Theoretical Studies on the Conformational Properties and Pharmacophoric Pattern of Several Bipyridine Cardiotonics

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Conformational features of two bipyridine cardiotonics *viz.*, amrinone and milrinone have been examined by an *ab initio* (STO-3-21G level) method. The calculated results suggest a twisted conformation for both molecules. Molecular electrostatic potential calculations have been carried out on various conformers of these molecules to visualize their pharmacophoric patterns. The results suggest that the twisted conformers of these molecules are responsible for their observed cardiotonic properties.

In recent years considerable interest has been focused on the conformational and electronic aspects of bipyridine cardiotonics because of their inotropic and peripheral vasodilatory properties.[1–3] Amrinone [5-amino-3,4′-bipyridin-6-(1*H*)-one] **1** and its analogue milrinone (1,6-dihydro-2-methyl-6-oxo-3,4′-bipyridine-5-carbonitrile) **2** are two such bipyridine cardiotonics and they are commonly used as a new class of drug having both inotropic and vasodilatory properties. Compared to amrinone, milrinone has been reported to be more potent.[3]

Recent crystallographic studies on the structure of amrinone[4] show that it is approximately planar, the torsion angle χ defining the plane difference of the two rings being 1.3°. In the case of milrinone, the results of crystallographic analysis show[4] that the bipyridine ring is considerably twisted and the torsion angle β between the two rings is 52.2°. NMR spectroscopic results on these molecules[4] suggest that in solution there is also a large structural difference between these two molecules. Although several experimental studies[4,5] have been carried out earlier to rationalize the conformational changes on the basis of crystal forces, only preliminary theoretical work has been reported[6] to justify the experimental results from a theoretical point of view.

It is well known that the molecular electrostatic potential (MEP) provides a highly informative means of assessing the electronic structure of molecules,[7,8] particularly when biological recognition processes are involved.[9–12] Such an electrostatic potential profile leads to direct inferences about the nature of the corresponding receptor site and about interaction between these sites and an approaching drug molecule. The present paper discusses the results of *ab initio* calculations on the conformational properties of two cardiotonics, *viz.*, amrinone and milrinone which produce significant effects on the heart and blood pressure. The calculations have been carried out at the 3-21G level. The pharmacophoric patterns of these molecules have been interpreted from the electrostatic potential maps.

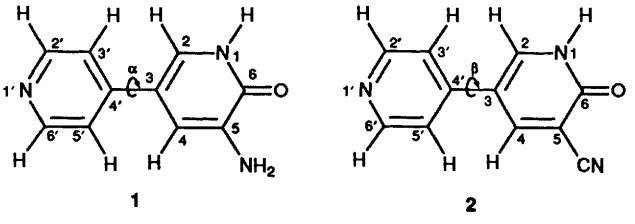

Fig. 1 Molecular structures of amrinone **1** and milrinone **2**

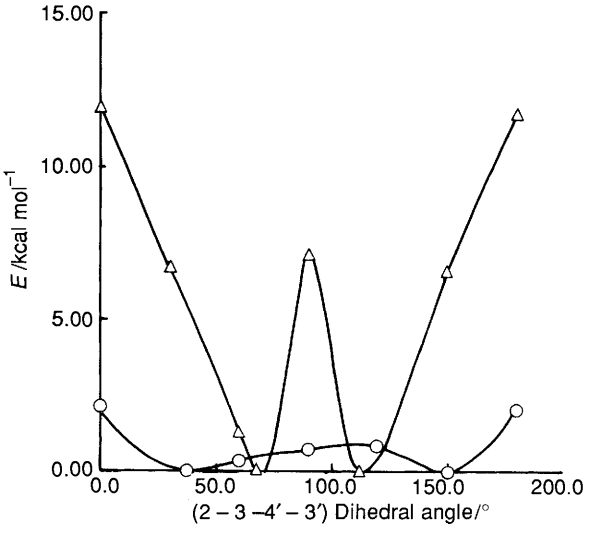

Fig. 2 Calculated energy profile as a function of torsional angle [C(3′)–C(4′)–C(3)–C(2)] for amrinone (○) and milrinone (△)

