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October, 1998

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A Kinetic Analysis of the Conformational Flexibility of Steroid Hormones

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Abstract

For a set of 10 androgen steroids and estradiol (E_2), the kinetic feasibility of conformation flexibility of the cyclic moieties was studied under the constraint of maintaining the B/C trans and C/D trans ring fusion of the natural and biologically active enantiomer. To this end, the conformational energy surface was quantified using the semiempirical quantum chemical AM1 model. The computational analysis included the location of Conformational transition states with associated barriers, and intrinsic reaction coordinate (IRC) calculations to characterize the trajectories of the rotations and the relationships of the transition states to neighbouring chair and twist conformations. Conformational transformations were observed only for the A and B rings except for E_2, which yielded corresponding transformations for the B and C ring, respectively. Interestingly, the rotation barriers starting from the lowest-energy conformations differed substantially, ranging from below 10 kJ/mol for four compounds to 18–20 kJ/mol for another five compounds. Moreover, chair and twist conformations were found only for steroids with higher saturated rings, while semichairs and semitwists occurred for steroids with aromatic or partly unsaturated rings, and B-ring transformations lead to kinetically unstable conformations with very flat energy minima. Although the rotation barriers for most of the transitions are clearly above the thermal energy (kT) at room temperature when evaluated relative to the lowest-energy conformations, the associated energy demands are well below the gain in energy from ligand-receptor binding. The results suggest that conformer interconversion are feasible from both a thermodynamic and kinetic perspective, and support previous investigations in which conformer distributions rather than lowest energy conformations were considered when assessing hormone receptor topography and the biological activity of ligands.

1 Introduction

With conventional 3-dimensional (3-D) structure activity relationship (SAR) methods, the molecular structure of each chemical under study is represented by a single lowest-energy conformer, as defined by quantum chemical or force field methods. However, the most stable conformations may be less likely to interact with a solvent or macromolecule [1]. To systematically evaluate conformational flexibility within the context of relevant biological environments (e.g., partitioning within a complex solution, substrate-receptor complex formation) a dynamic SAR approach [2] has been developed that assumes a molecule exists as a variety of conformers with solvation and binding interactions compensating for energy differences among the conformers. Biological activity is subsequently modeled as an electronic property of specific conformers, rather than a property derived from the lowest-energy gas-phase conformer of a compound.

As recently reviewed [3], this approach has been used to develop quantitative structure–activity relationships (QSARs) for selection of active conformers and an active analogue search method (originally introduced by [4]) which were employed for the elucidation of ligand binding to the estrogen (ER) and androgen (AR) receptors [5–7]. Rather than employing the lowest-energy conformation for each ligand under study, these investigations analyzed distributions of energetically-reasonable conformers, where the range in heats of formation for conformer sets were comparable to the free-energy of binding of estradiol (E_2) to

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Key words: steroids, estradiol, conformational flexibility, conformational energy surface, quantum chemical AM1 model

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the ER [8–10]. As a result, modelling was undertaken under the assumption that a ligand could exist as a suite of conformers, with the energy required for interconversions provided by the ligand binding domain of the receptor. These assumptions in an SAR modelling approach are consistent with both theoretical and empirical studies that indicate receptors undergo a progressive and substantial conformational change upon binding to ligands. The underlying assumption is that receptor-ligand binding reflects an induced fit, where the receptor adapts to the shape of the ligand, while the ligand's conformation can be altered to that of the ligand binding pocket [11]. The multiple conformer approach in SAR modelling of ligand binding affinities is also being undertaken in an attempt to establish a framework consistent with current views in tripartite receptor pharmacology, which propose that ligands of different structure acting upon a receptor should give rise to conformationally-different ligand-receptor complexes with the potential to differentially react with the suites of effector systems, ultimately resulting in different patterns of gene regulation and expression [11].

While current theory on receptor-ligand interactions suggest that flexible ligands or flexible substructures within a ligand (e.g., the B-ring of E_2; [8, 12, 13]) would be expected to adapt to the shape of the ligand binding pocket, most QSAR and receptor/pharamcophore mapping methods, such as CoMFA [14] and GRID [15], use X-ray structures or lowest-energy gas-phase structures in model development. In an attempt to include conformational diversity in modelling E_2 and related derivatives, Wiese and Brooks [8] used molecular dynamics and a quench reannealing method to systematically undertake conformational searches. With E_2, these workers identified one conformer that corresponded to the X-ray structure and a second conformer whose energy was 13.8 kJ/mol greater. Using MMP2, these workers also established that energies for the transition barriers between conformers of E_2 and several E_2 derivatives were typically lower than 42 kJ/mol. It was then suggested that the realization of conformers different from the lowest-energy structure could be compensated by receptor binding energy. However, it should be noted that conformational energies and barriers substantially above the thermal energy (kT, where k is the Boltzmann constant, and T — absolute temperature) of 2.48 kJ/mol at 298 K would indeed require a significant gain in energy from the subsequent ligand-receptor binding to make the conformational change feasible from a thermodynamic and kinetic standpoint, respectively.

In studies of ER and AR ligands, an exhaustive conformer generation algorithm, followed by quantum chemical optimization and elimination of conformers whose energies exceed the free energy of binding, was used to assess electronic and steric properties associated with receptor binding affinity (see [3] for a recent review). Consequently, the use of multiple conformers in modeling receptor binding affinity was in accord with the thermodynamic boundary conditions. However, to date these studies have not investigated the energy barriers associated with conformer conversions. To assess the kinetic feasibility of the multiple conformer approach [3, 5, 7], the energy barriers between multiple conformers of 10 androgen derivatives and estradiol steroids were quantified in the current study. The results of this investigation indicate that from both a thermodynamic and kinetic perspective the incorporation of conformer distributions appears useful when assessing hormone receptor topography and the biological activity of receptor ligands.

2 Methods

2.1 Steroid Ligands

Conformer interconversion barriers were assessed for the 11 steroid ligands depicted in Figure 1. Their AR binding activity was based on a competitive binding assay using [3H]RI881 (a radiolabled synthetic androgen; [16], [17]). The names of the studied steroids are listed in Table 1.

2.2 Conformer Generation

Conformers were generated using a combinatorial procedure [18] that generates all conformers from molecular topology, consistent with steric constraints (e.g., distances between non-bonded atoms, ring-closure limits, torsion resolution) and expert rules (e.g., the likelihood of intramolecular hydrogen bonds, cis/trans or +/− isomers). The procedure incorporates the conformational flexibility of saturated cyclic molecular fragments, as opposed to other techniques which explore conformational space formed by rotations around acyclic single bonds only. With strained cyclic structures, less restrictive geometric constraints are used to generate conformers. As a result some of the conformers can be distorted with respect to reference (e.g., force field) geometric parameters. In such cases a strain minimization technique (pseudo-molecular mechanics, PMM) is applied based on a simple energy-like function, where only the electrostatic terms are omitted [18]. Geometry optimization can then be completed by more accurate force field or quantum chemical methods.

