The AAPS Journal 2006; 8 (4) Article 76 (http://www.aapsj.org).

Themed Issue: Drug Addiction - From Basic Research to Therapies
Guest Editors - Rao Rapaka and Wolfgang Sadée

Conformational Characteristics of the Interaction of SR141716A With the CB1 Cannabinoid Receptor as Determined Through the Use of Conformationally Constrained Analogs

Submitted: February 6, 2006; Accepted: July 17, 2006; Published: October 27, 2006

Brian F. Thomas,[1] Yanan Zhang,[1] Marcus Brackeen,[1] Kevin M. Page,[1] S. Wayne Mascarella,[1] and Herbert H. Seltzman[1]

Research Triangle Institute, Research Triangle Park, NC 27709-2194

ABSTRACT

Interest in cannabinoid pharmacology increased dramatically upon the identification of the first cannabinoid receptor (CB1) in 1998 and continues to expand as additional endocannabinoids and cannabinoid receptors are discovered. Using CB1 receptor (CB1R) systems, medicinal chemistry programs began screening libraries searching for cannabinoid ligands, ultimately leading to the discovery of the first potent cannabinoid receptor antagonist, SR141716A (Rimonabant). Its demonstrated efficacy in treating obesity and facilitating smoking cessation, among other impressive pharmacological activities, has furthered the interest in cannabinoid receptor antagonists as therapeutics, such that the number of patents and publications covering this class of compounds continues to grow at an impressive rate. At this time, medicinal chemistry approaches including combinatorial chemistry, conformational constraint, and scaffold hopping are continuing to generate a large number of cannabinoid antagonists. These molecules provide an opportunity to gain insight into the 3-dimensional structure-activity relationships that appear crucial for CB1R-ligand interaction. In particular, studies in which conformational constraints have been imposed on the various pyrazole ring substituents of SR141716A provide a direct opportunity to characterize changes in conformation/conformational freedom within a single class of compounds. While relatively few conformationally constrained molecules have been synthesized to date, the structure-activity information is often more readily interpreted than in studies where entire substituents are replaced. Thus, it is the focus of this mini-review to examine the structural properties of SR141716A, and to use conformationally constrained molecules to illustrate the importance of conformation and conformational freedom to CB1R affinity, selectivity, and efficacy.

Corresponding Author: Brian F. Thomas, Research Triangle Institute, PO Box 12194, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194. Tel: (919) 541-6552; Fax: (919) 541-6499; E-mail: bft@rti.org

KEYWORDS: cannabinoid receptor, CB1, antagonists, SR141716A, structure-activity relationships, conformational analysis, molecular modeling

INTRODUCTION

With the discovery of the diarylpyrazole CB1 receptor (CB1R) antagonist/inverse agonist SR141716A (Rimonabant or Accomplia), researchers obtained a long-awaited and highly desirable molecular tool with which to further explore cannabinoid receptor function and signal transduction mechanisms. The remarkable phase 3 studies with SR141716A, demonstrating efficacy in both smoking cessation and obesity trials in humans, has continued to heighten interest in this class of compounds.[1] Several hundred analogs of SR141716A have since been designed, synthesized, and tested in a plethora of pharmacological assays, and compounds are continuing to be described with improved affinities, varying efficacies, differing receptor selectivities or unique pharmacological properties and clinical applications (see recent reviews by Muccioli and Lambert,[2] Padgett,[3] and Lange and Kruse[4]). These studies have helped characterize the conformational properties of SR141716A and the structure-activity relationships of cannabinoid antagonists and inverse agonists. Of particular relevance to the determination of the conformational requirements for receptor binding, receptor selectivity, and efficacy are studies where conformational constraints have been imposed on the various pyrazole ring substituents of SR141716A. Such studies can often define key conformational properties and relate changes in conformation/conformational freedom to ligand-receptor binding and G-protein coupling.