Results and Discussion

Theoretical Conformation Studies of Amrinone and Milrinone.—The molecular formulae of amrinone and milrinone are shown in Fig. 1. The geometry optimization of these molecules was carried out using the STO-3-21G basis.[13] The calculations were carried out using a GAUSSIAN 82 program package. Initial geometries of these molecules were constructed from the standard geometries of pyridine and 6-methyl-2-pyridone.[14] The salient features of the optimized geometries of these molecules are presented in Table 1. The results indicate that both molecules are considerably twisted. The energy profiles of the compounds along the α and β axes (Fig. 1) are shown in Fig. 2. The results indicate that the minimum energy conformers of amrinone (α = 36.9°) and milrinone (β = 66.95°) are *ca.* 2.0 and 12.0 kcal mol^{-1} more stable than their respective planar conformers. The low rotational barrier of amrinone (Fig. 2) indicates that this molecule is highly flexible. The structural parameters of the planar form of this molecule, Table 1,

Table 1 Topographical features of the minimum energy and planar conformations of amrinone and milrinone in the STO-3-21G basis

Structural parameters	Amrinone		Milrinone	
	Minimum energy	Planar	Minimum energy	Planar
Bond length[a]				
	1.511	1.515	1.513	1.536
C(3)–C(4′)	—	—	1.532	1.538
C(2)–C	1.081	1.082	—	—
C(2)–H	1.085	1.083	1.084	1.081
C(4)–H	1.079	1.076	1.081	1.077
C(3′)–H	1.079	1.077	1.081	1.061
C(5′)–H				
Bond angle[b]				
C(3)–C(2)–C	—	—	126.10	133.25
C(3)–C(2)–H	124.51	124.80	—	—
C(3)–C(4)–H	117.84	118.75	117.60	118.95
C(4′)–C(3′)–H	121.12	122.00	120.95	122.15
C(4′)–C(5′)–H	121.20	122.08	121.05	124.80
Torsional[b] angle (α/β)	36.9	0.0	66.95	0.0
Total energy[c]	−560.724 687	−560.721 451	−599.308 166	−599.289 062

[a] Bond lengths/Å. [b] Bond angles and torsion angles/°. [c] Total energies/a.u.

Table 2 Non-bonded interaction energies for the various conformers of amrinone and milrinone

Molecule	Conformers in terms of φ/°	Total non-bonded interaction energy/kcal mol^{-1}
Amrinone	0.0	15.04
	36.9	5.04
	120.0	2.07
Milrinone	0.0	42.59
	66.95	6.82
	90.0	1.15

Table 3 Potential constants (V_n and V'_n/kcal mol^{-1}) for internal rotations

Molecule	V_1	V_2	V_3	V'_1	V'_2
Amrinone	41.33	−21.09	4.76	−0.661	0.658
Milrinone	−532.72	327.09	−112.52	0.124	−0.360

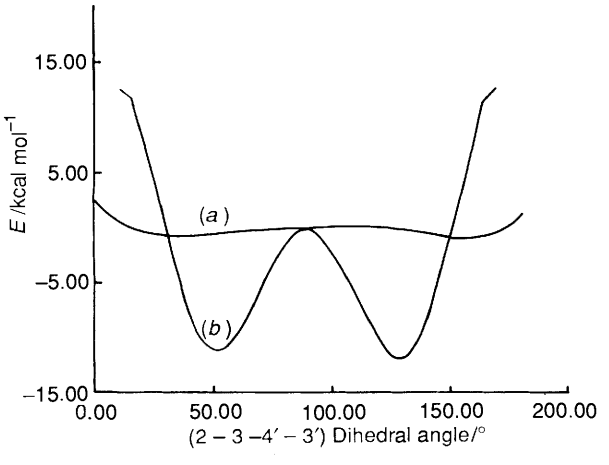

Fig. 3 Potential energy function $V(\varphi)$, describing the internal rotation of (*a*) amrinone and (*b*) milrinone