Due to the configurational rigidity of the steroids here, less restrictive geometric constraints for ring closures (1.0 and 2.5 and torsion resolution of 60°) were imposed for cyclic moieties to generate a sufficiently large initial set of conformations. All conformers maintained the appropriate stereochemistry (of the natural enantiomer with B/C trans

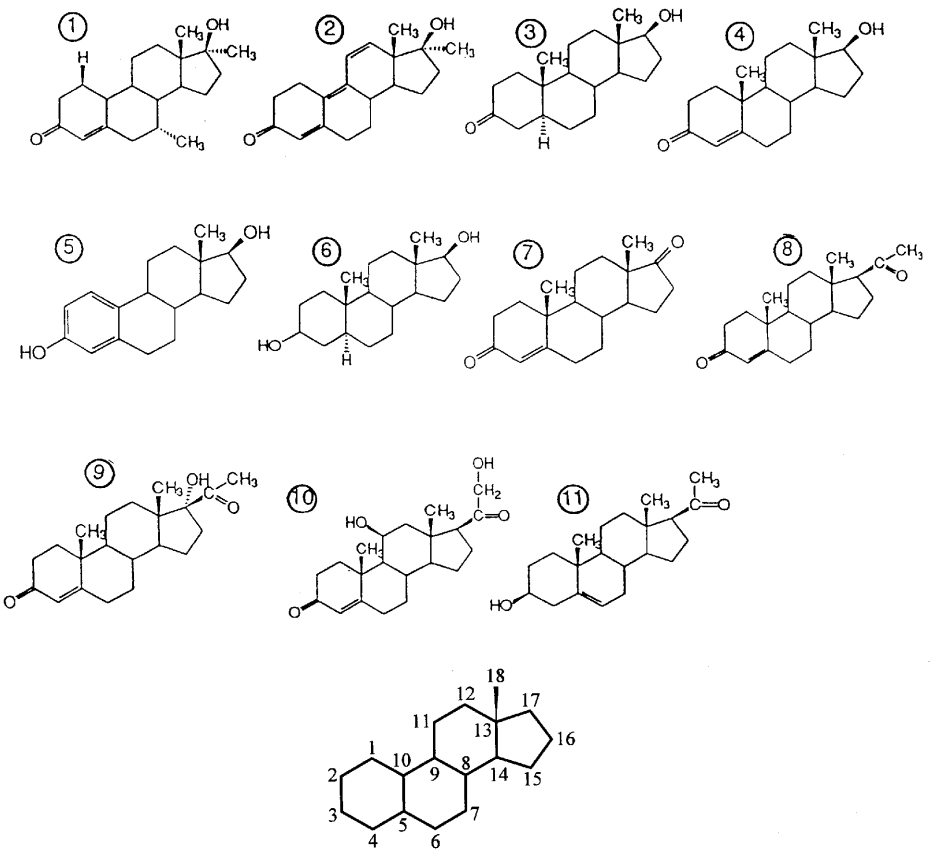


Figure 1. Steroid structures used to assess conformational flexibility; see Table 1 for compound names; for the IUPAC numbering see the last structure.

and C/D trans ring fusion). Subsequent geometry optimization of the conformers was employed using the semi-empirical AM1 Hamiltonian [19] as implemented in the MOPAC 93 package [20]. As a result of the optimizations, some of the conformations quenched into the same energy minima, which reduced the number of conformers.

2.3 Energy Profile Nomenclature for Cyclohexane Ring Conversions

As shown in Figure 2, the conversion of cyclohexane is a multi-step process. Although model manipulations might suggest a boat-type intermediate, force field calculations showed that the twist-type structure is energetically more favourable [1]. According to these results, the latter is more stable by 4−6 kJ/mol and can be obtained from the boat by a slight deformation, and the chair conformation is 20−26 kJ/mol lower in energy than the twist form. The calculations indicated further that the boat form is as an energy maximum in the interconversion of two distinct twist conformations. The existence of a twist form has been experimentally validated by infrared spectroscopy [21, 22]. The twist-to-chair activation energy has been determined by the evaluation of the rate of disappearance of the twist conformer.

2.4 Conformational Energy Surfaces: Transition State Modelling

Using ground state geometries, a semi-chair (semi-boat) structure was initially hypothesized for transition state (TS) calculations (Figure 3). This structure was obtained by placing atom #1 in the plane of atoms #2, 3, 5 and 6. This initial molecular model is subsequently used for locating the TS structure, i.e., the geometry that lies at a saddle point of the potential energy surface.

For all optimized TS structures, evaluation of the Hessian matrix confirmed the occurrence of only one negative eigenvalue [23]. To further ensure that the calculated transition geometries were indeed connecting the relevant pairs of conformers by steepest-descent paths, intrinsic reaction coordinate (IRC) calculations were performed [24] as implemented in MOPAC 93 [20]. An IRC represents the

Table 1. The steroids under investigation, generated conformers with associated ring conformations along with the corresponding ground state AM1 energies (ΔH_f^o in kJ/mol).

#	Steroid	Conformer #	Ring Conformations			ΔH_{f^o}
			A-Ring	B-Ring	C-Ring	kJ/mol
1.	Mibolerone	1	semichair	twist	chair	−483.0
		2	semitwist	chair	chair	−499.2
		3	semitwist	twist	chair	−461.7
		4	semichair	chair	chair	−507.5
2.	Methylrienolone	1	over[a]	under[a]	under	−286.2
		2	under[a]	under[a]	under	−284.4
		3	under[a]	over[a]	under	−265.1
3.	5-α-Dihydrotestosterone	1	twist	twist	chair	−563.1
		2	twist	chair	chair	−593.2
		3	chair	chair	chair	−600.6
4.	Testosterone	1	semichair	chair	chair	−494.3
		2	semichair	twist	chair	−479.7
		3	semichair	chair	chair	−484.3
5.	Estradiol	1	—	chair	chair	−450.5
		2	—	twist	chair	−444.1
		3	—	semichair/twist	twist	−406.0
6.	5-α-Androstane-3-α, 17β-diol	1	chair	chair	chair	−684.4
		2	twist	twist	chair	−641.1
		3	twist	chair	chair	−674.5
7.	δ^1-Androstenedione	1	semichair	chair	chair	−407.8
		2	semichair	twist	chair	−393.6
		3	semichair	chair	chair	−397.9
8.	Progesterone	1	semichair	chair	chair	−448.4
		2	semichair	twist	chair	−433.7
		3	semichair	chair	chair	−438.4
9.	17-α-Hydroxyprogesterone	1	semichair	chair	chair	−612.5
		2	semichair	twist	chair	−598.2
		3	semichair	chair	chair	−602.5
10.	Corticosterone	1	semichair	chair	chair	−792.8
		2	semichair	chair	chair	−804.0
		3	semichair	twist	chair	−792.6
11.	Pregnalone	1	chair	semichair	chair	−533.9
		2	chair	semichair	chair	−523.3
		3	twist	semichair	chair	−517.1

[a]see Results and Discussions for an explanation of the terms.

classical trajectory of the chemical system's motion from the reactant (in our case: conformation with chair configuration of a ring) to the products (in our case: conformers with a twist configuration), and it is located starting from the previously calculated TS structures by incrementally displacing the system in the direction of the negative (imaginary) vibration. Finally, the conformational energy surfaces around the TS barriers were obtained by plotting potential energies versus the according torsional angles of the IRC-calculated geometries.

