

Research article

Calculated conformer energies for organic molecules with multiple polar functionalities are method dependent: Taxol (case study)

Ami Lakdawala¹, Minmin Wang¹, Neysa Nevins¹, Dennis C Liotta¹, Danuta Rusinska-Roszak², Marek Lozynski² and James P Snyder*¹

Address: ¹Department of Chemistry, Emory University, Atlanta, Georgia 30322, USA and ²Department of Chemical Technology, Poznan University of Technology, 60-965 Poznan, Poland

E-mail: Ami Lakdawala - ami@flash.chem.emory.edu; Minmin Wang - Wang_Minmin@Lilly.com; Neysa Nevins - nevensn@etown.edu; Dennis C Liotta - diotta@emory.edu; Danuta Rusinska-Roszak - Danuta.Roszak@fct.put.poznan.pl; Marek Lozynski - Marek.Lozynski@fct.put.poznan.pl; James P Snyder* - snyder@euch4e.chem.emory.edu

*Corresponding author

Published: 1 October 2001

Received: 6 May 2001

BMC Chemical Biology 2001, 1:2

Accepted: 1 October 2001

This article is available from: <http://www.biomedcentral.com/1472-6769/1/2>

© 2001 Lakdawala et al; licensee BioMed Central Ltd. Verbatim copying and redistribution of this article are permitted in any medium for any non-commercial purpose, provided this notice is preserved along with the article's original URL. For commercial use, contact info@biomedcentral.com

Abstract

Background: Molecular mechanics (MM) and quantum chemical (QM) calculations are widely applied and powerful tools for the stereochemical and conformational investigations of molecules. The same methods have been extensively used to probe the conformational profile of Taxol (Figure 1) both in solution and at the β -tubulin protein binding site.

Results: In the present work, the relative energies of seven conformations of Taxol derived from NMR and X-ray analyses were compared with a set of widely used force fields and semiempirical MO methods coupled to a continuum solvent treatment. The procedures not only diverge significantly in their assessment of relative conformational energies, but none of them provide satisfactory agreement with experiment.

Conclusions: For Taxol, molecular mechanics and semiempirical QM methods are unable to provide a consistent energetic ranking of side-chain conformations. For similar highly polar organic structures, "energy-free" conformational search methods are advised.

Background

Conformational and structural analysis of complex organic molecules has been significantly advanced by the development of molecular mechanics schemes parameterized for a wide variety of organic functionalities. For small organic molecules, the Allinger family of programs has served the community very well for many years.[1] One widely used package that incorporates a range of force fields and features of the Allinger protocols, solvation continuum models and conformational searching options is MacroModel.[2] Two studies by Liljefors and

colleagues devoted to an evaluation of quantitative aspects of conformational analysis using the MacroModel force fields demonstrate them to perform rather well for a wide range of organic structures with few polar substituents.[3] A more recent investigation by Halgren on similar structures points out that there is still work to be done to accurately and completely map conformational energy profiles for organic molecules. [4] An area in which molecular mechanics and conformational analysis are critical is in the evaluation of drug candidates and in the molecular design of novel analogs. A case in point is

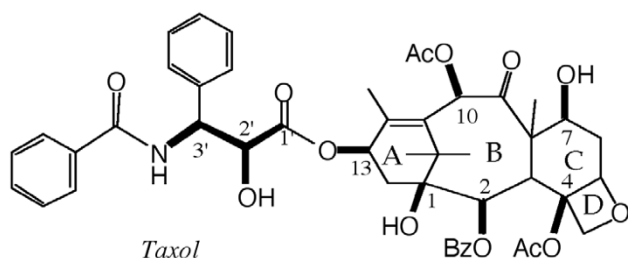


Figure 1
Topological structure of Taxol (paclitaxel).

the intense activity around Taxol (paclitaxel), one of the more clinically effective chemotherapeutic agents against a range of otherwise intractable cancers. During the past decade, numerous NMR studies in solution coupled to conformational analysis have led to suggestions that either the polar[5] or nonpolar[6] conformations represent the bioactive one. The recent electron crystallographic 3.7 Å resolution structure of $\alpha\beta$ tubulin and Taxol[7] has stimulated a range of suggestions for both the binding mode and the bioactive conformation[8] including a T-Taxol (butterfly) conformer.[9] Many of the proposals arise from molecular mechanics or constrained molecular dynamics conformational searching focused on low energy conformers of the molecule. Our long-standing interest in the binding forms of Taxol, epothilone and analogs[10] has led to a concern for the ability of current computational methodologies to accurately treat the conformational energy manifolds of such molecules either in solution or at a protein binding center.

