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Conformational Ensemble of Digoxin and Digitoxin and its Interamolecular Energy in Torsional Space

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ABSTRACT

Conformational ensemble of Digoxin and Digitoxin, the two prominent cardiac glycosides was developed by employing force fields based geometry optimization and energy minimization methods to find lowest energy conformers. The interamolecular energies of each lowest energy conformer obtained by various approaches were computed. The torsional space explored by the lowest energy conformers were calculated by measuring its rotatable bonds angle. Root mean squared deviation (RMSD) over all atoms in conformational space interspersed over lowest energy conformers was estimated to study the confidence of obtaining lowest energy conformers over converged iterations. We emphasized the role of force-fields and multiple steps of iterations for conformational space exploration in determining the lowest energy conformers.

KEYWORDS: *Conformational ensemble, Digoxin, Digitoxin, torsional space, force fields, RMSD.*

INTRODUCTION

Plant-derived digitalis cardiac glycosides such as Ouabain, Digoxin, Digitoxin, Stevioside, etc has been known for its application in congestive heart failure. These glycosides by binding to Na^+/K^+ - ATPase, the enzyme that maintains the normal gradients of Na^+ and K^+ across the plasma membrane in cardiac myocytes which in turn increases force of contraction of failing cardiac muscle and thereby reduces conduction rate [1, 2]. There are several pharmacokinetic properties related to Digoxin and Digitoxin. For example, Digoxin is completely absorbed in the gastrointestinal tract compared to Digitoxin [3] and Digitoxin has a large elimination half-life than Digoxin [4]. Another contrasting feature in the elimination scheme is that Digoxin is excreted from the body via kidneys whereas Digitoxin is eliminated through liver [5].

These structurally similar compounds were investigated by conformational analysis and energy minimization methods. We used molecular mechanics force fields by convergence at specific iteration steps to mine the lowest energy conformer in comparison with the possible optimized molecules generated using Monte Carlo search and distance geometry approaches. These conformational ensembles were cross validated by interamolecular energies and rotatable bond angular values distribution.

MATERIALS AND METHODS

Small molecule dataset and computational environment

The 2D structures of Digoxin (CID 2724385) and Digitoxin (CID 441207) were retrieved from NCBI Pubchem database in structure data format (SDF) [6]. All computational studies were carried out in a single machine running on Intel Core™ 2 Duo processor with 2 GB RAM and 148 GB hard disk with Microsoft Windows™ 7 Ultimate as the operating environment.

Geometry optimization using molecular mechanics force fields

The ligand dataset was subjected to geometry optimization using molecular mechanics (MM)

force fields using a utility in PyRx software from Scripps Research Institute [7]. The 2D configurations of Digoxin and Digitoxin were optimized using Merck Molecular Force Field 94 (MMFF94) [8] and Ghemical (a Tripos 5.2 like force field) force fields [9], respectively. The geometrical configurations were simultaneously evaluated using first-derivative technique, steepest descent to confirm the molecular motion progressions downhill on the energy surface. MMFF94 was primarily derived from high-quality computational quantum chemistry data and determined in a mutually consistent fashion which offers its applicability to a wide variety of chemical systems whereas Ghemical force field similar to Tripos 5.2 from Sybyl is an all atom force field and known for their computational speed compared to classical ones. Steepest descent optimization was introduced to monitor the following two conditions: the number of steps for update (one is chosen as this algorithm will update configurations approaching local minima and restricted to jump over local energy barriers) and the optimization terminates when energy difference of current configuration is less than 0.1 KJ/mol in comparison with previous configurations (this low energy barrier was chosen to oscillate motions down the barrier hill). We tried to investigate the preference of more than one local minimum (i.e., the occurrence of more than one conformer with large energy difference) in the above configurations by changing the number of iteration to 200, 500, 1000 and 10000 steps. All the optimized molecules were exported to hard disk in protein data bank (pdb) format using Open Babel format conversion program engineered in PyRx.

Monte Carlo approach of conformational search

The ligand dataset in simplified molecular input line entry specifcation (SMILES) format was specified as input to FRee Online druG conformation (Frog ver 2) server hosted at Mobyle portal [10]. Frog2 generates 3D conformation through graph decomposition method and searches for disambiguation isomers if chiral centers were unspecified followed by ring detection using DisambiGuate Automated Molecular Mechanics Optimization for *in silico* Screening (DG-AMMOS) [11] and assignment of protonation states using Open Babel [12]. Subsequently, the conformational ensemble was generated with the help of two stage Monte Carlo procedure. Monte Carlo, also known as random search in its first stage explores the conformational flexibility relied upon the limited number of representative atom type predefined dihedral angular values and checks for atomic positional and dihedral values specific redundancy to evade the biased conformational exploration thereby new conformers were stored and introduced in second stage. The combinatorial exploration was pruned by considering small atomic rotations within the stage one stored conformers and the lowest energy conformers were only requested to return in pdb format. The disambiguate run was enabled with number of maximum conformations to 1000 and an energy threshold of 50 Kcal/mol with default settings of other optional parameters.