3 Results and Discussion

The number of generated conformers and configuration of their rings are provided in Table 1 with the corresponding ground state AM1 energies (formation enthalpies, ΔH_f^o in

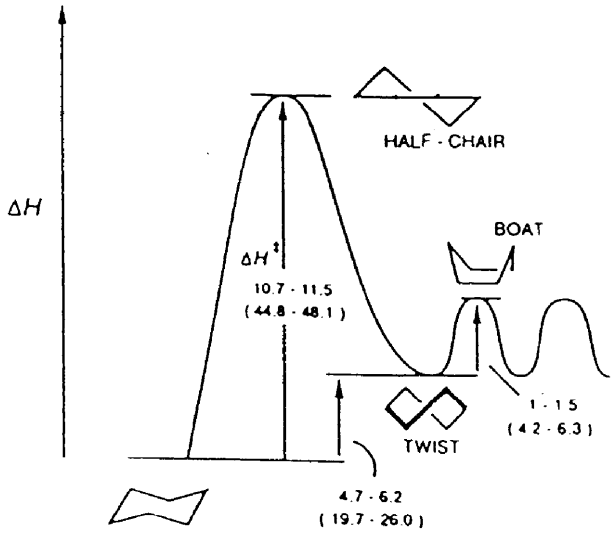


Figure 2. Energy profile ($H_{f°}$ in kJ/mol) for the cyclohexane ring reversal (modified from [1]).

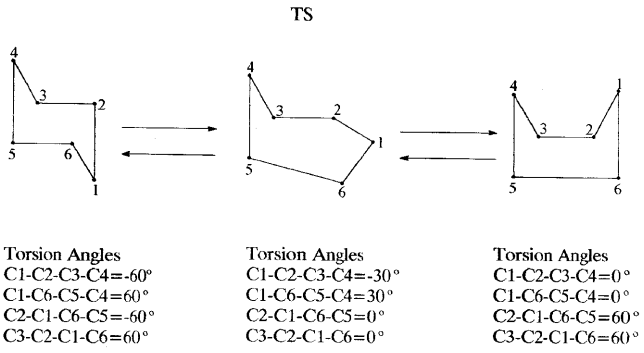


Figure 3. Hypothesized initial model for deriving transition states.

kJ/mol). The total number of conformers of the 11 steroids is smaller than reported previously [7] due to more restrictive degeneracy criteria and optimization constraints. Typically, three to four isomers were obtained for each steroid under investigation. However, a larger number of conformers were generated for chemicals #8−11 due to structural variations in the acyclic parts of these molecules. While these conformers should be taken into account when receptor topography is investigated, they are not the subject of the present study. Conformer interconversion barriers associated with structural transition in the cyclic skeleton of steroids were evaluated assuming that these barriers are, in general, significantly larger than the barriers corresponding to conformational changes in the acyclic substituents.

Chairs and twists were observed only for steroids with higher saturation of cyclic skeleton (#3, 6 and 11). Semichairs and semitwists appeared in the steroids with aromatic (E_2) or partly unsaturated A-rings. In the latter case, the structures have an A-ring with a double bond and Csp^2-atom adjacent to the double bond. In such a ring, five of the atoms are in a plane, with the sixth atom out of this plane. Apparently, such a ring can not be a pure chair or twist. The difference between the semichairs and the semitwists is that in the first case the 6th atom is over the plane whereas in the second case, it is beneath this plane. The conformation of the rings in compound #2 is more difficult to describe. The three double bonds in rings A, B and C define a plane. If the structure is oriented in such a way that the CH_3 side chain to cyclic atom 13 (CH_3-13) is over this plane, the conformation of ring A can result in atom 2 and 18 either being both above or below the plane. These will be termed as A-"over" and A-"under", respectively. Similarly, rings B and C are classified as "over" and "under" if atoms 7 and 18 are over and under this plane, respectively.

Interestingly, conformational variation of the C-ring was observed for E_2 only. This result could be due to internal limitations of the conformer generation algorithm or the values of associated geometric constraints. To evaluate the likelihood of these results being an artifact of the algorithm, conformations were produced by hand using the interactive 3D molecular design option of the OASIS packet [26] where the C-rings were modified from chair to boat for all of the conformers (see Table 1) except for those of chemicals #2 and #5 (E_2). After AM1 ground-state optimization, all hand-built C-ring boat conformers reverted to C-chairs. In another attempt to generate isomers with a boat (twist) conformation of the C-ring, compound #6 was re-analyzed using geometric constraints that were relaxed, thus allowing generation of a larger initial number of conformations. By reducing the torsion resolution from 60° to 30°, more than 700 conformations were generated as a result of the initial combinatorial step of the algorithm (see the Method section). After the force field optimisation, 197 conformers were obtained. Some of these conformers did have a twist conformation in the C-ring, however, after AM1 ground-state optimization, the conformers quenched into structures with a chair conformation. Efforts in progress are addressing features in the conformer generation algorithm in an attempt to isolate stable conformers with a C-ring twist.

Finally, two conformers with one and the same ring conformations of all cycles were generated for compound #11 (with all three conformers). The only significant difference in these structures was associated with A-ring geometries. Though having the same twist conformations, the A-rings differed in the values of the C2-C3-C4-C5 dihedral angle; 48.3° and −53.3° for conformers 2 and 3, respectively.

The TS energies, $\Delta H_f^{o\#}$, for all possible conformer interconversions are listed in Table 2 along with the energy

Table 2. The TS energies ($\Delta H_f^{o\#}$ in kJ/mol) for all possible conformer inerconversions and energy differences ($\Delta\Delta H_f^o$ in kJ/mol) to the associated energy minima (conformers).