The seven-conformer dataset

The Taxol molecule is a complex diterpenoid with a conformationally immobile core consisting of the fused A-D rings. Side chains critical for bioactivity of the molecule and analogs are those emanating from the core at C2, C4 and C13. To evaluate the energetic performance of various computational schemes, seven Taxol conformations derived from experimental data with differing torsional angles in the C13 fragment were examined. The diversity of C3' side chain orientations is illustrated in Figure 2, in which the diterpenoid core A-C rings have been superimposed, and amplified with reference to specific torsions in Table 1. The first five are those with the highest estimated populations (4 to 35%) with a ΔG range of 0.0–1.3 kcal/mol from an NMR/NAMFIS analysis in $CDCl_3$ solution. [11,12] Structure **1** corresponds to the non-polar conformation observed as the predominant species in $CDCl_3$ or CD_2Cl_2 as depicted in Figure 3. Characteristic of the form is the "hydrophobic collapse" [13] of the benzamido phenyl at C3' and the benzoyl phenyl at C2. The point is illustrated by the short 5.4 Å distance between

the centers of the corresponding phenyl rings. Conformer **2** experiences a similar collapse with a somewhat shorter ring-to-ring distance derived from an alternative set of torsions along the C13 side chain (Table 1). With respect to the close approach between pendant hydrophobic centers, **1** and **2** resemble the semisynthetic analog of Taxol, Taxotere (docitaxel). The latter compound differs constitutionally from Taxol in that the C10 acetate becomes an OH and the $NHC(=O)Ph$ benzamido group is replaced by $NHC(=O)O-t-Bu$. The single crystal X-ray structure[14] and the 2-D NMR ($CDCl_3$) [6d] of Taxotere likewise demonstrate hydrophobic association, in this case between the *tert*-butyl and the C2 phenyl group. Structure **5** is the polar form frequently found in $DMSO-d_6/D_2O$. It too exhibits hydrophobic collapse, but between the Taxol phenyl ring attached directly to C3' and the C2-benzoyl ring (Figure 2). Isomers **3** and **4** are extended rotamers in which the C2 benzoyl phenyl ring is distant from both C3' terminal rings. Rotamer **4** corresponds to the recently proposed bioactive conformation of Taxol bound to β -tubulin.[9] The perspective given as **4'** illustrates the "T" relationship between the three phenyl rings of the molecule (Figure 2). The final two Taxol conformers included in our dataset, **6** and **7**, appear together in the unit cell of an X-ray crystal structure determination.[15] The latter is a C13 side chain extended structure, while hydrophobically collapsed **6** is very similar to **5**. Table 1 complements Figure 3 in providing a selected set of dihedral angles to illustrate explicitly the conformational variation among the seven Taxol torsional isomers.

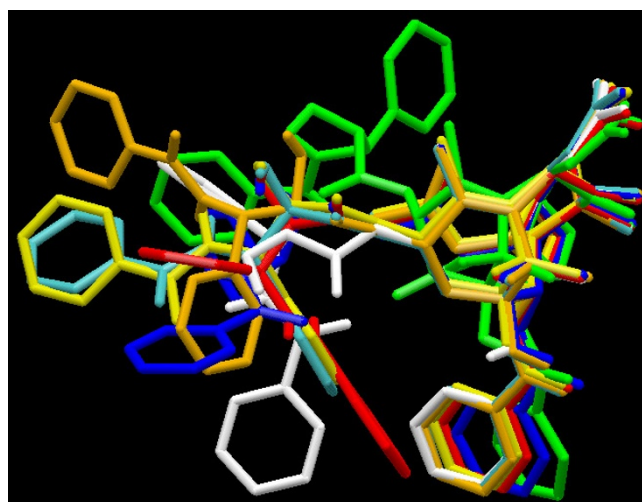


Figure 2

Seven conformations of Taxol superimposed within the diterpenoid core shown at the right. The diversity of C13 side-chain orientations with respect to the torsionally rigid A-C ring core is illustrated at the left.

Results

Five conclusions are immediately evident from Tables 2,3,4. 1) For a given medium (gas, CHCl₃, H₂O), there is no consistency in energy ranking within the molecular mechanics methods. MM3(96) and MM3 (2000), performing similar to MM3*, offer no exceptions. 2) None of the force-field/ solvation models posit non-polar **1** to be favored in CHCl₃, while only MM3* predicts polar **5** and **6** to be most stable in the water continuum model (Table 2). 3) About half of the force field protocols in Table 2 suggest the extended (uncollapsed) conformer **4** to be lowest in energy. 4) Use of 6-31G*-quality ESP charges as recommended for the continuum solvation models

[2b,16] leads to conformer **2**, a non-polar type conformer distinct from **1**, as the uniform global minimum for both gas phase and solution models (Table 3). The next lowest conformer is predicted to be 15–25 kcal/mol higher in energy, while overall conformer ranking between the different force fields generally inharmonious. Thus, incorporation of ESP charges amplifies and alters the energy rankings. 5) AM1 and PM3 calculations in the gas phase vary in suggesting **2**, **6** and **7** as most stable (Table 4). While H₂O solvation correctly identifies polar **6**, the CHCl₃ model also favors either **6** or extended **7** rather than **1** or **2**.

Table 1: C13 side chain dihedral angles for Taxol conformations 1–7 used as starting points for the optimization results recorded in Tables 2-5, deg.

	C12-C13-O-C	C13-O-C1'-C2'	O-C1'-C2'-C3'	C1'-C2'-C3'-N	C2'-C3'-N-C	C1-C2-O-C(O)
1	-155	175	76	73	-89	-98
2	-100	-160	105	-48	-65	-88
3	-122	154	78	93	-149	-88
4	-100	-170	73	79	-89	-89
5	-99	-167	94	164	-168	-89
6 ^a	-101	-177	103	179	-155	-86
7 ^a	-104	180	159	176	-117	-86

^a Taxol conformations determined in the solid state;¹⁵ optimized using AMBER* with all non-terpenoid core dihedral angles frozen; **6** polar; **7** extended.