There are 4 torsion angles of rotation in SR141716A that can represent most of its key conformational characteristics (Figure 1). Using quenched molecular dynamics simulations, it was possible to discern and compare the variety of conformational energies allowed by the structure of the molecule. For example, Figure 2 shows that for the torsion τ_1, the molecule is confined energetically to an s-trans configuration of the carboxamide oxygen group as it connects to the pyrazole. While other conformational minima are possible,

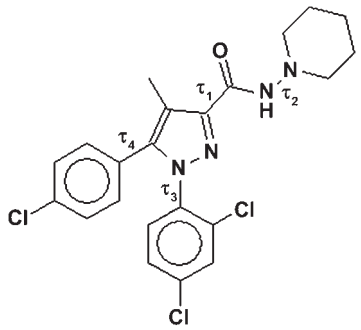

Figure 1. Structure of the first potent CB1 cannabinoid receptor antagonist, SR141716A. The torsion angles of interest are labeled τ_1-τ_4.

they are of considerably higher energy [~40 kJ/mol]. Torsion τ_2 describes the orientation of the amino-piperidine ring and reveals that 4 energetically preferred geometries exist, with torsion values of ~60º, 120º, 240º, and 300º. With these conformations, the energy differences are such that all 4 conformations are equally likely, but the symmetry of the piperidine ring makes the 60º and 240º, and the 120º and 300º conformations, identical. Despite this symmetry, this torsion angle, combined with the flexibility of the piperidine ring substituent, represents one of the areas of greatest conformational freedom for this molecule. The other 2 ring substituents are rigid aromatic ring systems, with each aryl substituent having 4 low energy conformations: torsion τ_3's energy minima are at ~40º, 140º, 220º, and 320º; τ_4's energy minima are at ~60º, 120º, 240º, and 300º. Because of the symmetry of the p-chloro ring system, the τ_4 torsion angle at 60º is equivalent to 240º, and 120º is the equivalent of 300º. These 2 torsion angles, τ_3 and τ_4, are "cogged," such that if τ_4 is at 60º/240º, τ_3 falls into energy minima at 40º or 220º, but if τ_4 is at 120º/300º, τ_3 has its energy minima at 140º and 320º. These interdependent conformational characteristics are quite apparent from the molecular dynamics simulations, and the energy plot obtained through a grid search of these 2 torsion angles (Figure 3). In the 3-dimensional plot, the 2 torsions have energetically unfavorable interactions when the torsion angles result in coplanar ring systems with one another (any combination of 0º or 180º). Of interest, τ_4 appears to be the most energetically constrained system, despite its lacking the larger chlorine substituent in the ortho position as in the τ_3 system, pointing to a steric effect of the lone methyl group substituent on the pyrazole ring.

Using x-ray crystallography, s-trans geometry of τ_1 was observed in SR141716A crystals (telephone conversation from Clifford George, June, 2004),[5] as it was in crystals of an aryl ring constrained analog of SR141716A synthesized in our laboratory.[6] The importance of the carboxyhydrazine heteroatoms and the trans configuration of τ_1 in SR141716A for recognition and inverse agonist activity has been investigated by Reggio and colleagues through the use of molecular modeling, conformationally restricted analogs, and site-directed mutagenesis.[5] Molecular modeling and receptor docking studies suggested a critical interaction with lysine K3.28(192) and this substituent. Based on this hypothesis, synthesis of a vinyl-cyclohexyl analog and 4 additional compounds differing in the presence, the orientation, or both of potential hydrogen-bond forming heteroatoms was performed. In studies using wild-type receptors, the binding affinity of the vinyl-cyclohexyl SR141716 analog (VCHSR) was reduced as compared with SR141716A. A similar decrease in binding affinity was observed with SR141716A when the lysine K3.28 was mutated to a nonhydrogen bonding residue (K3.28A) in the CB1R. However, an additive effect (or greater) was not observed when the VCHSR analog was tested in the K.328A mutation, consistent with a single (H-bonding) interaction between the carboxyhydrazine and the K3.28(192) residue. Although their modeling results suggest that it is the carboxamide oxygen of SR141716A that interacts with K3.28(192), the mutant cycle calculations could not identify the specific K3.28(192) hydrogen bonding site within the C3 substituent of SR141716A. It does seem apparent that the piperidine nitrogen is not necessary for high affinity binding, because the cyclohexyl analog has been shown to possess affinity similar to SR141716A. The authors further hypothesized that hydrogen bonding of the SR141716A C3 substituent with K3.28 is responsible for its higher affinity for the inactive receptor state, leading to its inverse agonism. Consistent with this hypothesis, VCHSR acted as a neutral antagonist at the wild-type CB1R. Additional conformational constraint and structural modifications of this region were recently reported.[7] Of interest, these molecules constrained the carboxyamide oxygen atom in the s-cis position and still retained reasonable affinities. The most analogous molecule to SR141716A, compound 2b (Figure 4), which has only hydrogen bond accepting capability, exhibited surprisingly high affinities despite the poor overlap of the piperidinyl groups in the low-energy conformer of SR141716. These results suggest that this analog may bind differently than SR141716 in hCB1-R, with the piperidinyl group in 2b occupying a separate hydrophobic pocket, distinct from that for the 1-piperidinyl group in SR141716, and the hydrogen bond accepting groups forming different networks of hydrogen bond interactions. Alternatively, the conformationally constrained molecules may induce conformational changes in the receptor to accommodate the piperidinyl rings in the same hydrophobic pocket as well as allow alternative hydrogen bond formation.