	Transitions					
Steroid #	Conformers	Ring	Dihedral angle	ΔH_f^0 kJ/mol	$\Delta\Delta H_f^0$-to left conformer kJ/mol	$\Delta\Delta H_f^0$-to right conformer kJ/mol
1.	I−III	A	C_{10}-C_1-C_2-C_3	−459.4	23.7	2.6
	III−II	B	C_5-C_6-C_7-C_8	−453.7	8.0	45.5
	II−IV	A	C_{10}-C_1-C_2-C_3	−490.3	8.9	17.2
	IV−I	B	C_5-C_6-C_7-C_8	−481.4	26.1	1.6
2.	I−II	A	C_{10}-C_1-C_2-C_3	−278.3	7.8	6.0
	II−III	B	C_5-C_6-C_7-C_8	−265.0	19.4	0.1
3.	III−II	A	C_{10}-C_1-C_2-C_3	−589.7	10.9	3.4
	II−I	B	C_5-C_6-C_7-C_8	−561.4	31.8	1.7
4.	III−I	A	C_{10}-C_1-C_2-C_3	−475.1	9.2	19.2
	I−II	B	C_5-C_6-C_7-C_8	−474.4	19.9	5.3
5.	II−I	B	C_9-C_{11}-C_{12}-C_{13}	−443.9	0.2	6.6
	I−III	C	C_5-C_6-C_7-C_8	−405.9	44.6	0.1
6.	I−III	A	C_{10}-C_1-C_2-C_3	−661.5	22.9	13.0
	III−II	B	C_5-C_6-C_7-C_8	−640.4	34.1	0.7
7.	III−II	A	C_{10}-C_1-C_2-C_3	−388.5	9.4	19.3
	I−II	B	C_5-C_6-C_7-C_8	−388.1	19.7	5.4
8.	III−I	A	C_{10}-C_1-C_2-C_3	−429.2	9.2	19.3
	I−II	B	C_5-C_6-C_7-C_8	−428.4	20.0	5.3
9.	III−I	A	C_{10}-C_1-C_2-C_3	−593.6	9.0	18.9
	I−II	B	C_5-C_6-C_7-C_8	−592.7	19.8	5.5
10.	I−II	A	C_{10}-C_1-C_2-C_3	−781.9	13.9	22.0
	II−III	B	C_5-C_6-C_7-C_8	−784.8	19.2	7.8
11.	I−II	A	C_{10}-C_1-C_2-C_3	−505.4	28.5	17.9
	II−III	A	C_{10}-C_1-C_2-C_3	−507.5	15.9	9.6
	III−I	A	C_{10}-C_1-C_2-C_3	−514.7	2.4	19.1

differences of the formation enthalpies ($\Delta\Delta H_f^{o\#}$) between the conformer energy minima. The conformer interconversions were found to be restricted to the A- and B-rings except in the case of E_2, where B- and C-ring conversions were observed. For some of the steroids, more than one conformer interconversion was associated with the same ring. For example, for chemical #1 two interconversions were related with the A-ring, and another two with the B-ring. Interestingly, all conformer interconversions of chemical #11 were associated with the A-ring. The energy surfaces associated with conformer interconversions in the studied steroids are presented in Figures 4a−4k.

In general, the imaginary frequencies were in the range of $400-2000\,cm^{-1}$. In few cases frequencies were less than $100\,cm^{-1}$, however, the associated TSs were validated by IRC calculations. For example, the first eigenvalue for the TS of 5-α-Dihydrostestosterone was found to be $-83.0\,cm^{-1}$. This TS is responsible for the twist à chair transition of cycle "A" (i.e., the transition between conformers II and III). After IRC calculation of the two reaction directions proceeding from the TS, two different conformations were obtained. These geometries were subsequently submitted to ground state (AM1) optimization. After optimization, these structures were compared with conformers II and III of the structure, by RMS. The resulting comparisons showed matching greater than 99%, which suggested that the TS is responsible for the twist à chair transition of cycle "A", despite the associated frequency of $-83.0\,cm^{-1}$.

The analysis of data included in Tables 1 and 2, as well as of the conformational energy surfaces illustrated in Figure 4, shows that the differences in formation enthalpies (ΔH_{fo}) between the conformers of each steroid are generally less than 42 kJ/mol and, in most cases, less than 21 kJ/mol. The exception is the enthalpy difference of 44.5 kJ/mol between conformers 1 and 3 of E_2 (#5). Similarly, it was found that