Table 2: NMR/NAMFIS and X-ray structure determined conformations of Taxol evaluated energetically by six force fields in the gas phase and two solvation continuum models; Relative energies, kcal/mol.a

	MMFF			AMBER*			MM2*			MM3*			MM3(96)	MM3(2000)
	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	Gas
1	0.0	4.2	1.1	4.1	4.4	4.5	4.8	3.1	0.0	5.9	2.0	1.6	4.2	3.5
2	5.8	8.9	7.5	4.0	8.1	8.0	6.6	2.4	5.4	0.0	0.0	2.5	0.0	0.0
3	1.5	6.6	6.6	8.1	2.0	0.0	8.8	6.0	4.7	10.4	9.1	6.1	16.0	15.2
4	0.0	0.0	0.0	2.5	0.0	3.9	0.0	0.0	5.8	2.1	0.6	2.4	4.9	3.6
5	3.6	6.0	1.7	1.2	0.1	0.5	3.5	0.6	3.9	3.3	1.4	0.0	1.5	0.9
6 ^b	2.5	6.9	6.0	0.0	1.4	1.5	4.8	1.9	6.2	5.6	2.7	1.4	1.8	1.2
7 ^b	2.1	5.6	6.3	5.4	5.6	7.3	11.3	5.0	11.9	12.8	8.5	8.3	4.3	6.8

^a Each structure was optimized with the indicated force field and the accompanying GBSA solvation model. [^{2b}] ^b Taxol conformations determined in the solid state;¹⁵ optimized using AMBER* with all non-terpenoid core dihedral angles frozen; **6** polar; **7** extended.

Table 3: NMR/NAMFIS and X-ray structure determined conformations of Taxol evaluated energetically by four force fields in the gas phase, two solvation continuum models with the use of scaled ESP atomic charges; Relative energies, kcal/mol.a

	MMFF			AMBER*			MM2*			MM3*		
	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O
1	60.0	57.8	57.5	76.0	74.8	88.5	47.8	43.2	40.1	46.3	41.9	41.9
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	66.1	62.6	61.5	81.6	80.9	86.6	39.2	29.7	25.8	33.2	30.1	30.1
4	22.6	18.0	24.9	21.7	21.5	24.5	24.3	15.2	21.7	28.1	16.4	16.4
5	36.5	31.6	35.3	33.4	30.9	37.8	33.0	28.9	28.5	32.0	27.4	27.4
6 ^b	23.7	23.0	28.1	20.0	24.9	27.2	30.0	22.6	24.6	28.6	22.9	22.9
7 ^b	50.2	49.9	60.6	38.8	44.2	53.6	72.4	63.2	72.3	81.3	68.0	68.0

^a Each structure was optimized with the indicated force field and the accompanying GBSA solvation model. [2b] ^b Taxol conformations determined in the solid state; ¹⁵ optimized using AMBER* with all non-terpenoid core dihedral angles frozen; **6** polar; **7** extended.

Table 4: NMR/NAMFIS and X-ray structure determined conformations of Taxol evaluated energetically by semiempirical methods; Relative energies, kcal/mol

	AM1 ^a						PM3 ^a					
	AM1//AMBER*			AM1//AM1			PM3//AMBER*			PM3//PM3		
	gas	CHCl ₃	H ₂ O	gas	CHCl ₃	H ₂ O	gas	CHCl ₃	H ₂ O	gas	CHCl ₃	H ₂ O
1	3.7	4.0	5.3	4.0	2.0	2.5	4.6	5.8	7.6	0.2	3.8	2.1
2	1.8	4.7	5.1	0.0	0.8	0.2	2.4	6.6	7.7	0.3	6.9	4.3
3	9.3	9.4	11.5	6.7	4.7	5.9	8.5	9.2	11.5	2.6	5.7	4.4
4	1.7	1.4	4.7	0.4	2.1	0.3	2.8	2.8	6.1	2.0	4.4	4.1
5	1.4	1.9	2.2	4.1	2.5	1.8	1.3	2.1	2.4	1.4	4.6	1.3
6	0.0	0.0	0.0	3.1	0.9	0.0	0.0	0.0	0.0	1.2	3.7	0.0
7	2.8	0.3	4.3	4.7	0.0	3.2	3.8	1.4	5.4	0.0	0.0	0.4

^a AMSOL, PM3/SM5.4a [18] Solvation energies calculated at AMBER* geometries.