indicate that amrinone is capable of adjusting the destabilization due to rotation by a relatively small increase in central C(3)–C(4′) bond length and *ortho* hydrogen bond angles. The high rotational barrier of milrinone indicates (Fig. 2) that in comparison to amrinone it is a rigid molecule and exists solely in a twisted form. The structural parameters indicate that the rotation of this molecule from twisted (β = 66.95°) to planar form involves considerable increase in C(3)–C(4′) bond length and *ortho* hydrogen bond angle. The steric hindrance of the methyl group also shortens the *ortho* hydrogen lengths (Table 1). These structural features clearly account for the higher stability of the twisted form.

At this stage it is tempting to make some comments regarding the formation of a barrier at α = 120° for amrinone and β = 90° for milrinone. An inspection of the molecular structure of both these molecules indicates that the barriers cannot originate due to non-bonded interactions since these would be at a minimum in these regions of the molecules. For a quantitative estimate of such interactions we calculated the non-bonded interactions for the various conformers of these molecules using the method of Scheraga *et al.*[15] The estimation of non-bonded interactions for both these molecules has been carried out in the regions containing atoms 2, 3, 4, 3′, 4′ and 5′ (Fig. 1). The interactions due to hydrogen atoms and the methyl group (for milrinone) connected to these atoms have also been taken into account. The results (Table 2) clearly show that the non-bonded interactions have no role regarding the formation of the observed energy barrier in these molecules. We then tried to reproduce the energy profile of these molecules using a truncated Fourier series.[16] A five-term truncated Fourier series of the following type [eqn. (1)] has been found to reproduce the energy profiles quite satisfactorily. The parameter φ in eqn. (1)

$$V(\varphi) = \frac{1}{2}\sum_{n=1}^{3} V_n[1 - \cos(n\varphi)] + \sum_{n=1}^{2} V'_n \sin(n\varphi) \quad (1)$$

represents the dihedral angle around the single bond (3–4′) of the molecules concerned. Calculated energy profiles and the derived potential constants V_n and V'_n are shown in Fig. 3 and Table 3, respectively. As an aid to understanding the decomposition of the potential function, the components $V_n(\varphi)$ =