Distance geometry based conformational search

The distance geometry based conformational search and mining of lowest energy conformers were executed using AMMOS program available at Mobyle portal [13]. AMMOS accept molecular inputs only in Sybyl Mol2 format and hence, we used Open Babel to convert the ligand dataset into Mol2 format by inclusion of hydrogens and Gasteiger charges. This preprocess step was performed due to inability of AMMOS to perform this task and this inclusion is required as parameters prior to submission. AMMOS is inspired by AMMP (Another Molecular Mechanics Program), a full-featured molecular mechanics, dynamics and modeling program [14] implemented with Gauss-Siedel Distance Geometry (GSDG), a distance geometry algorithm which takes into account bond, angle, hybridization, torsion, non-bonded atom electrostatics and van der Waals potential terms as its background. AMMOS take advantages of this algorithm to generate initial 3D conformations using conjugate gradient

optimization with AMMP force field with the following conditions: number of maximum iterations: 500 steps and a convergence scheme by energy threshold of 0.02 Kcal/mol/Å. Realistic conformers were only recovered and unrealistic configurations generated owing to gradient based optimization method were discarded as the ligand dataset constitute aromatic rings and inability of this optimization method to deal with rings.

Interamolecular energy calculation and molecular graphics

The interamolecular energy was calculated for lowest energy conformers recovered from Frog2 and AMMOS programs using Abalone software (release 2007) from Agile Molecule [15] whereas the lowest energy conformers obtained by other approaches mentioned above were reported by the software itself was considered for study. Interamolecular energy was calculated using AMBER94 force field [16] on a static environment with boundary conditions in vacuum. The lowest energy conformers were superimposed using YASARA View (academic license) [17] by chemically flipping equivalent functional groups to return root mean squared deviation (RMSD) value and graphs were produced using MS Excel plotting functions [18]. All the computed energies were manually converted into KJ/mol for ease of comparison.

RESULTS AND DISCUSSION

Geometry optimization using molecular mechanics force fields

The ligand dataset comprised of Digoxin and Digitoxin were geometrically optimized using MM force fields including MMFF94 and Ghemical, respectively. The interamolecular molecular energies obtained from lowest energy conformers with convergence obtained in 200, 500, 1000 and 10000 iterative steps were examined (**fig. 1**).

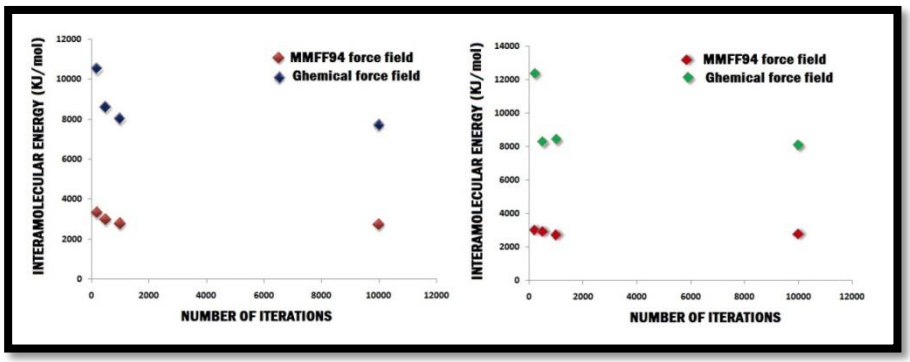

Fig. 1: Interamolecular energies of lowest energy conformers iterated at 200, 500, 1000 and 10000 steps for Digoxin (left) and Digitoxin (right)

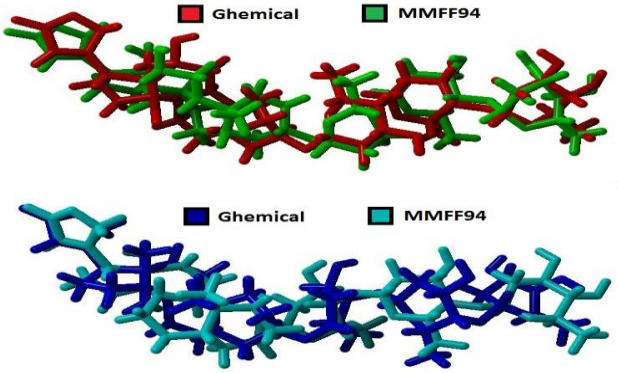

Fig. 2: The lowest energy conformer iterated at 10000 steps for Digoxin (up) and Digitoxin (bottom) using MM force fields