Ring constraint of torsion τ_2 has not been fully characterized. Instead, investigators have often elected to replace the piperidinyl ring system entirely. Even though the plane of symmetry in the piperidine ring simplifies the system, it is relatively flexible and can occupy an extended area of space

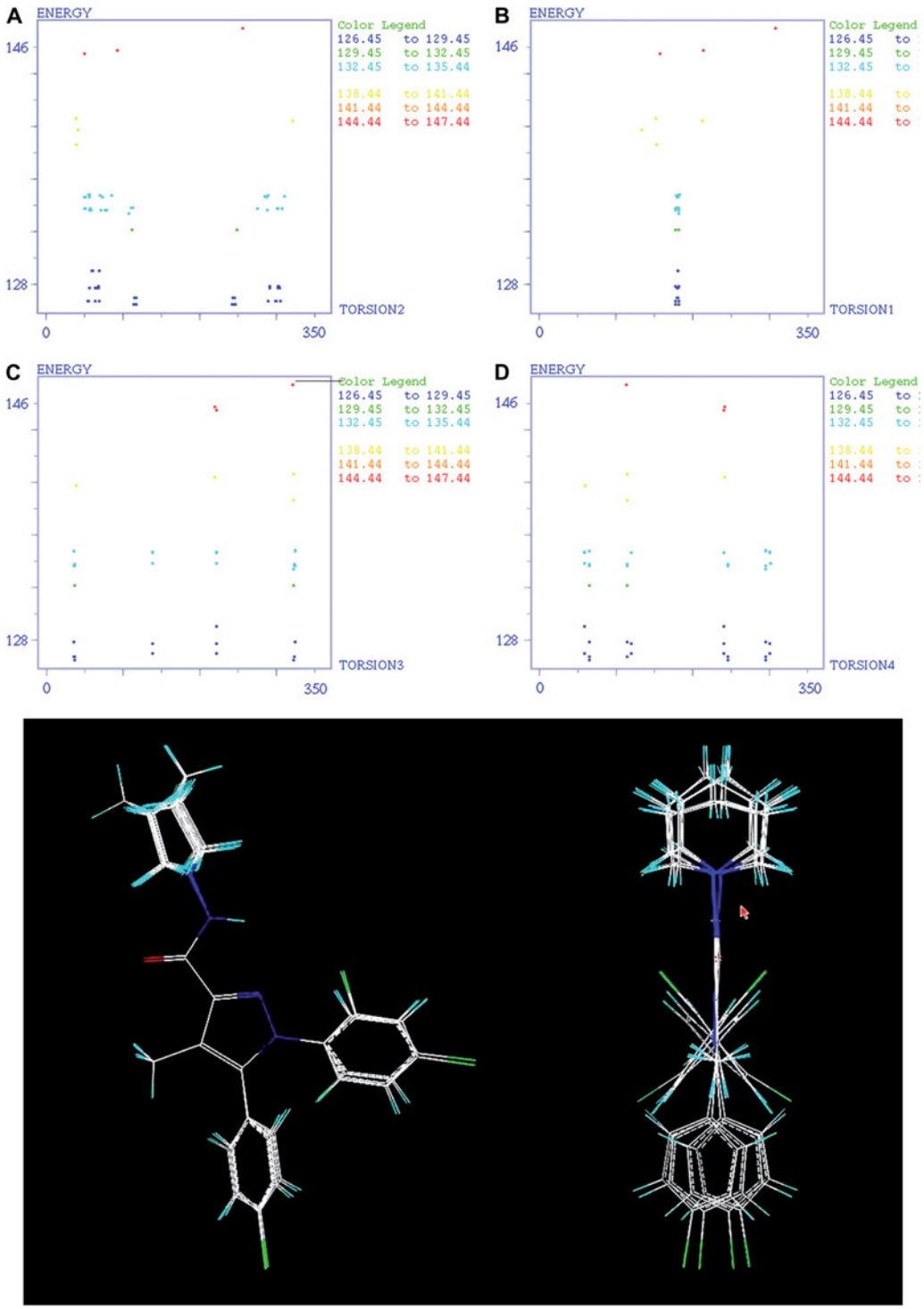

Figure 2. (Top) Results of quenched molecular dynamics analysis of SR141716A plotted as graphs of conformational energy and torsion angle (τ_1, (B); τ_2, (A); τ_3, (C); and τ_4, (D)). (Bottom) Molecular dynamics were run up to 2000ºK and snapshot conformations quenched using the MMFF94 force field within SYBYL (Tripos, St Louis, MO). This approach involves molecular dynamics simulations on each energy-minimized analog at 2000°K. During the molecular dynamics simulation, the molecule was heated from 0°K to 2000°K at 100°K steps lasting 10 picoseconds each, with snapshot conformations taken at 10-ps intervals. Upon reaching 2000°K, the molecule was held at this temperature for 1000 ps, while additional snapshots were acquired at 10-ps intervals. Each of the snapshot conformations obtained for a particular analog was energy minimized again using a conjugate gradient of 0.01 kcal/mol or a maximum of 100 000 