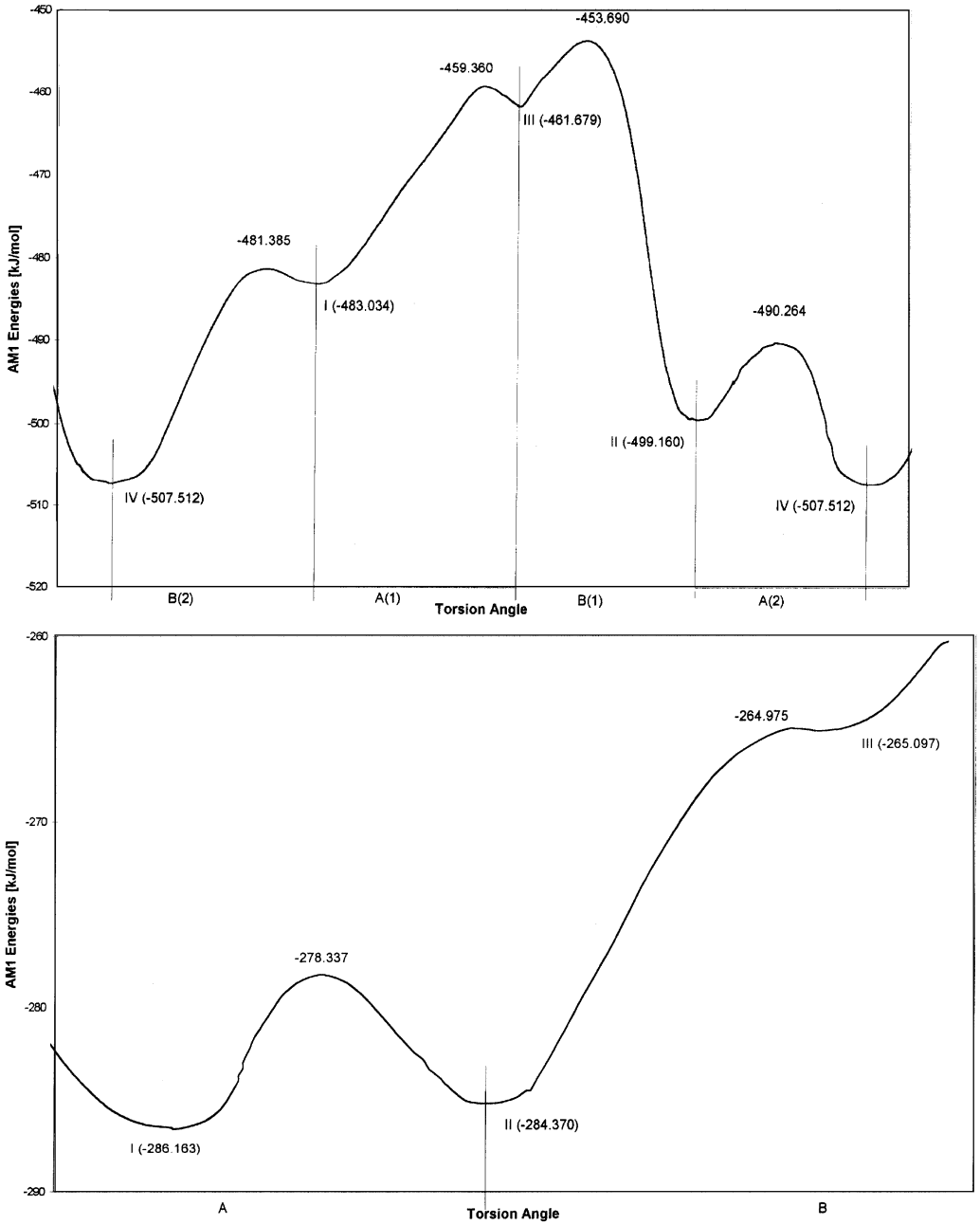


Figure 4a & b. Conformational energy surfaces associated to steroids under investigation. Generated conformations are denoted by Roman numbers (see Table 1). The area notations A, B and C on x-axis correspond to conformer transformations in rings A, B, and C, respectively. When more than one transformation occurs with one and the same cycle, the transition number is indicated in brackets. The adjacent point estimates were typically within 1°, hence no interpolation was required to generate conformational energy surfaces. Torsion angles used in dihedral driving experiments were: (a) comp. #1: A(1), C10-C1-C2-C3 from −53.3° (I) to 52.9° (III) with TS at 37.1°; B(1), C5-C6-C7-C8 from 56.0° (III) to 51.3° (II) with TS at 56.0°; A(2), C10-C1-C2-C3 from −55.6° (II) to 56.0° (IV) with TS at 14.3°; B(2), C5-C6-C7-C8 from −54.4° (IV) to 51.9° (I) with TS at −34.3°; (b) comp. #2: A, C10-C1-C2-C3 from 44.3° (I) to −48.5° (II) with TS at −2.0°; B, C5-C6-C7-C8 from −45.6° (II) to 54.4° (III) with TS at −38.0°; (c) comp. #3: A, C10-C1-C2-C3 from 30.1° (III) to −50.2° (II) with TS at −7.3°; B, C5-C6-C7-C8 from −39.7° (II) to 56.5° (I) with TS at −15.9°; (d) comp. #4: A, C10-C1-C2-C3 from 57.7° (III) to −55.7° (I) with TS at 15.5°; B, C5-C6-C7-C8 from −45.7° (I) to 53.8° (II) with TS at −8.8°; (e) comp. #5: B, C5-C6-C7-C8 from 41.0° (II) to −27.1° (I) with TS at −14.0°; C, C9-C11-C12-C13 from −52.1° (I) to 29.5° (III) with TS at 21.0°; (f) comp. #6: A, C10-C1-C2-C3 from 39.1° (I) to −53.7° (III) with TS at −8.5°; B, C5-C6-C7-C8 from −36.9° (III) to 57.0° (II) with TS at −19.0°; (g) comp. #7: A, C10-C1-C2-C3 from 57.7° (III) to −55.8° (I) with TS at 18.8°; B, C5-C6-C7-C8 from −45.8° (I) to 53.7° (II) with TS at −8.7°; (h) comp. #8: A, C10-C1-C2-C3 from 57.6° (III) to −55.8° (I) with TS at 15.1°; B, C5-C6-C7-C8 from −45.6° (I) to 53.8° (II) with TS at −8.9°; (i) comp. #9: A, C10-C1-C2-C3 from 57.8° (III) to −55.6° (I) with TS at 15.8°; B, C5-C6-C7-C8 from −46.0° (I) to 53.9° (II) with TS at −9.0° (j) comp. #10: A, C10-C1-C2-C3 from −55.6° (I) to 57.4° (II) with TS at 15.4°; B, C5-C6-C7-C8 from 53.1° (II) to −48.3° (III) with TS at −6.6°; (k) comp. #11: A(1), C10-C1-C2-C3 from −57.8° (I) to 63.7° (II) with TS at 8.9°; A(2), C10-C1-C2-C3 from −39.5° (II) to 63.7° (III) with TS at −5.8°; A(3), C10-C1-C2-C3 from 48.5° (III) to −53.3° (I) with TS at 15.2°.

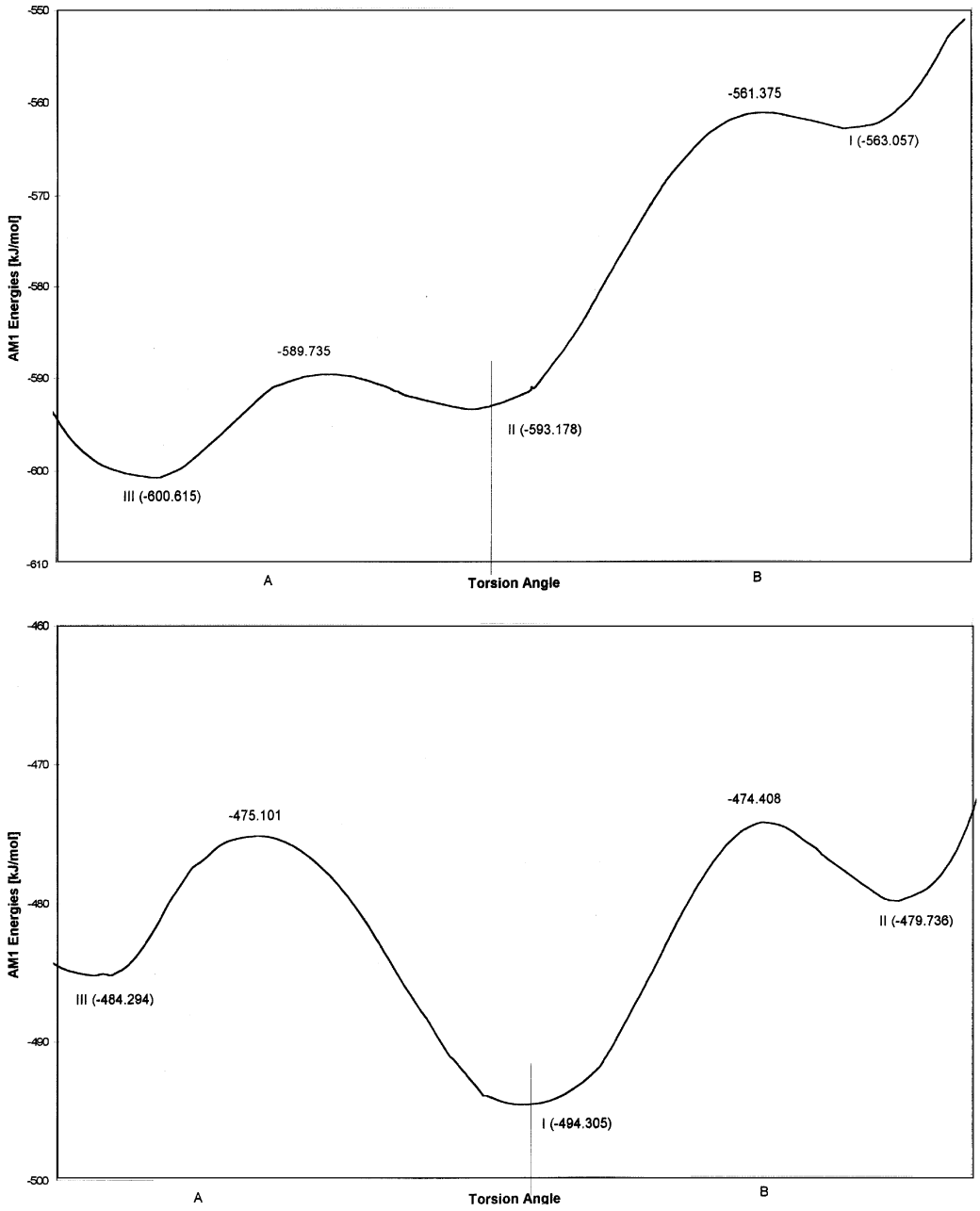
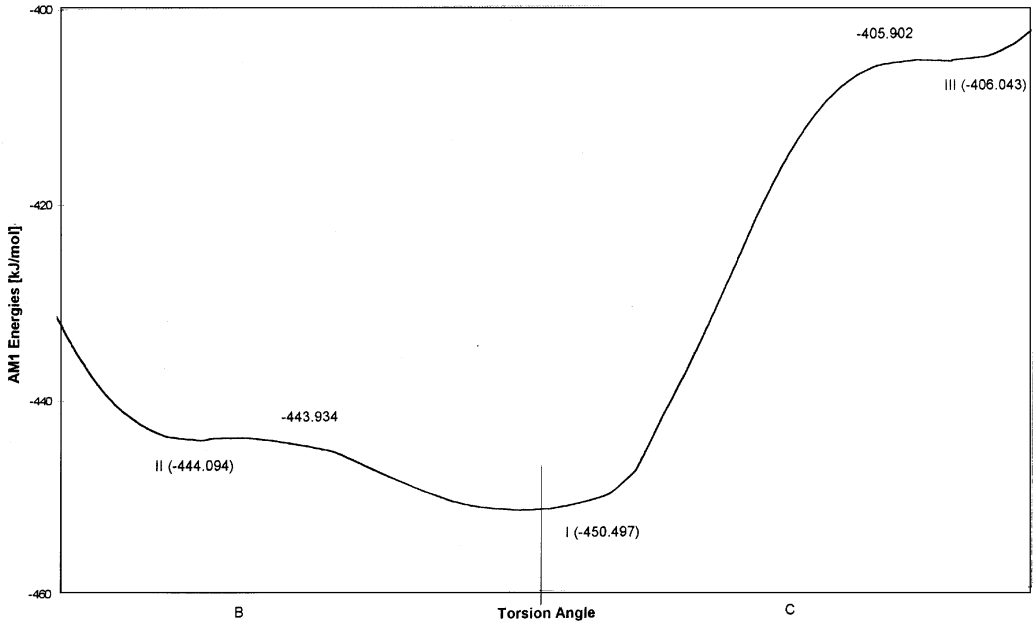