Discussion

Electrostatic interactions dominate and differ across methods

For Taxol, application of commonly applied force fields as well as the semiempirical methods AM1 and PM3 results in an ordering of conformational energies that is method dependent and mostly inconsistent with experimental data. In an attempt to understand this behavior within the force field framework, we examined the various energy contributions for each conformation and method. In most cases the overwhelming factor is the electrostatic term, a component that is seriously amplified by incorporating ESP charges. The point is likewise

illustrated by damping rather than magnifying intramolecular electrostatics. Taxol includes ten polar functionalities: five 3-atom units (four esters, one amide) and five 1- or 2-atom units (three OHs, one ether, one C9 carbonyl). In one set of calculations, the latter five groups were converted to the hydrocarbon analogs (CH₃, CH₂ and C=CH₂, respectively). The optimized structures (Table 5) are very similar to those cited in Tables 2 and 3, but the individual global minima are shifted to different conformers. When the five C(=O)-X units are converted to trans butene moieties, now removing all heteroatoms and most of the electrostatics in the molecule, the energy minima shift once again and the average energy spread

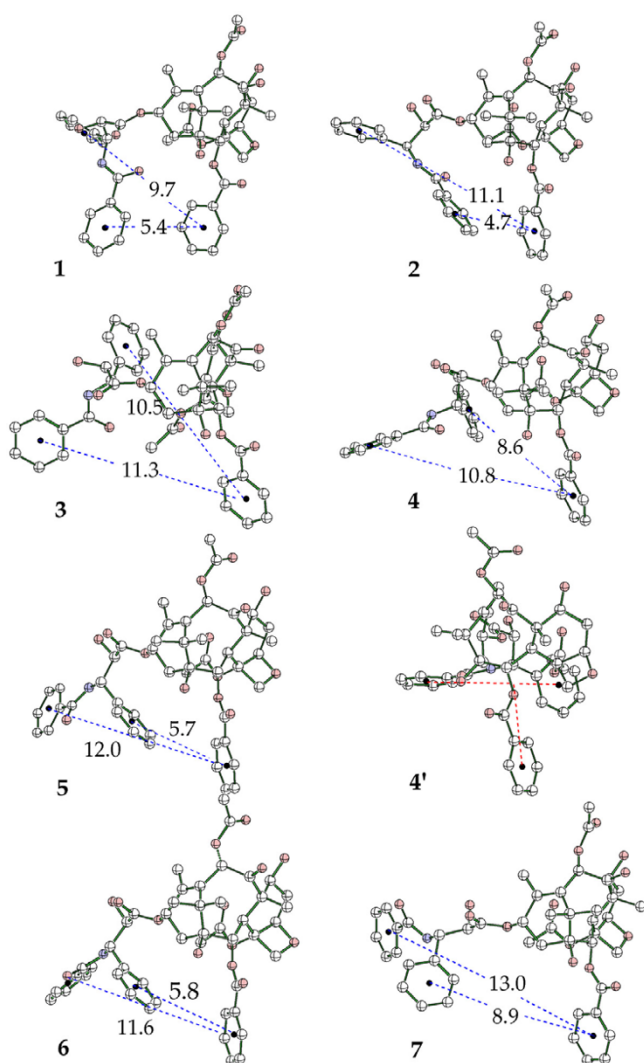


Figure 3

Seven conformations of Taxol showing distances (Å) between the centroids of the C3' phenyl rings and the C2 terminus. The T-Taxol or butterfly conformation (**4** and **4'**) is pictured in two views; **4'** illustrates the "T" relationship among the three aromatic rings.

between high and low unconstrained energy conformations (i.e. **1-5**) diminishes to an average of 2.8 kcal/mol (Table 5). The latter is to be compared with an average of 7.5 and 57.6 kcal/mol for the fully optimized structures of Tables 2 and 3, respectively.

The molecular mechanics results might be ascribed to the presence of "low quality"[17] parameters in the force fields employed. Indeed MM3* and MMFF involve 74 and 62 such interactions out of a total of 439 and 555 (17 and 11%), respectively. However, MM2* and AMBER* incorporate only 2 and 6% such parameters, respectively, yet their ability to accurately predict the purported ex-

perimental forms is not improved. The two semiempirical methods each locate 2–6 conformations with energies no more than 2.5 kcal/mol above the lowest, but none of the calculations selects the same set of low energy conformers.

Modern force fields with stretch, bend, torsional, hydrogen bond and cross-terms are parameterized primarily within three-bond units (A-B-X-Y). Of the two additional key terms, the van der Waals component operates with a very short range force and essentially prevents atom-atom interpenetration. In the absence of damping, the single long-range interaction that persists from one end of the molecule to the other is the electrostatic contribution. While individual bond dipoles are characteristic of the Allinger MM family of force fields, other molecular mechanics methods employ atomic charges. In either case, no general scheme for parameterizing the electrostatic interaction among multiple polar groups in three-space in terms of conformational energies has yet emerged.