iterations as termination criteria, yielding a group of 139 energy-minimized conformers per compound. After these simulations had been performed, all conformations from each of the analogues were overlaid using a single template molecule. Because the pyrazole ring system of SR141716 had a reasonably close corresponding central ring system in most of the analogues, the pyrazole ring atoms were used for atom-by-atom root mean square distance minimization. This alignment positioned all of the molecules in the same 3-dimensional space and superposed the central ring systems to as great an extent as possible.

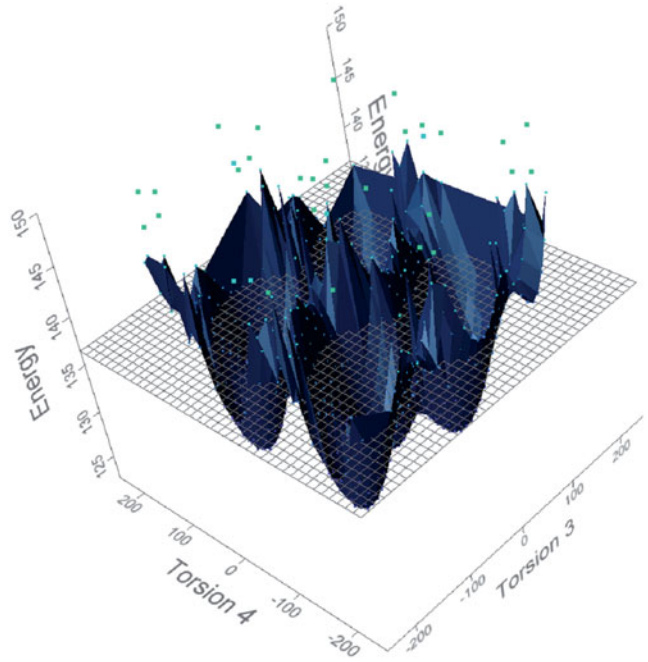

Figure 3. Conformational energy profile for rotations about τ_3 and τ_4 of SR141716A. Energy minimization of the various permutations of 10º increments of τ_3 and τ_4 performed in SPARTAN using the MMFF94 force field (Wave Function).

through several conformational minima. Thus, it remains to be determined if there is a benefit to be gained by reducing the conformational mobility of this particular substituent, an approach that might be considered promising, because modification of this system has been shown to produce large changes in affinity as well alter receptor selectivity between CB1 and CB2.[6,8-11]