Figure 4c & d.

the transition barriers between conformers were generally below 42 kJ/mol. Here, exceptions were noted for E_2 (#5) with transition barriers of 44.6 kJ/mol between conformer 1 and 3, and for Mibolerone (#1) with a transition barrier of 45.5 kJ/mol between conformers 2 and 3.

Analysis of the conformational energy surfaces reveals interesting differences between the individual steroids. With compounds #1,2,4 and 5, the barrier between the minimum-energy conformation and the next-lowest conformation is below 10 kJ/mol, while barriers above 18 kJ/mol are observed for #7–11, and barriers between 10 and 13 kJ/mol for #3 and 6. These findings suggests that at least for the steroids #1–6, the conformational barriers are well below the gain in energy through binding at the receptor. In this context it should be noted, however, that previous investigations of the performance of AM1 to predict rotation barriers have revealed some deficiencies for certain groups of chemical structures [27], which are different from the present compound set. Moreover, a more definite assessment would also have to address the effect of solvation [28], which can be calculated on both the ab initio and semiempirical level using continuum-solvation models, keeping in mind that there may be substantial



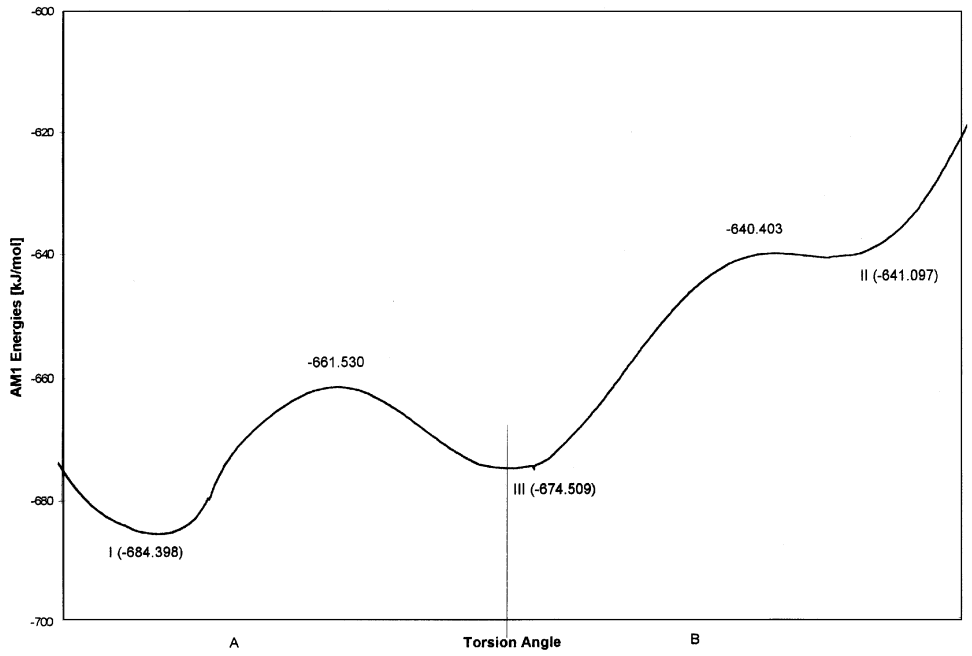


Figure 4e & f.

differences in performance for different classes of chemical compounds [23, 29–30].

With compounds #1, 2, 3, 5 and 6 there are one or two conformations with energies very close to neighbouring TS energies. For example, energy surfaces of chemicals #2,5 and 6 are with transition barriers smaller than 0.7 kJ/mol, while a barrier of 2.3 kJ/mol was calculated for steroids #1 and 3. The conformers with enthalpies close to TS energies should readily slide to more stable conformational states, and thus can be considered as reaction intermediates that are kinetically unstable at room temperature.