Similarly, within a quantum chemical semiempirical framework, charge polarization notwithstanding, conformational energies are sensitive to method and charge distribution. Inspection of Table 4 reveals that, unlike the force-field results described in Tables 2 and 3, all four semiempirical recipes predict polar conformers **5** and **6** to be the low and "global" minimum energy forms, respectively, when the aqueous solvation continuum model is employed. The one exception, AM1//AM1 suggests both polar and nonpolar forms **6** and **2** to be equally populated. This limited success is negated, however, by the incorrect prediction that the same polar species are also the low energy forms in the gas phase and in the CHCl₃ solvation model. In no instance do the semiempirical calculations predict either nonpolar forms **1** or **2** to be dominant in CHCl₃. As mentioned above, the AM1//AM1 calculations posit equal populations for **2** and **6** within the latter regime, but they simultaneously relegate nonpolar **1** to a higher energy. The remaining semiempirical methods predict the empirically verified nonpolar conformers **1** and **2** to be unobservable in the chlorocarbon solvent with energies ranging from 3.8 to 6.9 kcal/mol above the polar collapsed conformation. It might be concluded that the water model is robust, while the chloroform model is ill-parameterized, although the AMSOL literature on solvation[18] gives little basis for this contention. Alternatively, the few inerrant predictions for **6**, combining both structure and continuum aqueous solvation energies, might well be fortuitous. The likelihood that this is correct is accentuated by the fact that Taxol conformational analysis by NMR has never been performed in pure water, but always as a mixture of DMSO-d₆ and D₂O.[5] In addition, in anhydrous methanol, a

Table 5: MM2* energetics of Taxol conformations denuded of polar groups; Relative energies; kcal/mol.a

Conf	Taxol ^b			Taxol-HCl ^c			Taxol-HC2 ^d			Taxol-HC3 ^e		
	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O	Gas	CHCl ₃	H ₂ O
1	4.8	3.1	0.0	0.1	2.1	0.0	0.5	0.2	0.0	1.4	0.0	1.3
2	6.6	2.4	5.4	0.0	1.2	1.6	1.3	2.6	3.4	2.6	3.3	2.4
3	8.8	6.0	4.7	7.4	6.1	6.7	1.8	2.4	2.5	1.7	0.1	2.0
4	0.0	0.0	5.8	1.8	2.0	0.5	0.0	0.0	0.4	0.0	0.0	0.0
5	3.5	0.6	3.9	0.6	0.0	0.3	1.3	1.0	1.7	0.6	0.0	1.0
6 ^f	4.8	1.9	6.2	3.6	3.2	3.4	6.2	5.4	7.2	9.1	8.2	9.5
7 ^f	11.3	5.0	11.9	7.5	4.2	6.5	9.1	6.7	11.8	8.6	7.2	9.9
ΔE ^g	8.8	6.0	5.8	7.4	6.1	6.7	1.8	2.6	3.4	2.6	3.3	2.4

^a The number of low quality MM2* parameters for the hydrocarbon (HC) analogs are very few (HCl 1.0, HC2 1.0, HC3 0.0%). Thus, the energetic changes are primarily electrostatic in origin. ^b MM2* relative energies as presented in Table 1. ^c C1, C7 and C2' OHs in **1** were replaced with CH₃; C5 ether with CH, and C9=O with C=CH₂. ^d C2, C4, C10 and C13 esters and C3' amide were replaced with *trans*-CH=CH. ^e All ten polar groups replaced with hydrocarbon as in b and c. ^f Taxol conformations determined in the solid state¹³; optimized using AMBER* with all non-terpenoid core dihedral angles frozen; **6** polar; **7** extended. ^g The energy spread (kcal/mol) between the highest and lowest unconstrained energy conformations; i.e. **1-5**.

solvent with a high dielectric and the capacity for explicit hydrogen bonding as in water, the polar form goes undetected. [5b]

T-Taxol (the butterfly conformation)

For the unsuspecting organic or medicinal chemist, however, either interpretation is equally unfortunate. Faced with a large, poly-polar organic structure and a solvation-equipped semiempirical package, the user is limited by the proposition that the aqueous solvation model might provide the correct global minimum, but that other conformers may or may not be assigned an appropriate relative energy. In less polar chlorocarbon solvents, the Taxol structure and other molecules with similar complexity do not appear to be capable of even qualitative ranking by the various methods with respect to energy. An interesting but fortuitous outcome in this respect is that conformer **4**, the T or butterfly conformer of Taxol, is predicted to be the dominant conformation by MMFF, AMBER* and MM2* under various protocols (Table 2). Semiempirical models likewise predict it to be of low energy, though not the most stable (Table 4). This conformer was assigned a low population in the multi-conformational analysis of Taxol in chloroform[12], used in the fitting of the electron crystallographic density of the β-tubulin/Taxol complex in zinc-stabilized sheets, and ultimately assessed as the binding conformation for Taxol in this structure. [9]. A unique, but difficulty observed conformer, computational methodology overestimates its stability and might have led to its discovery by misrepresentation. While the operation of serendipity is

much to be desired in the drug seeking process, errant methodology is the least desired path to discovery.

Conclusions

As a consequence of the above considerations, for highly polar molecules like Taxol it is overoptimistic to expect that conformational searching based on a *computational energy criterion* will yield results faithful to experiment. The use of solvent continuum models clearly does not ameliorate the situation. Our observations and those of Halgren[4] suggest that a given force field or quantum chemical method selected for potential application to a highly-polar molecular system be carefully validated to assure that conformational energy comparisons are not spurious. Until this problem can be addressed at a fundamental theoretical level, it would seem prudent to employ an "energy-free" conformational search protocol. We find the NAMFIS approach valuable in this respect;[11,12,19] however there are a number of equally attractive alternatives[20] that, in principle, can also bypass the energy catastrophe. Future work will be devoted to examining a range of functionalized organic molecules in order to define the boundary of molecular polarity within which standard conformational search methodology can be applied with confidence.