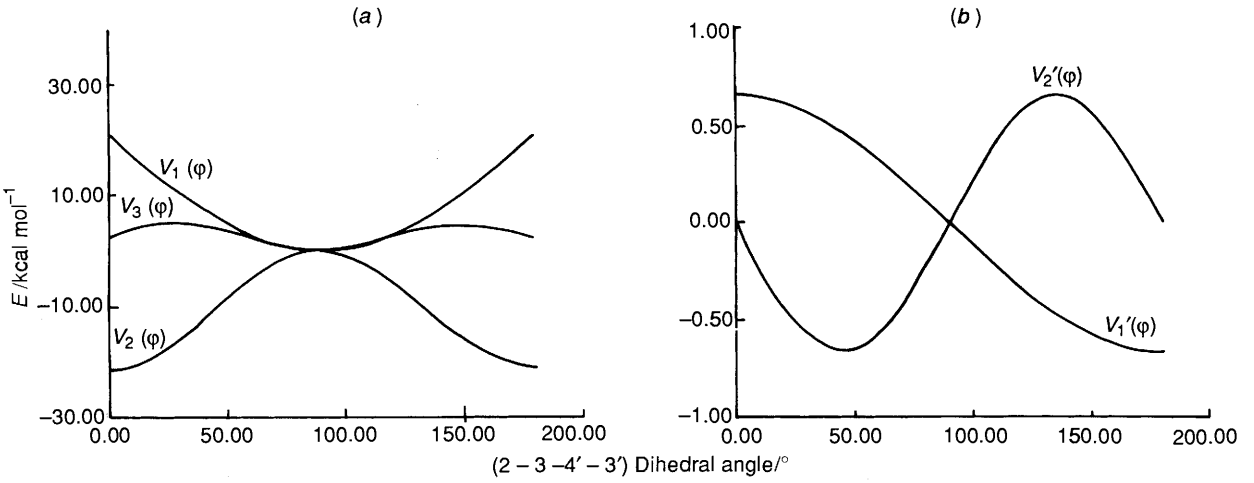

Fig. 4 Fourier decomposition functions for amrinone; (*a*) represents $V_1(\varphi)$, $V_2(\varphi)$, $V_3(\varphi)$; (*b*) represents $V'_1(\varphi)$, $V'_2(\varphi)$

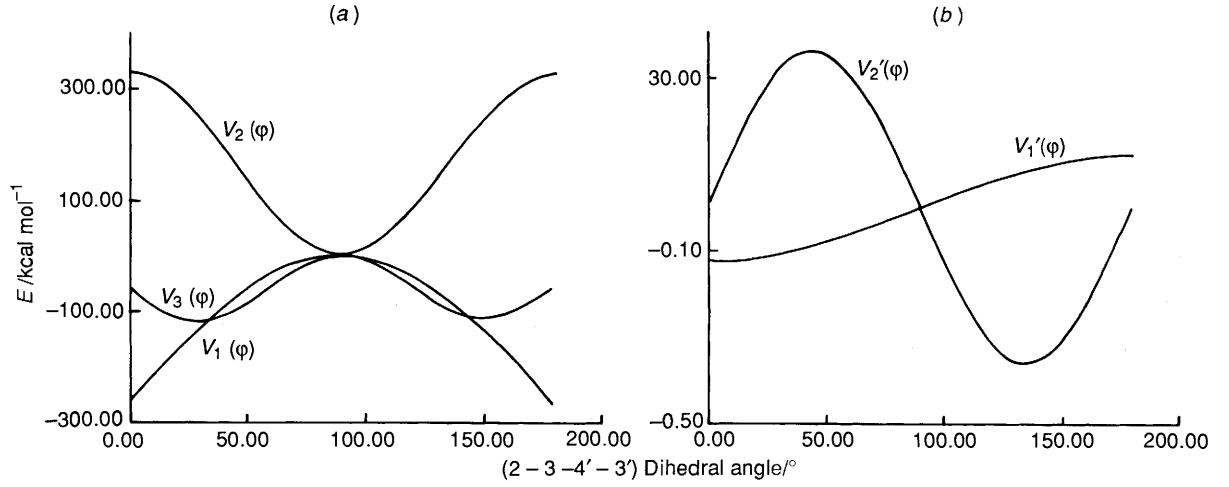

Fig. 5 Fourier decomposition functions for amrinone; (*a*) represents $V_1(\varphi)$, $V_2(\varphi)$, $V_3(\varphi)$; (*b*) represents $V'_1(\varphi)$, $V'_2(\varphi)$