In the case of E_2 (#5), one stable conformer was derived that corresponds to the structure found in the solid phase by X-ray crystallography (denoted as conformer 1 in Table 1 and Figure 4e). The next-lowest conformer belongs to the above-mentioned group of kinetically unstable structures and is separated from the lowest-energy conformation by a barrier of only 6.6 kJ/mol. A further conformer has a flat

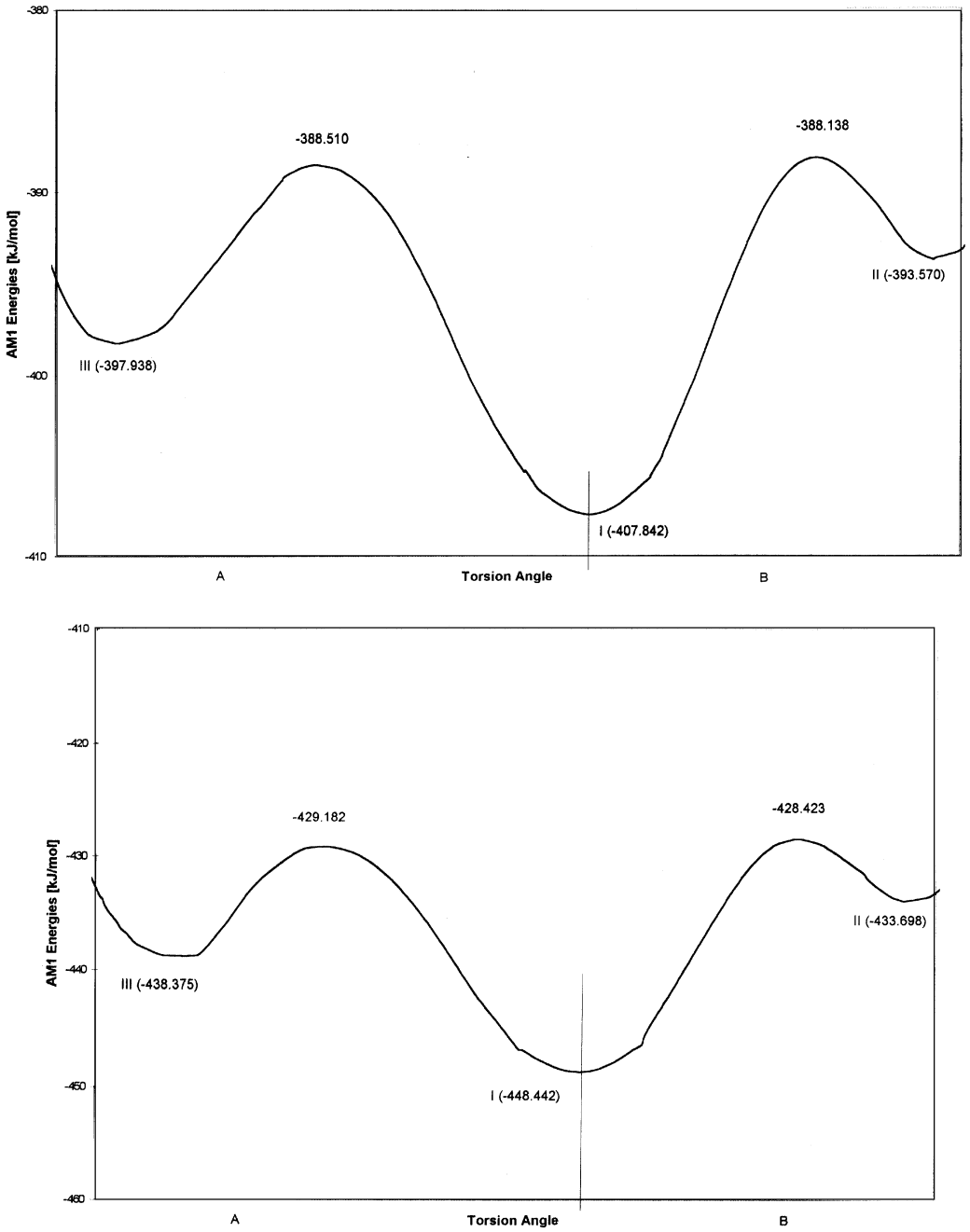


Figure 4g & h.

minimum with a heat of formation greater by 44.6 kJ/mol, and an associated barrier of only 0.1 kJ/mol.

Similarly, for chemical #3, an energy barrier of 1.7 kJ/mol was calculated between conformer 1 and conformer 2. In turn, a barrier of 3.4 kJ/mol was noted between conformer 2 and the most stable conformer 3. Thus, with this compound there are no significant energy obstacles for the conformers 1 and 2 to convert into the most stable conformer, which apparently is the single structure that can be observed experimentally in solid/liquid phase.

Although the formation enthalpies of these kinetically unstable conformers (i.e., reaction intermediates) are very close to associated rotation barriers, we should emphasize that the theoretical analysis clearly shows that their structures fulfil the ground-state requirements. Conformers with correspondingly flat energy minima are typically observed for steroids that could have neighbouring rings with the same twist (or semitwist) ring conformations (steroids #1, 3, 5 and 6; see Figure 4 and Tables 1 and 2). Alternatively, steroids (#4, 7–11) have conformers in which the neighbouring rings do not have the same twist