Materials and Methods

Force fields and semiempirical methods applied to Taxol

The study was conducted by employing four popular force fields in MacroModel6.5 (MM2*, MM3*, AMBER* and MMFF).[2] Each was used in the "gas phase" with

two continuum solvation models (CHCl₃, H₂O) [2b] with default MacroModel atomic charges (Table 2). To provide a comparison with MacroModel, we also tested solvated MM3(96) and polarization enhanced MM3(2000).[21] In addition, the seven AMBER* conformations were fitted with MNDO electrostatic potential (ESP) charges scaled to the 6-31G* level[22,23] and the calculations repeated (Table 3). The structures were also fully optimized with the AM1 and PM3 semiempirical methods[22] and supplemented by AMSOL SM5.4a solvation energies (Table 4). [24]

References

- Allinger N, Burkett U: **Molecular Mechanics**. Washington, D.C. ACS 1982 b) N Allinger, C Kuohsiang, J-H Lii: **An improved force field (MM4) for saturated hydrocarbons**. *J Comp Chem* 1996 17: 642-668; c) N Nevins, C Kuohsiang, N Allinger: **Molecular mechanics (MM4) calculations on alkenes**. *J Comp Chem* 1996 17: 669-694; d) N Nevins, J-H Lii, N Allinger: **Molecular mechanics (MM4) calculations on conjugated hydrocarbons**. *J Comp Chem* 1996 17: 695-729; e) N Nevins, N Allinger: **Molecular mechanics (MM4) vibrational frequency calculations for alkenes and conjugated hydrocarbons**. *J Comp Chem* 1996 17: 730-746; f) N Allinger, C Kuohsiang, JA Katzenellenbogen, SR Wilson, GM Anstead: **Hyperconjugative effects on carbon - carbon bond lengths in molecular mechanics (MM4)**. *J Comp Chem* 1996 17: 747-755.
- Mohamadi F, Richards NGJ, Guida WC, Liscamp R, Lipton M, Caufield C, Chang G, Hendrickson T, Still WC: **MacroModel - an integrated software system for modeling organic and bioorganic molecules using molecular mechanics**. *J Comput Chem* 1990, 11:440 b) WC Still, A. Tempczyk, RC Hawley, T Hendrickson: **Semianalytical Treatment of Solvation for Molecular Mechanics and Dynamics** *J Am Chem Soc* 1990 112: 6127-6129; c) <http://www.schrodinger.com/Products/macromodel.html>
- Gundertofte K, Liljefors, Norrby P-O, Pettersson I: **A Comparison of Conformational Energies Calculated by Several Molecular Mechanics Methods**. *J Comp Chem* 1996, 17:429-449 b) I Pettersson, T Liljefors: in *Rev. Comp. Chem.* (Edited by KB Lipkowitz, DB Boyd) v. 9, VCH Pub, Inc NY, 1196, 167-190.
- Halgren TA: **MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular-interaction energies and geometries**. *J Comp Chem* 1999, 20:730-748
- Polar form*: a) Refs. 6d,e,i; b) DG Vander Velde, GI Georg, GL Grunewald, CD Gunn, LA Mitscher: **"Hydrophobic Collapse" of Taxol and Taxotere Solution Conformations in Mixtures of Water and Organic Solvent**. *J Am Chem Soc* 1993 115: 11650-11651; c) LG Paloma, RK Guy, W Wrasidlo, KC Nicolaou: **Conformation of a Water-Soluble Derivative of Taxol in Water by 2D-NMR Spectroscopy**. *Chem Biol* 1994 1 107-112; d) J Jimenez-Barbero, AA Souto, M. Abal, IBarasoain, JA Evangelio, AU Acuña, JM Andreu, F Amat-Guerri: **Effect of 2'-OH acetylation on the Bioactivity and Conformation of 7-O-[N-(4'-fluoresceincarboxyl)-L-alanyl] Taxol**. *Biorg Med Chem* 1998 6: 1857-1863; e) I Ojima, S Chakravarty, T Inoue, S Lin, L He, SW Horwitz, SD Kuduk, SJ Danishefsky: **A Common Pharmacophore for Cytotoxic Natural Products that Stabilize Microtubules**. *Proc Nat Acad Sci USA* 1999, 96, 4256-4261.
- Nonpolar form*: a) JK Baker: **Nuclear Overhauser Effect Spectroscopy (NOESY) and Dihedral Angle Measurements in Determination of the Conformation of Taxol in Solution**. *Spectroscopy Lett.* 1992 25: 31-48; b) BD Hilton, GN Chmurny, GM Muschik: **Taxol: Quantitative Internuclear Proton-Proton Distances in Deuterated Chloroform Solution from nOe data: 2D NMR ROESY buildup rates at 500 MHz**. *J Nat Prod* 1992 55: 1157-1161; c) CJ Falzone, AJ Benesi, JTJ Lecomte: **Characterization of Taxol in Methylene Chloride by NMR Spectroscopy**. *Tet Lett* 1992 33: 1169-1172; d) J Dubois, D Guénard, F Guéritte-Voegelein, N Guedira, P Potier, B Gillet, J-C Beloeil: **Conformation of Taxotere and Analogues Determined by NMR Spectroscopy and Molecular Modelling Studies**. *Tetrahedron* 1993 49: 6533-6544; e) HJ Williams, AI Scott, RA Dieden, CS Swindell, LE Chirlian, MM Francl, JM Heerding, NE Krauss: **NMR and Molecular Modeling Study of the Conformation of Taxol and its Side Chain Methyl