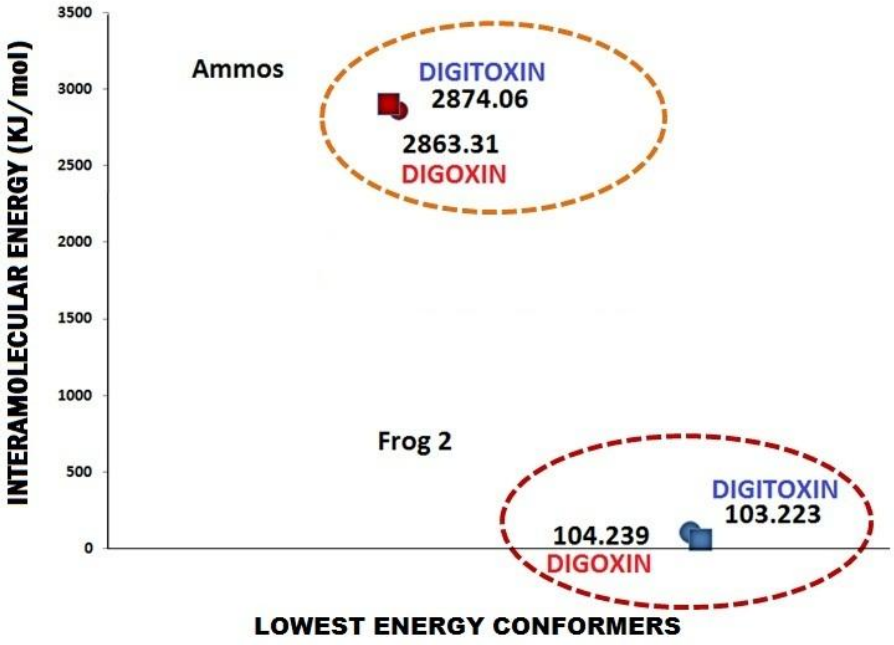

Fig. 3: Interamolecular energy clustered by cross force fields

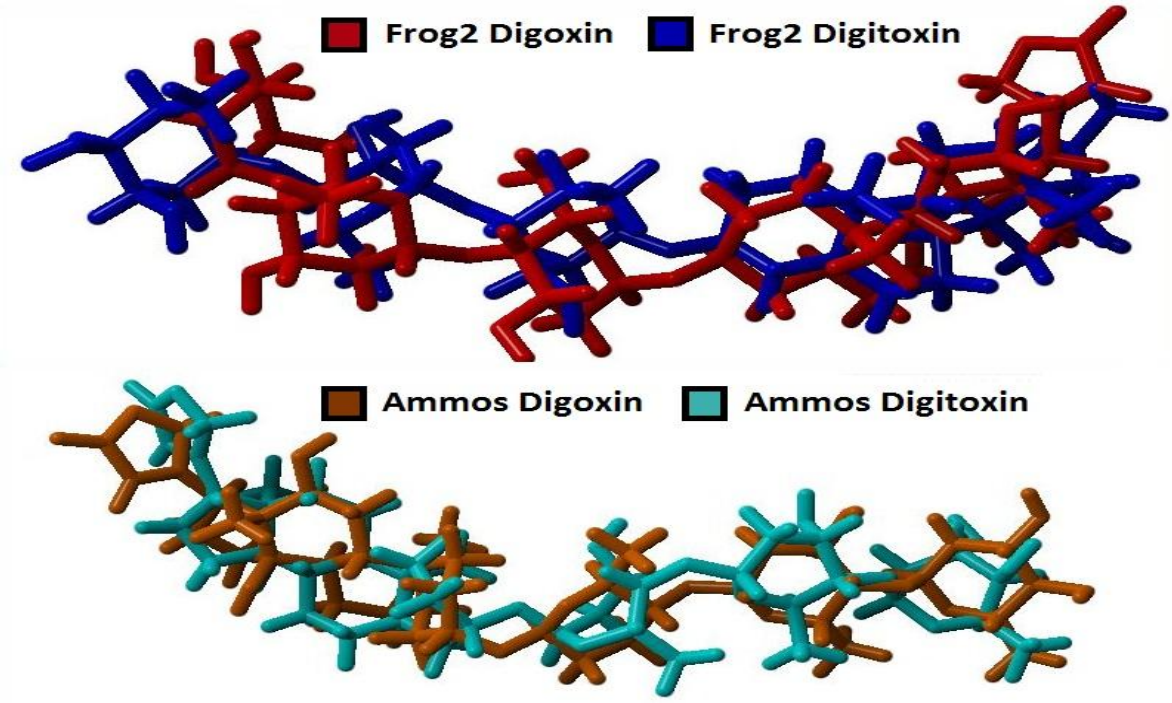

Fig. 4: Comparison of lowest energy conformers obtained via Frog2 (up) and Ammos programs (bottom)