While τ_3 has 4 low energy torsion angles, and τ_4 has only 2 due to symmetry, these ring systems show coordinated movement such that modifications of the conformational freedom of one system typically have some implicit or quantifiable effect on the other. In addition, because of the symmetry of the molecule, several low energy nonsuperimposable mirror image conformations are possible, and these are not necessarily equivalent with regard to biological activity. It is possible that the chiral environment of the receptor binding site may preferentially permit high affinity interactions with only one of the mirror image conformations. Thus, constraint of these 2 aryl ring systems would be expected to provide additional information as to the nature of the interaction of these rings with the CB1R. Coplanarity of the diaryl ring systems can be forced via the structure shown in Figure 5. This molecule was synthesized in our laboratory via a photocyclization reaction.[6] Its reduced affinity suggests that coplanarity is not an optimal configuration of the systems for interaction with CB1R, and/or that the o-chlorine contributes to the high affinity of SR141716A. An alternative approach to constrain τ_4 involved fusing the central pyrazole group of SR141716A with its 5-(4-chlorophenyl) to form a central indazole ring (Figure 6).[12] However, this molecule does not constrain the overall geometry or conformation of the monochloro ring to a position that closely approximates that occupied by the same ring in SR141716A; thus its relatively low affinity (~500 nM) cannot be solely attributed to constraining the ring in the plane of the pyrazole. It would be interesting to examine additional analogs, such as the one proposed in Figure 6, to better approximate the structure of SR141716A. The Stoit,[13] Mussinu,[11] and Murineddu[14,15] research groups used carbon bridges to reduce the conformational mobility of τ_4 (Figure 7, Table 1). Initially, Stoit et al[13] reported the 3 carbon-bridged compound and found that it had lower affinity for the CB1R than SR141716A. However, Pinna and colleagues[11,13,14] varied the bridge length from 1 carbon to 3 carbons and reported that the 3 carbon bridged compound, NESS-0327, had fentomolar (fM) affinity as compared with the nanomolar (nM) affinity of SR141716A. Despite the large discrepancy between affinities, each group ascribed the changes in affinity to the conformational differences of the molecules as compared with those of SR141716A. Thus, in this particular instance, any interpretation must be tempered by the need to establish a more robust, reproducible estimation of the affinity and activity of these compounds, particularly the 3 carbon-bridged

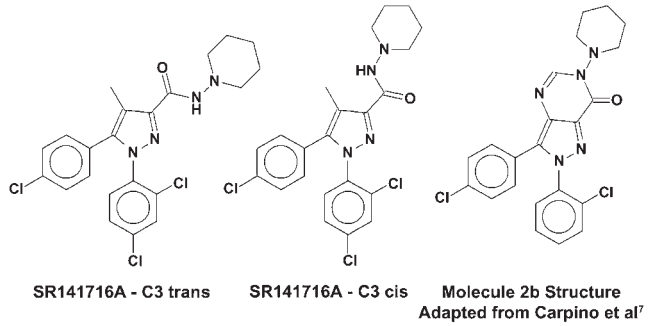

Figure 4. S-trans (left) and s-cis (center) conformations of SR141716A and molecule 2b structure adapted from Carpino et al[7] (right).

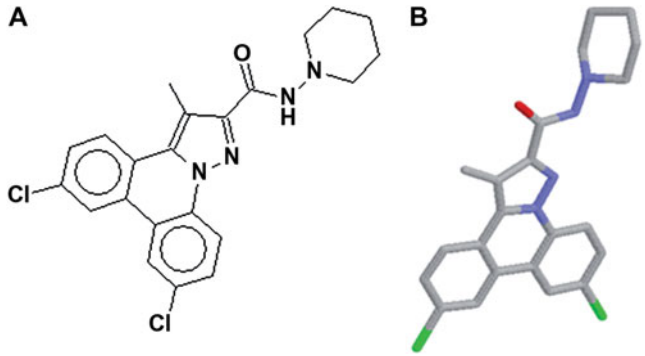

Figure 5. Structure (A) and x-ray crystal structure (B) of pyrazole [1,5 f] phenanthridine analog adapted from Francisco et al.[6] Note the x-ray shows the transconfiguration of τ_1.

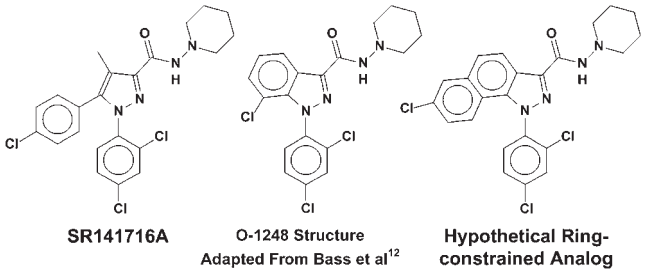

Figure 6. Structure of SR141716A (left), O-1248 structure adapted from Bass et al[12] (center), and a hypothetical ring-constrained molecule (right).