Ester in Aqueous and Non-Aqueous Solution**. *Tetrahedron* 1993 49: 6545-6560; f) SV Balasubramanian, JL Alderfer, RM Straubinger: **Solvent and Concentration Dependent Molecular Interactions of Taxol (Paclitaxel)**. *J Pharm Sci* 1994 83: 1470-1475; g) H Williams, AI Scott, RA Dieden, CS Swindell, LE Chirlian, MM Francl, JM Heerding, NE Krauss: **NMR and Molecular Modeling studies of Active and Inactive Taxol Analogs in Aqueous and Nonaqueous Solution**. *Can J Chem* 1994 72: 252-260; h) RE Cachau, R Gussio, JA Beutler, GN Chmurny, BD Hilton, GM Muschik, JW Erickson: **Solution Structure of Taxol Determined Using a Novel Feedback-Scaling Procedure for NOE-Restrained Molecular Dynamics**. *Supercomputer App High Perform Comput* 1994 6: 24-34; i) I Ojima, SD Kuduk, S Chakravarty, M Ourevitch, J-P Bégué: **A Novel approach to the Study of Solution Structures and Dynamic Behavior of Paclitaxel and Docetaxel Using Fluorine-Containing Analogues as probes**. *J Am Chem Soc* 1997 119: 5519-5527; j) M Milanesio, P Ugliengo, D Viterbo, G Appendino: **Ab Initio Conformational Study of the Phenylisoserine Side Chain of Paclitaxel**. *Med Chem* 1999 42: 291-299.
- Nogales E, Wolf SG, Downing KH: **Structure of the $\alpha\beta$ Tubulin Dimer by Electron Crystallography**. *Nature* 1998, 391:199-202.
- Rao S, He L, Chakravarty S, Ojima I, Orr GA, Horwitz SB: **Characterization of the Taxol binding site on the microtubule. Identification of Arg(282) in beta-tubulin as the site of photoincorporation of a 7-benzophenone analogue of Taxol**. *J. Biol. Chem* 1999, 274:37990-37994 b) Y Li, B Poliks, L Cegelski, M Poliks, Z Gryczynski, G Piszczek, PG Jagtap, DR Studelska, DGI Kingston, J Schaefer, S Bane: **Conformation of Microtubule-Bound Paclitaxel Determined by Fluorescence Spectroscopy and REDOR NMR**. *Biochemistry* 2000 39: 281-291; c) P Giannakakou, R Gussio, E Nogales, K H Downing, D Zaharevitz, B Bollback, G Poy, D Sackett, KC Nicolaou, T Fojo: **A Common Pharmacophore for Epothilone and Taxanes: Molecular Basis for Drug Resistance Conferred by Tubulin Mutations in Human Cancer Cells**. *Proc Natl Acad Sci USA* 2000 97: 2904-2909; d) L He, PG Jagtap, DGI Kingston, H-J Shen, GA Orr, SB Horwitz: **Common Pharmacophore for Taxol and the Epothilones Based on the Biological Activity of a Taxane Molecule lacking a C-13 Side Chain**. *Biochemistry* 2000 39: 3972-3978.
- Snyder JP, Nettles JH, Cornett B, Downing KH, Nogales E: **The Binding Conformation of Taxol in β -Tubulin: A model based on electron crystallographic density**. *Proc. Natl. Acad. Sci. USA* 2001, 98:5312-5316
- Snyder JP: **A Unified Taxol, Epothilone, Discodermolide Minireceptors for Molecular Design**. *Alfred Benzon Symposium 42 Copenhagen, Denmark, 1997, abstracts*: b) JM Jansen, KF Koehler, MH Hedberg, AM Johansson, U Hacksell, G Nordvall, JP Snyder: **Molecular Design Using the Minireceptor Concept**. *J Chem Inf Comput Sci* 1997 37: 812-818; c) JP Snyder, X Xia, MA Schestopol, Y Kirn, D Bray, M Cain, D Liotta, KF Koehler, JM Jansen: **Minireceptors for Molecular Design**, in *Rational Molecular Design in Drug Research* (Edited by T. Liljefors, F. S. Jorgensen, P. Krosggaard-Larsen) Munksgaard, Copenhagen, 1998, pp 115-135; d) M Wang, X Xia, Y Kirn, D Hwang, JM Jansen, M Botta, DC Liotta, JP Snyder: **A Unified and Quantitative Receptor Model for the Microtubule Binding of Paclitaxel and Epothilone**. *Organic Lett* 1999 1 43-46.
- Cicero DO, Barbato G, Bazzo R: **NMR Analysis of Molecular Flexibility in Solution: A new method for the study of complex distributions of rapidly exchanging conformations**. *J Am Chem Soc* 1995, 117:1027-1033 The NAMFIS protocol finds a small family of conformers from a much larger set that statistically best fits available NMR nOe-determined distances and J-derived dihedral angles.
- Snyder JP, Nevins N, Cicero DO, Jansen J: **The Conformations of Taxol in Chloroform**. *J Am Chem Soc* 2000, 122:724-725
- Wiley A, Rich DH: **Peptidomimetics derived from natural products**. *Med Res Rev* 1993, 3:327-384 b) DH Rich: **Effect of hydrophobic collapse on enzyme-inhibitor interactions. Implications for the design of peptidomimetics**. In *Perspectives in Medicinal Chemistry*, B Testa, E Kyburz, W Fuhrer, W Giger, eds. VCH, New York, 1993 15-25.
- Guéritte-Voegelein F, Mangatal L, Guénard D, Potier P, Guilhem J, Cesario M, Pascard C: **Structure of a Synthetic Taxol Precur-**