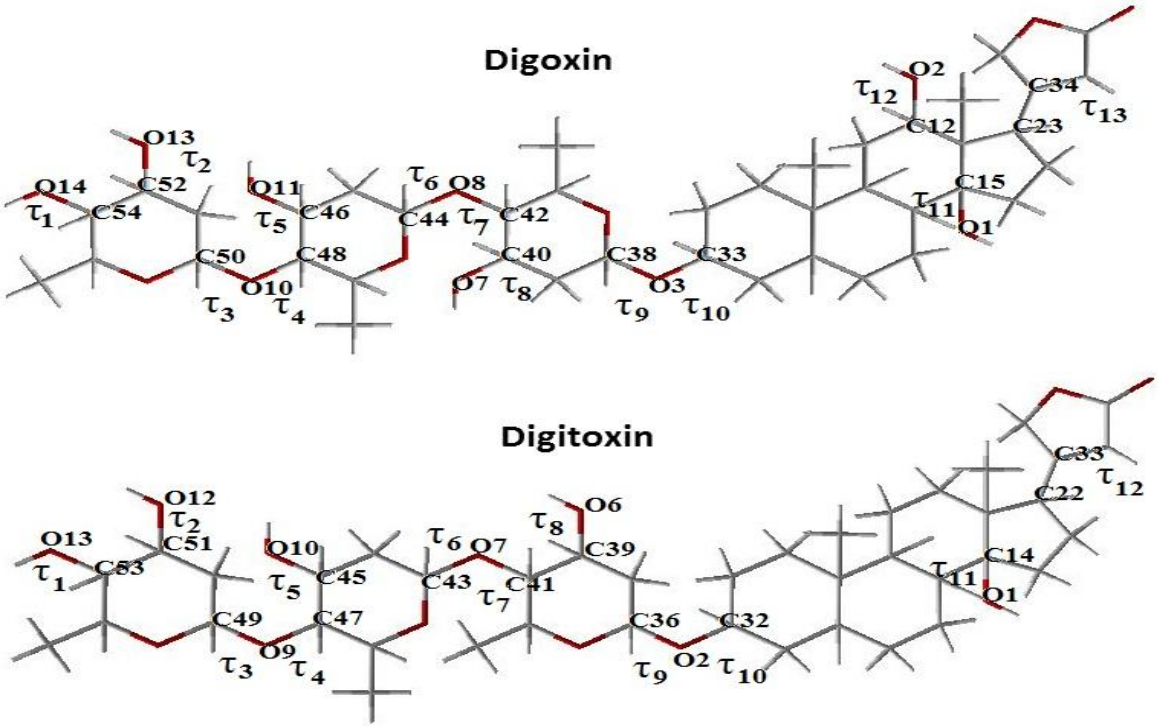

Fig. 5: Rotatable bond distribution of Digoxin (up) and Digitoxin (bottom)

