd_2(dba)_3$, PhMe, 100 °C (46%); (f) 4-pentenylzinc bromide, $Pd(PtBu_3)_2$, THF, 75 °C (38%); (g) HCl, Et_2O; (h) **7**, EDC, HOBt, TEA, DMF, 50 °C (63% for two steps); (i) Zhan IB, DCE, 40 °C (12%); (j) H_2, Pd/C, EtOAc (87%).

In conclusion, we found that *N,N*-disubstituted-1,4-diazepane orexin receptor antagonists exist in a U-shaped conformation as a result of favorable π-stacking interactions and the adoption of a twist-boat ring conformation. These effects result in a low-energy conformation that resembles the bioactive conformation, reducing the conformational entropy required to mold the structure into a binding orientation. The lessons learned herein provide inspiration for the synthesis of 'bridged' diazepane analogs in which a conformational constraint is installed within the core to enforce or lock-in the U-shaped conformation. Results of this effort will be the subject of a future report from our group.

Acknowledgments

The authors would like to thank Ms. Nancy Tsou for growing diffractable crystals of **1**, Dr. Rodney Bednar, Ms. Wei Lemaire, and Mr. Joe Bruno for determining binding potencies of key compounds, and Dr. Charles Ross and Ms. Joan Murphy for high resolution mass spectral analyses.

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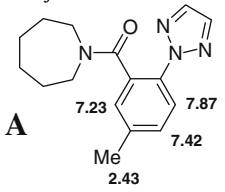
3. It is known that restricted rotation around exocyclic partial double bonds to heterocyclic six-membered rings can be observed on the NMR time scale; see Kleinpeter, E.; Spitzner, R.; Schroth, W. *Magn. Reson. Chem.* **1987**, *25*, 688. Additionally, conformational studies on compound **2** further supports hindered rotation about the exocyclic bond to the quinazoline as a complicating factor in the analysis of **1**..
4. To determine the inherent isotropic shift of the C6 phenyl and aromatic methyl protons, we synthesized and analyzed compound **A** in which no π-stacking interaction is available. The C6 phenyl and aromatic methyl protons appear at 7.23 and 2.43 ppm, respectively.



A

5. Molecular modeling studies utilized the conformational searching algorithm implemented in Maestro v8.5 (mixed torsional/low-mode sampling) with a constant dielectric constant of 4 in chloroform using the MMFFs force field. The conformation pictured in Figure 1C is 0.2 kcal/mol higher in energy than the global minima, a structure with a similar overall conformation, but in which the triazole ring is π-stacking with the quinazoline ring. There is no evidence to support the existence of the triazole π-stacked conformation in solution by either NOE correlations or resonance shifts.
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8. Examination of both computational and hand-held models of **2-rotamer B** indicate that geometrical constraints of this amide rotamer prevent the aryl rings from getting into the close proximity seen in **2-rotamer A**, in line with NMR data suggesting a weaker π-stacking interaction in **2-rotamer B**.
9. Compound **1**: $C_{23}H_{23}N_7O$, $M_r = 413.475$, monoclinic, $P2_1/c$, $a = 13.3019(2)$, $b = 19.7509(4)$, $c = 15.4723(3)$ Å, $\beta = 91.3620(10)°$, $V = 4063.80(13)$ Å^3, $Z = 8$, $D_x = 1.352$ g cm^{-3}, monochromatized radiation λ(Mo) = 0.71073 Å, $\mu = 0.09$ mm^{-1}, $F(0\,0\,0) = 1744$, $T = 100$ K. Data were collected on a Bruker CCD diffractometer to a θ limit of 28.31° which yielded 63565 reflections. There are 10056 unique reflections with 5738 observed at the 2σ level. The structure was solved by direct methods (SHELXS-97, Sheldrick, G.M. *Acta Crystallogr.*, **1990**, *A46*, 467–473) and refined using full-matrix least-squares on F^2 (SHELXL-97, Sheldrick, G.M. SHELXL-97. *Program for the Refinement of Crystal Structures.* Univ. of Göttingen, Germany). There are two independent molecules in the asymmetric unit, related by a pseudo inversion center, with no significant conformational differences between them. The final model was refined using 561 parameters and all 10056 data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are: $R = 0.074$ (based on 5738 reflections with $I > 2\sigma(I)$), $wR = 0.165$, $S = 1.02$ with $(\Delta/\sigma)_{max} = 0.12$. The maximum peak height in a final difference Fourier map is 1.284 eÅ^{-3}, located within the quinazoline ring, and is without chemical significance. **CCDC 723977** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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20. Sanders, J. The value provided in Ref. [18] is—4.1 kcal/mol, but was determined at a slightly lower level of theory.
21. A search of the CSD for 1,4-diazepanes with no substitution on the ring carbons and an exocyclic amide carbonyl at one of the ring nitrogens yields no hits. The same search, but with the exocyclic amide constraint relaxed, retrieves several structures, including ADASUU, ALAFUO, and QAFGUA in which the central ring adopts a chair conformation. This analysis excludes organometallic complexes in which the ring nitrogens are involved in a metal complex, forcing the structure to adopt a boat conformation. See for example: Guo, Y. M.; Du, M.; Bu, X.-H. *J. Mol. Struct.* **2002**, *610*, 27.
22. For instance, decreased molecular flexibility, as measured by the number of rotatable bonds, may lead to improved bioavailability. See: Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
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24. Interestingly, direct RCM of **11** is expected to result in a macrocycle with six alkyl carbons bridging the aryl groups, rather than the five carbon bridge present in **12**, but this product is not observed. A well known side reaction in difficult RCM reactions is olefin isomerization, possibly due to the presence of a ruthenium hydride species formed by decomposition of the catalyst, see, for example: (a) Hong, S. H.; Day, M. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **2004**, *126*, 7414; (b) Hong, S. H.; Sanders, D. P.; Lee, C. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **2005**, *127*, 17160. Apparently, the six carbon-bridged structure is unable to form under these conditions. On the other hand, the five-carbon bridged macrocycle is accessible, but its formation requires isomerization of one of the double bonds prior to ring closing, likely accounting for the low yield for this process. Attempts to cyclize analogs of **11** that would have led to a four- or seven-carbon bridged structure (i.e., one carbon-extended or two carbon-deleted versions of **11**) led to no isolatable RCM products, further supporting the conclusion that the five-carbon bridged structure is optimal.