$\frac{1}{2}V_n[1 - \cos(n\varphi)]$ (n = 1, 2, 3) and $V'_n(\varphi) = V'_n \sin(n\varphi)$ [n = 1, 2] of amrinone and milrinone are plotted in Figs. 4 and 5, respectively. The results clearly indicate that the one-, two- and three-fold components (V_n terms) of both amrinone and milrinone are responsible for the observed barrier. Since V'_n terms are very small, they make little contribution to the formation of a barrier [Figs. 4(*b*), 5(*b*)]. In the case of amrinone, the energy component analysis [Fig. 4(*a*)] shows that V_1 and V_2 are opposite in character and in the resulting energy profile (Fig. 3) their effects are not very prominent for the development of an energy barrier at α = 120°. The term V_3 is positive and small and is mainly responsible for the formation of a low energy barrier (*ca.* 1 kcal mol^{-1}). The energy component curves further indicate that the minimum energy points are generated by mutual cancellation of V_1, V_2 and V_3 terms. Thus one could conclude that barrier formation at α = 120° is mainly due to a bond–bond repulsion (V_3) term [16] and although this repulsion still contributes at α = 150 or 36.9°, the greater contribution of V_2, *i.e.*, stabilization due to interaction of π orbitals, causes these conformers to exist as a minimum energy form. The high energy form at α = 90 or 180° occurs mainly due to strong non-bonded interactions (Table 2). Similar effects have also been observed in the case of milrinone [Figs. 5(*a*) and (*b*)]. The difference is that the barrier occurs around the dihedral angle β = 90° and the energy profile is totally symmetric in nature. The bond–bond repulsion term in this case has a much greater contribution than amrinone and it is responsible for the formation of a much higher energy barrier (*ca.* 11 kcal mol^{-1}). Unlike amrinone, V_3 is also responsible for the stabilization of the milrinone conformer at β = 66.95 and 112.2°.

The theoretical conformational analysis of both amrinone and milrinone, as presented above, clearly indicates that these molecules prefer to exist in a twisted form. The molecular structure of milrinone[4] shows that this molecule exists in twisted form in the crystalline state, whereas the crystal structure of amrinone does not corroborate with the theoretical findings. If the greater cardiotonic potency of milrinone is attributed to its existence in a twisted form, the influence of this form on the cardiotonic potency needs to be explained. Also to be explained is whether the planar conformer, as shown by crystallographic data, has any effect on its cardiotonic property. The pharmacophoric patterns of the different conformations of these molecules might account for these factors. These points are discussed in the next section in terms of the molecular electrostatic potential of the molecules.

Molecular Electrostatic Potential of Amrinone and Milrinone.—Molecular electrostatic potentials (MEPs) have been calculated using the bond-increment technique of G. Náray-Szabó.[17] The π-coefficients for the unsaturated systems have been calculated by localizing the MNDO eigenvectors of suitable planar model molecules. The coefficients are supposed to be fully transferable. Calculated electrostatic potential maps generated for the planar and twisted conformers of amrinone are shown in Figs. 6–8. The contours have been drawn in terms of lines of equipotential energy in kcal mol^{-1} that would

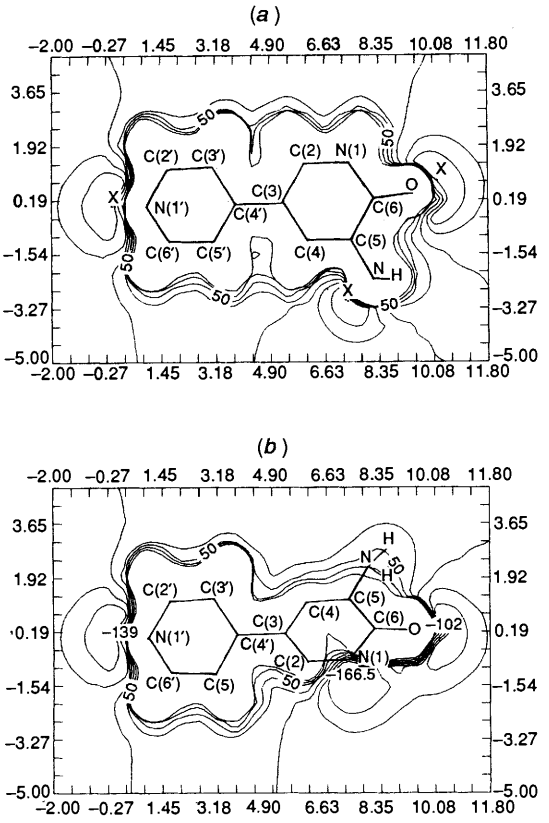

Fig. 6 MEP of amrinone for its (*a*) planar and (*b*) twisted conformer. The isoenergy contours (in kcal mol^{-1}) are in the plane of pyridine nucleus (z = 0.0). The crosses (×) indicate potential minima around N-1′ (−140), O (−105.7) and NH_2 nitrogen (−104), respectively.