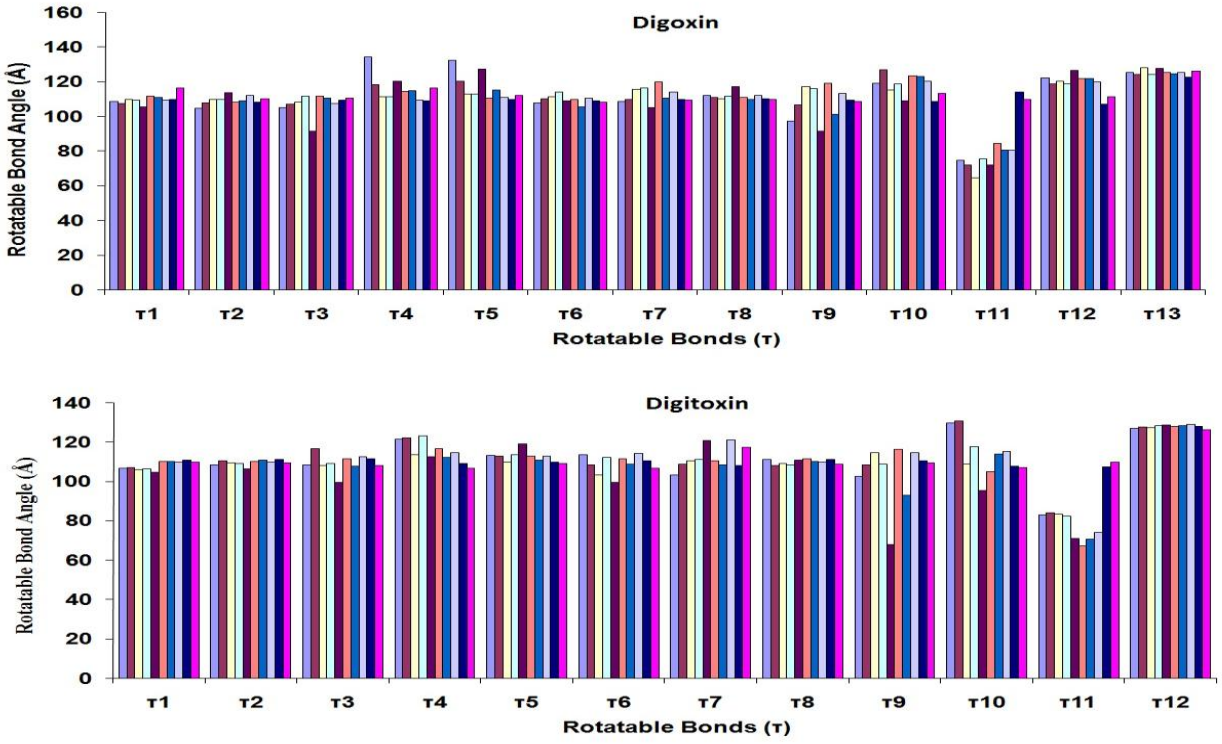

Fig. 6: Rotatable bond angular values of Digoxin (up) and Digitoxin (bottom) ensembles

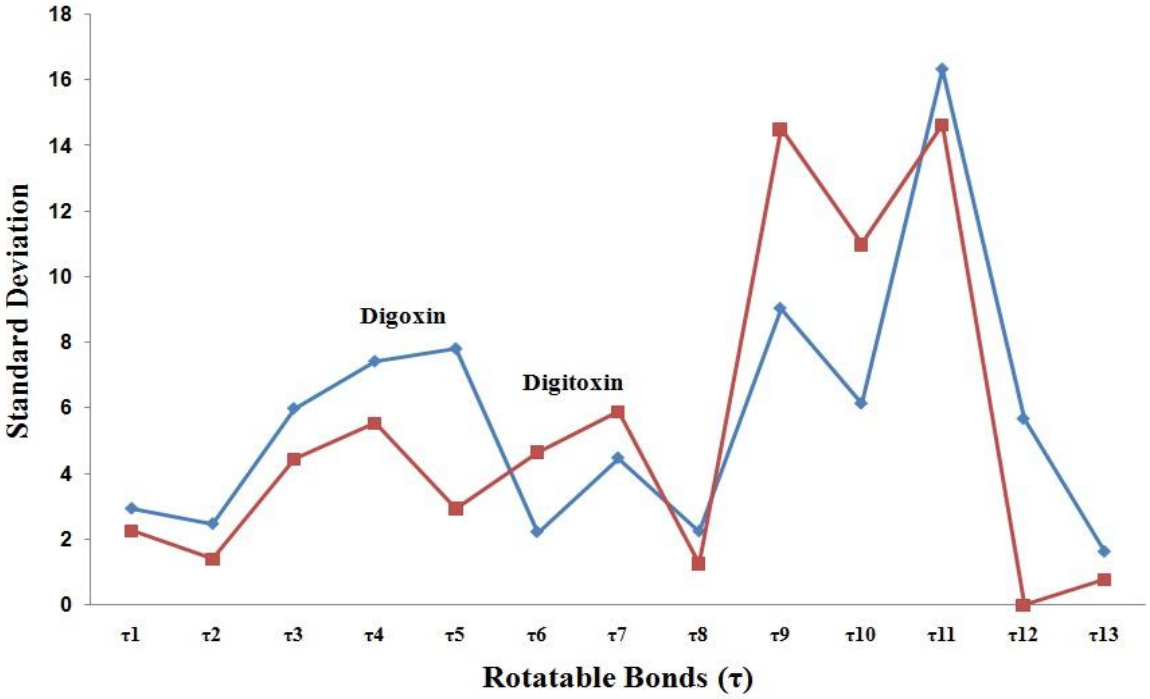

Fig. 7: Standard deviation of rotatable bond angular values of Digoxin and Digitoxin conformers