Table 1. Affinities of tricyclic pyrazole analogs for CB1 and CB2 receptors

X	K_i (CB1)	K_i (CB2)	Reference
1	2050 ± 90 nM	0.34 ± 0.06 nM	14
2	14.8 ± 0.43 nM	227 ± 5 nM	14
3	0.00035 ± 0.000005 nM	21 ± 0.5 nM	14
3	126 nM		13

*K_i indicates the concentration of the competing ligand that binds to half the binding sites at equilibrium.

molecule. It is interesting to note that the research reported by Stoit et al[13] included in vivo administration of the 3 carbon-bridged compound by intraperitoneal (ip) and oral (po) routes, and the authors reported that no activity was detected, which would be more consistent with a compound that had decreased affinity as compared with SR141716A. However, it is not clear in their report what measures of pharmacological activity were measured, and as the authors pointed out, the bioavailability of the compounds could be quite different. Regardless of these difficulties, the conformational considerations of these bridged compounds are quite interesting.

As shown in Figure 8, with 1 carbon atom as the bridge, the τ_4 torsion angle is drastically reduced in its range of motion, remaining almost coplanar with the pyrazole ring system. There is little range for torsional changes of τ_4, and the energy minima on each side are equivalent. Of interest, the one carbon-bridged molecule appears to pull the *p*-chloro-substituted aryl ring away from the dichloro aryl ring system, allowing free rotation across the entire 360 range of τ_3. The energy manifold shows a small energy barrier resulting from proton-proton steric interactions, and a somewhat larger energy barrier resulting from proton-chlorine interactions; however, these energy barriers are below 40 kJ/mol. Increasing the bridging carbon atoms to 2 appears to push the aryl ring systems closer together, making it energetically more difficult for τ_3 to rotate through its entire range. While it is clear that the energy barrier increases from the 1 to the 2 carbon-bridged compounds, particularly as the *o*-chlorine tries to pass by the *p*-chloro-substituted ring system, there is still a clear path for this transition that is below 40 kJ/mol. Finally, the barrier grows even further when the 3 carbon bridge is in place. Even here, however, there appears to be a path for transition (saddle point) at ~40 kJ/mol, so that τ_3 is in equilibrium across the full range of rotation at room temperature.

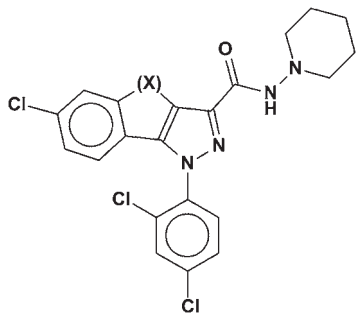

Figure 7. Tricyclic pyrazole analogs.

The effects on affinity and potency observed with these bridged compounds deserve further mention. First, the 1 carbon-bridged compound has significantly lower affinity (equilibrium dissociation constant (K_i) of ~2000 nM) than SR141716A (K_i of ~1 nM), or when the aryl rings are fused as in the pyrazole [1,5 f] phenanthridine analog (K_i ~50 nM).[6] Thus, constraining the rotation of τ_4 with the 1 carbon bridge, so that the ring is in the plane of the pyrazole ring system, is detrimental to binding affinity. However, if the constraint of τ_4 is done by forming the pyrazole [1,5 f] phenanthridine analog, the additional constraint of τ_3 appears to attenuate the decrease in affinity seen with the 1 carbon-bridged molecule. Second, in the 2 carbon-bridged compound, τ_4 has somewhat greater flexibility and deviates from coplanarity with the pyrazole ring, and τ_3 can still freely rotate at room temperature; the affinity of this compound is intermediate (~14 nM). Finally, in the 3 carbon-bridged molecule, τ_3 can obtain positions similar to those available for SR141716A, and τ_4 can access all of the energy minima at torsions also in close proximity to the minimum energy torsion angles for SR141716A. Thus, based on similarity of conformational shape to SR141716A, it might be expected that the 3 carbon-bridged analog would have similar affinity to SR141716A. However, if the conformational constraint restrains the ring system to a bioactive conformation around τ_4, a more potent compound would be anticipated. Alternatively, a less potent compound would be anticipated if τ_4 and τ_3 were being constrained to angles outside of the bioactive conformation, which would still be quite energetically accessible with SR141716A's free rotation of τ_4. Because the barrier for free rotation does not exceed 40 kJ/mol, a compound of roughly the same affinity might be expected. However, the fact that 2 laboratories