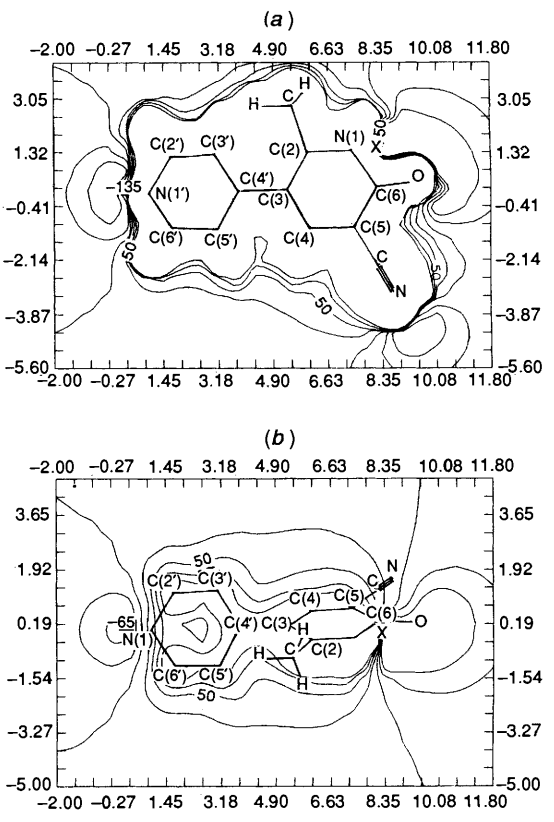

Fig. 7 MEP of milrinone for its (*a*) planar and (*b*) twisted conformer. The isoenergy contours (in kcal mol^{-1}) for the planar conformer is in the plane of the pyridine nucleus (z = 0.0) and the contours for the twisted conformer is drawn 0.9 Å above the pyridine plane. The crosses (×) indicate potential minima of −253 and −213 kcal mol^{-1} in Figs. (*a*) and (*b*), respectively.

be felt by a test positive charge placed at a specified point on the grid. Such an electrostatic field pattern can be called the 'interaction pharmacophore' and may be regarded as approximate measures of activity which are only valid at long range, since effects that occur upon close approach of an actual molecule *i.e.*, exchange repulsion, polarization and charge transfer are not considered.

MEP maps of the planar and twisted conformers of amrinone [Figs. 6(*a*) and 6(*b*)] indicate that they have distinct positive and negative potential zones separated by sinuous frontier lines. In the case of the planar conformer, there are three distinct negative potential zones around N-1′, the carbonyl oxygen at C-6 and the side chain amino nitrogen [C(5)–NH_2] atoms. In the case of the twisted conformer, the nature of the negative potential zone around N-1′ has not been changed, but the close proximity of the lone-pair electrons of the carbonyl oxygen around the amino nitrogen has broadened the negative potential zone around these atoms. The depth of the negative potential zone around the amino nitrogen has also been changed appreciably (−166.5 kcal mol^{-1}). These features clearly indicate that the electrostatic interaction in the case of the twisted conformer would be greater and thus this form would be more active than the planar one.