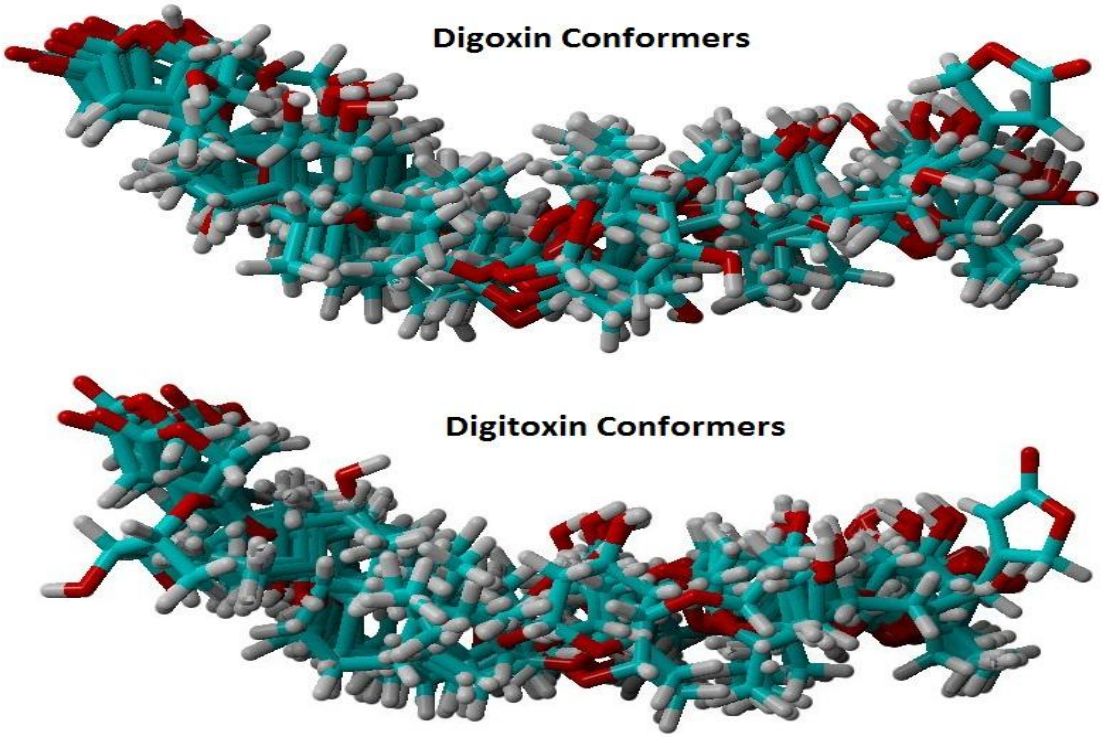

Fig. 8: Superimposed view of Digoxin and Digitoxin lowest energy conformers obtained by various Approaches

It can be observed that conformations obtained in small steps viz. 200 and 500 obtained by both force fields only moved a slight area along the energy barrier. Beside, the comparison of conformations for Digoxin and Digitoxin obtained at the 1000 and 10000

steps revealed the energy difference of 50.03 and 314.28 KJ/mol, respectively (**Table 1**). Hence, it is evident that a large iterative step is required to obtain lowest energy conformer irrespective of the force field employed. Superimposition of lowest energy conformers converged at 10000 steps using the above two force fields provided a RMSD value of 1.9793 Å for Digoxin and 2.1463 Å for Digitoxin, respectively. This cross force field comparison enabled to visualize the molecular similarity graphically (**fig. 2**) but no attempt was made to compare energy values obtained by using these force fields as the energy term is directly dependent upon the descriptors in the force field.

Monte Carlo approach of conformational search

The dataset in SMILES format representing 2D layout was subjected to conformational search using Monte Carlo approach implemented in Frog2 program and a single lowest energy conformer was retrieved for Digoxin and Digitoxin. The interamolecular energy of these two molecules were very close to each other as Digoxin energy was found to be 104.239 KJ/mol whereas Digitoxin energy was 103.223 KJ/mol and the energy difference was only 1.016 KJ/mol (**fig. 3**) and obtained a RMSD value of 3.1115 Å (**fig. 4**), respectively. It is apparent that close energies were observed due to molecular similarity and achieved larger RMSD (>3Å) due to chemical functionalities and its arrangements.