- source: **N-tert-Butoxycarbonyl-10-deacetyl-N-debenzoyltaxol.** *Acta Crystallogr* 1990, **C46**:781-784
15. Mastropaolo D, Camerman A, Luo Y, Brayer GD, Camerman N: **Crystal and Molecular Structure of Paclitaxel (Taxol).** *Proc Natl Acad Sci USA* 1995, **92**:6920-6924
 16. **MacroModel6.5 Technical Manual** 85-96
 17. **From the MacroModel 6.5 interactive output (Ref 14).**
 18. Giesen DJ, Gu MZ, Cramer CJ, Truhlar DG: **A Universal Computational model for Solvation Free Energies.** *J Org Chem* 1996, **61**:8720-8721 b) CC Chambers, GD Hawkins, CJ Cramer, DG Truhlar: **A Model for Aqueous Solvation Based on Class IV Atomic Charges and First-Solvation-Shell Effects.** *J Phys Chem B* 1996 **100**: 16385-16398; c) DJ Giesen, CC Chambers, CJ Cramer, DG Truhlar: **Solvation Model for Chloroform Based on Class IV Atomic Charges.** *J Phys Chem B* 1997, **101**: 2061-2069; d)see: [http://www.makolab.com/amsol/html/amsol_about.htm]
 19. Nevins N, Cicero DO, Snyder JP: **A Test of the Single-Conformation Hypothesis in the Analysis of NMR Data for Small Polar Molecules: A Force Field Comparison.** *J Org Chem* 1999, **65**:3979-3986
 20. Brüscheweiler R, Blackledge MJ, Ernst RR: **Multiconformational Peptide Dynamics Derived from NMR data: a new search algorithm and its application to antamanide.** *J. Biomol. NMR* 1991, **1**:3-11 b) C Landis, VS Allured: **Elucidation of Solution Structures by Conformer Population Analysis of NOE Data.** *J Am Chem Soc* 1991 **113**: 9493-9499; CP Casey, SL Hallenbeck, JM Wright, CR Landis: **Formation and Spectroscopic Characterization of Chelated d⁰ Yttrium(III)-Allyl-Alkene Complexes.** *J Am Chem Soc* 1997 **119**: 9680-9690; CR Landis, L Luck, JM Wright: **Multiconformational Analysis of Solution NOE Data for the Ac-(L)Proline-(D)Alanine-NHMe Dipeptide in a Nonprotic Solvent.** *J Magn Reson, Ser B* 1995 **109**: 44-59; c) MJ Blackledge, R Brüscheweiler, C Griesinger, JM Schmidt, P Xu, RR Ernst: **Conformational Backbone Dynamics of the Cyclic Decapeptide Antamanide – Application of a new multiconformational search algorithm based on NMR Data.** *Biochemistry* 1993 **32**: 10960-10974. d) JJ Wang, RS Hodges, BD Sykes: **Generating Multiple Conformations of Flexible Peptides in Solution on the Basis of NMR Nuclear Overhauser Effect Data: Application to Desmopressin.** *J Am Chem Soc* 1995 **117**: 8627-8634; e) DA Pearlman: **FINGAR: a new genetic algorithm-based method for fitting NMR Data.** *J Biomol NMR* 1996 **8**:49-66; f) ML Beckers, LM Buydens, JA Pikkemaat, C Altona: **Application of a genetic algorithm in the conformational analysis of methylene-acetal-linked thymine dimers in DNA: comparison with distance geometry calculations.** *J Biomol NMR* 1997 **9**: 25-34.
 21. Ma B, Lii J-H, Allinger NL: **Molecular polarizabilities and Induced Dipole Moments in Molecular Mechanics.** *J Comput Chem* 2000, **21**:813-825
 22. **MOPAC6.0 QCPE #455; JP Stewart, Stewart Computational Chemistry, 15210 Paddington Circle, Colorado Springs, CO 80921**
 23. Besler BH, Merz KM Jr, Kollman PA: **Atomic charges derived from Semiempirical Methods.** *J Comp Chem* 1990, **11**:431-439
 24. **Coordinates for the seven conformations are available upon request:** [snyder@euch4e.chem.emory.edu.]

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright



BioMedcentral.com

Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>

editorial@biomedcentral.com