The nature of the MEP map of the planar conformer of milrinone [Fig. 7(*a*)] is similar to that of amrinone. In the case of milrinone, however, the negative potential zones are much deeper. The MEP map for the twisted conformer of milrinone is shown in Fig. 7(*b*). In order to visualize the lone-pair interactions of the carbonyl oxygen at C-6 and the nitrogen atom of the C-5 carbonitrile group the contours have been drawn 0.9 Å above the pyridine plane. A comparison of the MEP maps of the planar and twisted forms of milrinone clearly indicates that in the case of the twisted form the negative potential

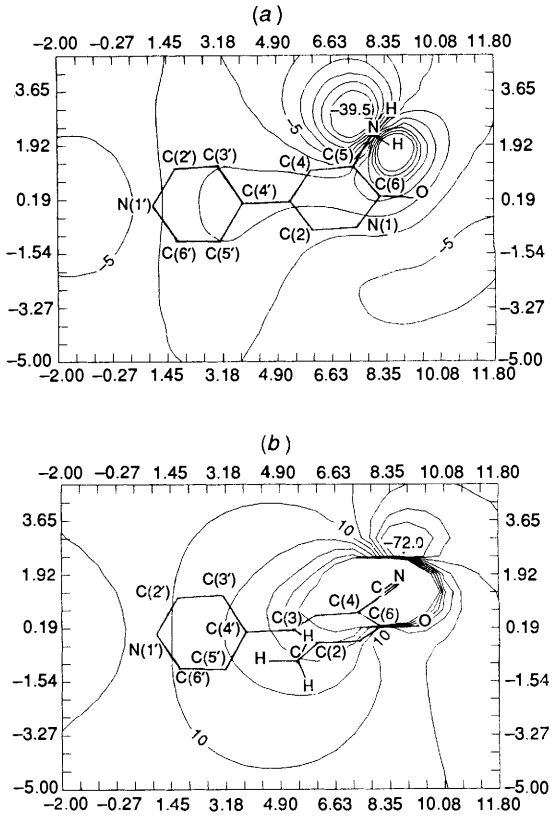

Fig. 8 MEP of (*a*) amrinone and (*b*) milrinone. The contours are drawn 3.0 Å below the pyridine plane (z = 0.0).

zone around the carbonyl oxygen and the cyano nitrogen is broadened due to the close proximity of the oxygen and nitrogen lone-pair electrons. The planar conformer of milrinone, cannot therefore be the pharmacologically active form because of its higher conformational energy (Table 1). The crystallographic results also show that milrinone exists solely in a twisted geometry. Thus MEP maps of the twisted conformers of amrinone and milrinone should be compared in order to account for their pharmacological properties.

From an examination of the 'interaction pharmacophore' representing the binding sites of the preferred conformations of the molecules under study, it is clear that the critical factor for the difference in potency between amrinone and milrinone is the depth of the negative potential zone around the carbonyl oxygen and the side chain nitrogen atom of the amino or cyano groups. The replacement of the NH_2 group by a C=N group at the C-5 position of milrinone increases the potential minima from -166.5 to -213.2 kcal mol^{-1}. The MEP map at -3.0 Å below the plane of the pyridine nucleus for the twisted conformers of both molecules [Figs. 7(*a*) and (*b*)] also shows that the depth of the negative potential zone around these atoms is greater for milrinone, indicating its higher potency.

Conclusions

The computational results of this theoretical investigation clearly indicate the greater flexibility of amrinone in comparison to milrinone. Thus, the greater cardiotonic potency of milrinone could be attributed to the reduced conformational flexibility due to the presence of a 2-methyl group. Further, if a molecular recognition pattern of these compounds is to be deduced from an electrostatic potential analysis so as to interpret the biological activity of these analogues, the effect of this torsional angle on the contour maps should be crucial. It has in fact been observed from MEP analysis that the torsion angle C(2)–C(3)–C(4′)–C(3′) affects the negative potential zone around the carbonyl oxygen and the amino/cyano nitrogen atoms at C-5. The MEP maps also confirm that the planar conformers of these molecules have little influence on the observed cardiotonic potency of these molecules.

The structural feature of the two bipyridine cardiotonics presented here is a schematic and qualitative one. Further sophisticated calculations including solvent effects are required to prove or disprove the proposed reasons for their biological potency. It also needs to be elucidated whether the model is sufficiently selective to distinguish between active and inactive compounds.

Acknowledgements

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