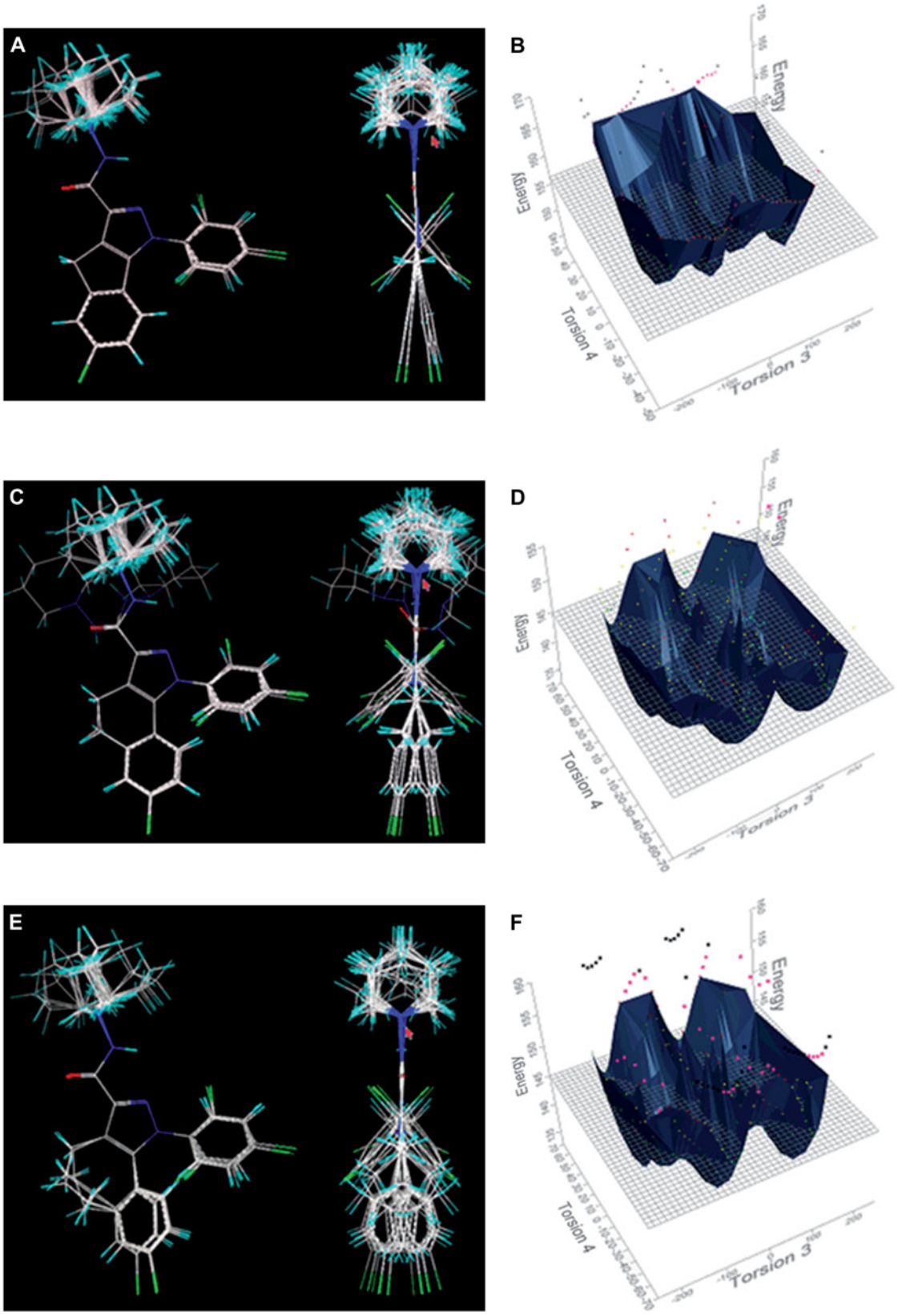

Figure 8. Orthographic views of the minimum energy conformations (A,C,E) and energy surface for the rotation of τ_3 and τ_4 (B,D,F) for 1 (A,B), 2 (C,D), and 3 (E,F) carbon-bridged analogs reported by Stoit et al[13] and Murineddu et al.[14]

have reported such divergent values for the affinity of the 3 carbon-bridged molecules complicates our ability to make firm conclusions on what is the bioactive conformation of SR141716A.