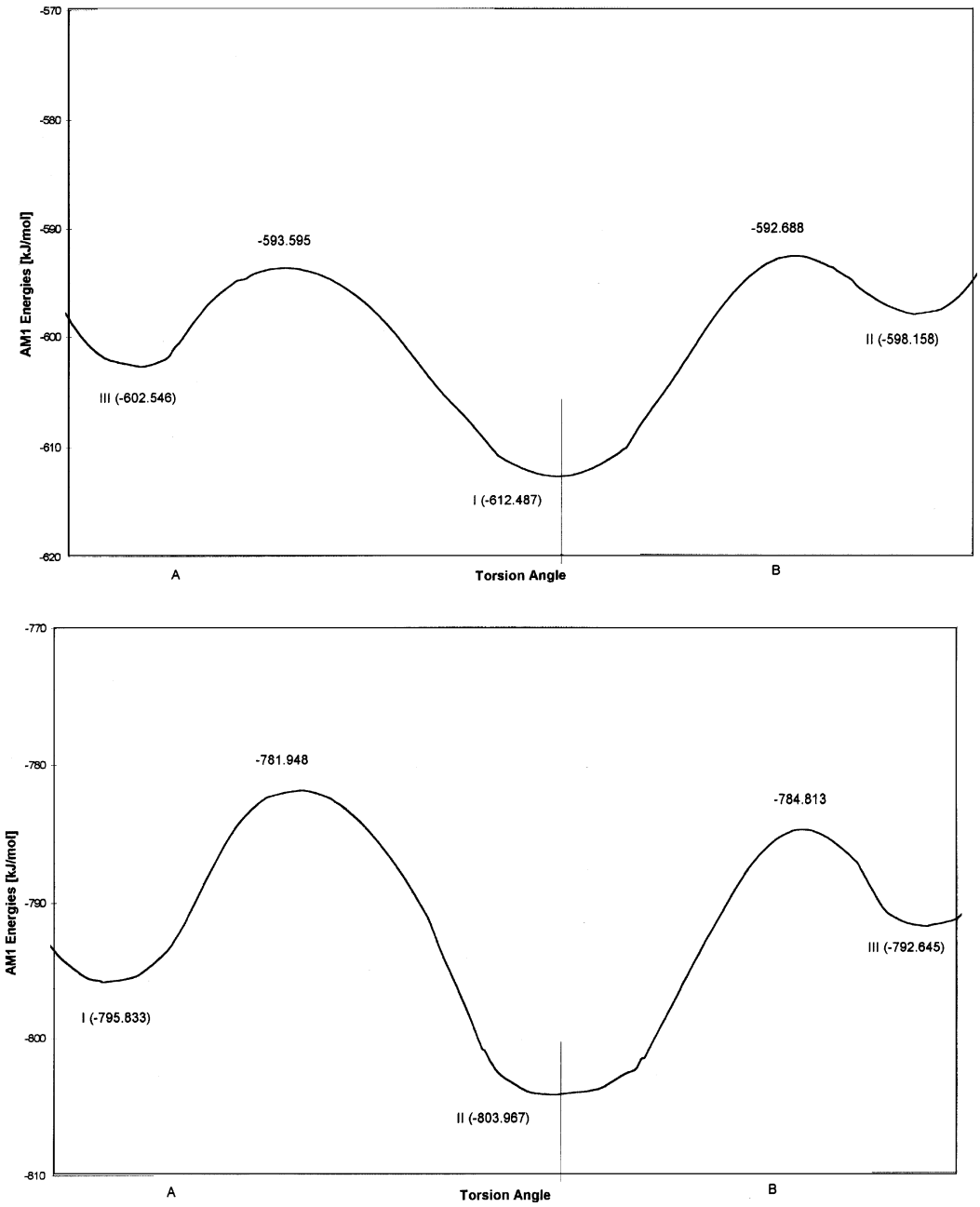


Figure 4i & j.

or semitwist conformations (though similar chair/semichair ring conformations were observed). Thus, kinetically unstable conformers that could be considered as reaction intermediates are associated with steroids whose structures allow conformers with neighbouring rings having the same twist (or semitwist) conformations. Steroids which do not permit such ring conformations have relatively stable conformers which would be predicted to be observed experimentally.

The results of this analysis showed that conformational variability of steroids is limited to structural transformation of A and B rings (B and C for E_2). With the exception of E_2, transformations in the C-ring always lead to a loss in the regional stereochemistry. Transformations in the A-ring generally appear to be energetically more favourable and are associated with lower TS energies. Thus, conformational transformations in the A-rings of the steroids typically produced stable conformers, whereas transformations in the "inner" B and C rings resulted in less stable conformers. The latter conformations are extremely unstable when the conformational states of the two neighbouring rings are both in a twist or semitwist conformations.

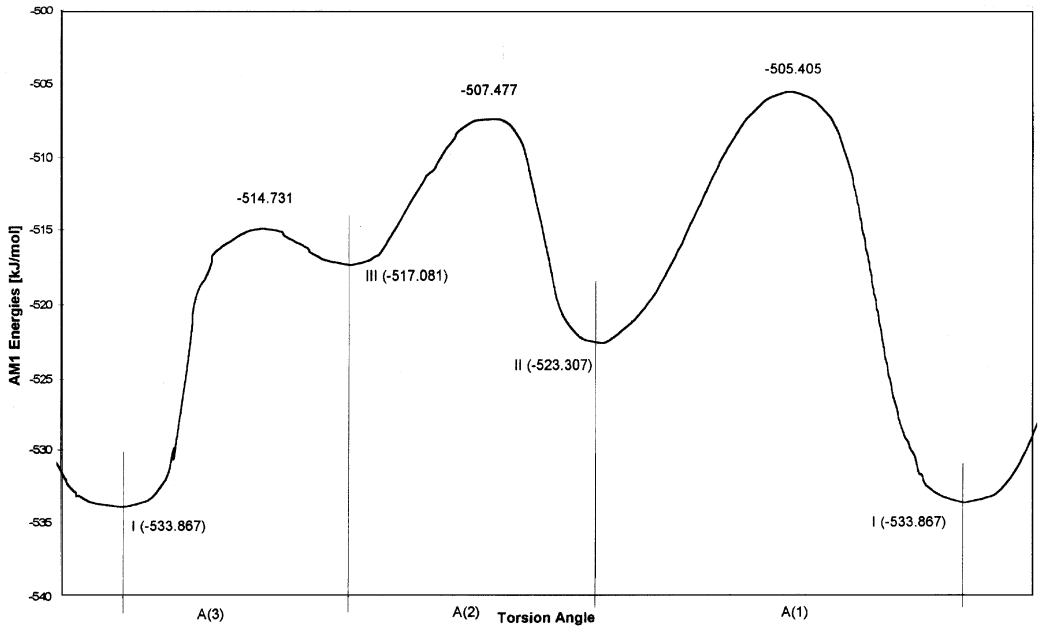


Figure 4k

The calculated conformational energy surfaces of a group of 11 steroids suggests that for this class of compounds, the elucidation of receptor topographies or ligand biological activities should take into account their conformational variability. According to the present computational analysis, potentially relevant conformational transformations are generally to be expected for the A and B rings, with the exception of E_2 where the B and C ring showed conformational flexibility.

While rotation barriers for A ring transformations starting from the lowest-energy conformation are often below 10 kJ/mol, the energy needed for changing the B ring conformation is typically higher and results in kinetically unstable conformers with very flat energy minima. Although the energetic demands are somewhat above RT at room temperature, they can be well compensated by the gain in binding energy from ligand-receptor interactions, which is at least the case for the A ring flexibility (B ring flexibility for E_2). It follows that the search for a mechanistic understanding of the biological activity of the steroids should go beyond an analysis of the lowest-energy conformers, keeping in mind that site-specific polarizabilities and reactivities may well change with the conformation of the chemical structure.

The findings from this study extend the investigations of Wiese and Brooks [8], who first proposed that E_2 derivatives could exist in two or more conformations within the ligand binding domain. The results of the current study establish the kinetic feasibility for more diverse group of androgen derivatives, in addition to E_2, to also exist as multiple conformers. In turn, the current investigations provides additional support to previous SAR studies on hormone binding affinity, which used thermodynamically-reasonable distributions of conformers [3, 7], because these findings suggest that in many cases the free-energy of binding to steroid hormones is also sufficient to overcome the energy barriers associated with TSs. Thus, the use of conformational distributions in SAR analysis of receptor binding affinity and associated biological activity may provide the means to ascertain the relative role of the lowest energy conformation versus higher energy structures [e.g., see 10] within a tripartite receptor pharmacology framework [11].

The present results lead to the question, whether and how the kinetically unstable conformers have any relevance for the three-dimensional mapping and interaction with the receptor, which may give rise to more detailed investigations. Finally, it will be interesting to quantify the effect of solvation on the conformational energy surfaces, which is the objective of a forthcoming study.

Acknowledgements

This research was supported, in part, by a U.S. EPA Cooperative Agreement (CR822306-01-0) with the Bourgas University "As. Zlatarov". The authors thank Dr. Stoyan Karabunarliev for fruitful discussions. One of the authors (J.I.) acknowledges a post-doctoral research grant from the UFZ Centre for Environmental Research, Germany.

Disclaimer

Mention of models or modelling approaches does not constitute endorsement on the part of the U.S. EPA.

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Received on April 29, 1998; accepted on July 4, 1998