TABLE 1. Interamolecular Energy of Lowest Energy Conformers Obtained by Various Approaches

Molecule / Software	Force Field	Interamolecular Energy (KJ/mol)	Settings
Digoxin			
PyRx	MMFF94	3330.28	200 iterations
PyRx	MMFF94	2982.08	500 iterations
PyRx	MMFF94	2783.31	1000 iterations
PyRx	MMFF94	2733.28	10000 iterations
PyRx	Ghemical	10524.28	200 iterations
PyRx	Ghemical	8610.09	500 iterations
PyRx	Ghemical	8034.36	1000 iterations
PyRx	Ghemical	7720.08	10000 iterations
Frog2	Monte Carlo search	104.239*	No convergence
Ammos	GSDG	2863.31*	No convergence
Digitoxin			
PyRx	MMFF94	3037.95	200 iterations
PyRx	MMFF94	2937.82	500 iterations
PyRx	MMFF94	2750.96	1000 iterations
PyRx	MMFF94	2791.23	10000 iterations
PyRx	Ghemical	12377.89	200 iterations
PyRx	Ghemical	8304.39	500 iterations
PyRx	Ghemical	8453.47	1000 iterations
PyRx	Ghemical	8108.86	10000 iterations
Frog2	Monte Carlo search	103.223*	No convergence
Ammos	GSDG	2874.06*	No convergence

*Calculated using Abalone package.

Distance geometry based conformational search

The distance geometry based conformational search was adopted using AMMOS program to map a lower energy conformer along the energy barrier. Customized ligand dataset in Mol2 format was specified as input and only realistic configurations were recovered. The interamolecular energy of these molecules were very far to each other as Digoxin and

Digitoxin energies were computed as 2863.31 and 2874.06 KJ/mol, respectively. The energy deviation of 10.75 KJ/mol (**fig. 3**) and RMSD value of 4.4589 Å (**fig. 4**) showed that a conformational space exist between the two analogues. Monte Carlo search provided two possible optimized conformers which were very similar to each other as evident from the energy difference and RMSD over matched atoms. Contradictory to this case, high energy difference (>5 KJ/mol) and large RMSD value (>3 Å) was reported by lowest energy conformers recovered from AMMOS program which clearly indicated that energy constraints and molecular similarity indices were secondary to the molecular features when a large conformation space exist. Hence, we step forwarded to observe the torsional space regulated by rotatable bonds in these molecule.

TABLE 2: Rotatable Bonds Distribution in Digoxin

Digoxin	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_7	τ_8	τ_9	τ_{10}	τ_{11}	τ_{12}	τ_{13}
200 Iterated MMFF4	108.42	104.69	105.25	134.15	132.55	107.79	108.44	112.15	97.36	119.00	74.85	122.38	125.38
500 Iterated MMFF4	107.32	107.64	107.13	118.23	120.45	110.22	109.88	111.05	106.58	126.96	71.85	118.78	124.32
1000 Iterated MMFF4	109.73	109.96	108.28	111.29	112.96	111.25	115.48	110.09	117.03	115.29	64.48	120.45	128.12
10000 Iterated MMFF4	109.34	109.97	111.66	111.15	112.81	113.92	116.28	111.57	116.11	118.78	75.45	118.70	124.15
200 Iterated Ghemical	105.53	113.72	91.39	120.30	127.32	108.94	104.98	117.30	91.67	109.01	72.21	126.46	127.54
500 Iterated Ghemical	111.77	108.34	111.57	114.63	110.47	109.65	120.08	110.84	119.22	123.30	84.37	121.69	125.29
1000 Iterated Ghemical	110.96	108.86	110.40	114.65	115.15	105.56	110.59	109.73	101.11	122.83	80.67	121.78	124.52
10000 Iterated Ghemical	109.53	112.01	107.29	109.41	111.08	110.41	114.03	112.24	113.41	120.40	80.64	120.05	125.27
Frog2	109.64	108.32	109.51	108.97	109.68	109.12	109.82	110.04	109.55	108.45	113.94	106.88	122.68
Ammos	116.56	110.23	110.75	116.59	112.30	108.29	109.27	109.91	108.76	113.40	109.90	111.44	126.20