Conclusion

The conformational properties of SR141716A are readily modeled and have been further characterized by the use of conformational constraint. However, the degree of conformational constraint that has been described to date has not been extensive. Some studies have indicated that the s-trans configuration of the amino-piperidine ring substituent is important in hydrogen bonding with the cannabinoid receptor, while others have illustrated the importance of the orientation of the aryl ring systems. Still, while some systematic changes in the conformation of the monochloro ring system have been imposed and tested, very little is known regarding the conformational considerations involved in the interaction of the dichloro ring system within the cannabinoid receptor binding site(s). It remains to be determined if molecules can continue to be synthesized that impose unique conformational constraints, that when tested, can further define the conformational requirements for optimal interaction with the cannabinoid receptor.

Acknowledgments

Research funding for this study was provided by a grant from the National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD (grant No. DA019217).

References

1. Cleland JG, Ghosh J, Freemantle N, et al. Clinical trials update and cumulative meta-analyses from the American College of Cardiology: WATCH, SCD-HeFT, DINAMIT, CASINO, INSPIRE, STRATUS-US, RIO-Lipids and cardiac resynchronisation therapy in heart failure. *Eur J Heart Fail*. 2004;6:501-508.

2. Muccioli GG, Lambert DM. Current knowledge on the antagonists and inverse agonists of cannabinoid receptors. *Curr Med Chem*. 2005;12:1361-1394.

3. Padgett LW. Recent developments in cannabinoid ligands. *Life Sci*. 2005;77:1767-1798.

4. Lange JH, Kruse CG. Keynote review: medicinal chemistry strategies to CB1 cannabinoid receptor antagonists. *Drug Discov Today*. 2005;10:693-702.

5. Hurst DP, Lynch DL, Barnett-Norris J, et al. N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) interaction with LYS 3.28(192) is crucial for its inverse agonism at the cannabinoid CB1 receptor. *Mol Pharmacol*. 2002;62:1274-1287.

6. Francisco ME, Seltzman HH, Gilliam AF, et al. Synthesis and structure-activity relationships of amide and hydrazide analogues of the cannabinoid CB(1) receptor antagonist N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716). *J Med Chem*. 2002;45:2708-2719.

7. Carpino PA, Griffith DA, Sakya S, et al. New bicyclic cannabinoid receptor-1 (CB(1)-R) antagonists. *Bioorg Med Chem Lett*. 2006;16:731-736.

8. Rinaldi-Carmona M, Barth F, Millan J, et al. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther*. 1998;284:644-650.

9. Wiley JL, Jefferson RG, Grier MC, Mahadevan A, Razdan RK, Martin BR. Novel pyrazole cannabinoids: insights into CB(1) receptor recognition and activation. *J Pharmacol Exp Ther*. 2001;296:1013-1022.

10. Thomas BF, Francisco ME, Seltzman HH, et al. Synthesis of long-chain amide analogs of the cannabinoid CB1 receptor antagonist N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyra zole-3-carboxamide (SR141716) with unique binding selectivities and pharmacological activities. *Bioorg Med Chem*. 2005;13:5463-5474.

11. Mussinu JM, Ruiu S, Mule AC, et al. Tricyclic pyrazoles. 1. Synthesis and biological evaluation of novel 1,4-dihydroindeno[1,2-c]pyrazol-based ligands for CB1 and CB2 cannabinoid receptors. *Bioorg Med Chem*. 2003;11:251-263.

12. Bass CE, Griffin G, Grier M, Mahadevan A, Razdan RK, Martin BR. SR-141716A-induced stimulation of locomotor activity: a structure-activity relationship study. *Pharmacol Biochem Behav*. 2002;74:31-40.

13. Stoit AR, Lange JH, Hartog AP, et al. Design, synthesis and biological activity of rigid cannabinoid CB1 receptor antagonists. *Chem Pharm Bull (Tokyo)*. 2002;50:1109-1113.

14. Murineddu G, Ruiu S, Loriga G, et al. Tricyclic pyrazoles. 3. Synthesis, biological evaluation, and molecular modeling of analogues of the cannabinoid antagonist 8-chloro-1-(2′,4′-dichlorophenyl)-N-piperidin-1-yl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide. *J Med Chem*. 2005;48:7351-7362.

15. Murineddu G, Ruiu S, Mussinu JM, et al. Tricyclic pyrazoles. 2. Synthesis and biological evaluation of novel 4,5-dihydro-1H-benzo[g]indazole-based ligands for cannabinoid receptors. *Bioorg Med Chem*. 2005;13:3309-3320.