Torsional space of Digoxin and Digitoxin

The torsional space acquired by molecule is dependent upon the number of rotatable bonds and its translation around chemical space and the number of possible conformers is dependent by this count too. The rotatable bonds in Digoxin and Digtoxin were graphically illustrated (**fig. 5**). We calculated 13 rotatable bonds in Digoxin and 12 in Digitoxin using standard structure visualizers and measured its angular values manually (**Table. 2 and 3**). The following bonds associated with rotation of Digoxin: C52-C54-O14, C51-C52-O13, C51-C50-O10, C46-C48-O10, C48-C46-O11, O9-C44-O8, C40-C42-O8, C42-C40-O7, O5-C38-O3, C32-C33-O3, C24-C15-O1, C18-C21-O2 and C23-C34-C36 while the bonds contributing to rotation of Digitoxin are as follows: C51-C53-O13, C50-C51-O12, C50-C49-O9, C45-C47-O9, C47-C45-O10, O8-C43-O7, C40-C41-O7, C38-C39-O6, C38-C36-O2, C28-C32-O2, C24-C14-O1 and C22-C33-C35. This analysis helped us to identify simulations in particular rotatable bonds. For instance, the τ_{11} bond in both Digoxin and Digitoxin ensemble varies in their bond angle simply due to its terminal location

and has greater degrees of freedom (**fig. 6**). The τ_4 bond in Digoxin conformations has a maximum angle of 134.15 Å and minimum of 108.97 Å and an averaged angle of 115.937 Å which makes its terminally attached aromatic ring to occupy variable conformations. The τ_9 and τ_{10} bonds in Digoxin series is very crucial as it can orient molecules into equally halved variable conformers as its angle can rotate a maximum of 117.03 Å (**fig. 6**). Similarly, the τ_9 and τ_{10} bonds in Digitoxin ensembles orients molecules into equally variable conformers. We estimated the standard deviation of all the rotatable bonds to measure the variability or diversity in the torsional space (**fig. 7**). The τ_9 and τ_{10} bonds in the ligand dataset with a standard deviation of 9.0354 and 6.13667 for Digoxin and 14.5156 and 10.9962 for Digitoxin and a superimposed ensemble of lowest energy conformers obtained from various approaches clearly indicates the chemical space acquired by it (**fig. 8**). Although the Taniomoto coefficient for Digoxin and Digitoxin is 0.898438, it is evident that torsional space and its interamolecular energies are variable. We also showed that conformational ensemble over large iterations and cross- force fields evaluation helps us to guide the degrees of freedom in the torsional space. By applying the force fields and interamolecular energies to the knowledge of isolating a lowest energy conformer is suggested here which also depicts the conformational perturbations in crucial rotatable bonds. We also suggest that when docking a protein with a 2D small molecule, the lowest energy conformer should be considered by interpreting the torsional roots as observed in AutoDock Tools version 4.2 as well as to apply the energy minimization or geometry optimization programs without any convergence to gain confidence. We also insist to evaluate different force fields and analyze all the resultant configurations to gain confidence of obtaining a lower energy conformer.

TABLE 3: Rotatable Bonds Distribution in Digitoxin

Digitoxin	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_7	τ_8	τ_9	τ_{10}	τ_{11}	τ_{12}
200 Iterated MMFF4	106.69	108.27	108.38	121.37	113.11	113.73	103.21	111.34	102.43	129.79	82.98	127.00
500 Iterated MMFF4	106.95	110.57	116.69	122.31	112.96	108.45	108.64	108.06	108.28	130.86	84.02	127.50
1000 Iterated MMFF4	106.05	109.45	108.07	113.75	109.76	103.24	110.50	109.25	114.64	108.78	83.29	127.29
10000 Iterated MMFF4	106.29	109.01	109.17	123.05	113.74	112.33	111.08	108.58	108.69	117.82	82.50	128.29
200 Iterated Ghemical	104.52	106.39	99.52	112.64	119.21	99.66	120.72	110.97	68.05	95.37	71.12	128.54
500 Iterated Ghemical	110.22	110.26	111.67	116.67	112.75	111.41	110.66	111.61	116.23	104.83	67.18	127.82
1000 Iterated Ghemical	109.99	110.86	107.75	112.25	110.88	108.80	108.28	110.03	93.16	114.08	70.54	128.18
10000 Iterated Ghemical	109.78	109.93	112.64	114.54	113.02	114.27	121.25	109.64	114.67	115.34	74.06	129.04
Frog2	110.89	111.15	111.38	109.16	109.65	110.50	108.01	111.12	110.51	107.68	107.24	127.82
Ammos	109.96	109.31	107.98	106.79	108.95	106.60	117.29	108.83	109.31	107.16	109.75	126.41

CONCLUSION

Conformational studies of Digoxin and Digitoxin was studied by force fields based geometry optimization and energy minimization methods to identify lowest energy conformers. The 3D configurations obtained were evaluated based on the interamolecular energies and its statistics beyond the torsional space. We suggest the application of cross force fields and optimization scheme along with maximum convergence rate to obtain a lower energy conformer and it can be easily interpreted by using RMSD or other parameters to conclude.

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