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Andrographolide Analogues: Pharmacophore Modeling and 3D QSAR analysis

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Subject: Medicinal chemistry

Abstract
Andrographolide a natural product obtained from *Andrographis paniculata* shows a wide range of Biological activity. Andrographolide analogues can inhibit α-Glucosidase enzyme. Using PHASE module of Schrödinger LCC a Pharmacophore Based 3D QSAR analysis was carried out for 23 of the Andrographolide analogues by dividing them in test and training set. A statistically significant PLS model r^2 = 0.9597, Q^2 = 0.9309, RMSE= 0.0937 and Pearson-R = 0.97 was selected to explain the structure activity relationship quantitatively.

Keywords: *Andrographis paniculata; Andrographolide; PHASE- Schrödinger; α-Glucosidase enzyme inhibitor; Pharmacophore; 3D QSAR*

Introduction

Andrographis paniculata (Burm.f.) Wall ex Nes (acanthaceae) are used as medicinal herb for the treatment of various disease [1], it is also used as a traditional medicine in various countries of Asia. In India it is known as kalmegh [2]. The crud extract of kalmegh shows anti-microbial, anti-inflamatory, hepato protective, anti tumor, anthelmintic and immunimodulatory activity. Recently *Wang et al. (2010)* found out the anti HIV activity of Andrographolide analogues [3]. α- Glucosidase is an enzyme plays an important role to reduce the blood glucose level in Type II diabetes mellitus. Acarbose, Miglitol, Voglibose are the drugs currently used as α-Glucosidase enzyme inhibitors [4]. But these drugs are not cost effective. This creates a necessity to discover new cost effective drugs for the treatment of Type II diabetes mellitus. Targeting α-Glucosidase enzyme may be an effective approach to design an effective drug for this purpose. The natural products Andrographolide possess α-Glucosidase inhibitory action. Some of its novel analogues possess more potent and safer α-Glucosidase inhibitory property. Efforts had been made to establish a relation between the structures of Andrographolide analogues and their biological activity to facilitate the drug discovery process [5-11]. In the present scenario Quantitative structure activity relation ship is a widely used tool to establish a relationship between the chemical structure and their biological properties. After suitable generation of a statistically significant QSAR model one can predict the biological activities of an untested compound. Now days QSAR technology producing more encourageous results for designing newer drugs against AIDS, Cancer etc [12]. PHASE module of Schrödinger LCC is a commercial program used to carry out this pharmacophore based 3D QSAR analysis, which high light the structural features required for α-Glucosidase inhibition which can be useful for further design of more potent α-Glucosidase inhibitors.

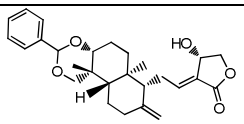
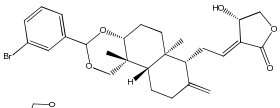
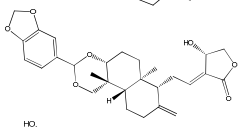
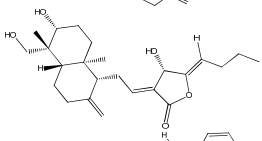
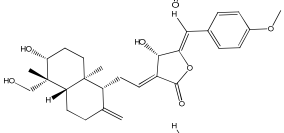
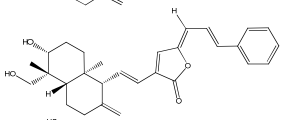
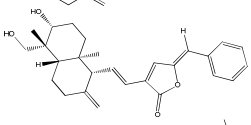
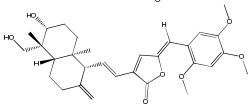
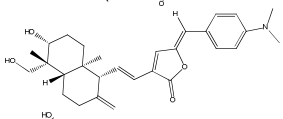
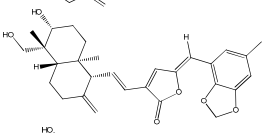
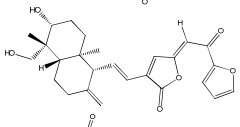
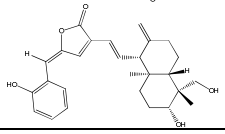
Materials and Methods

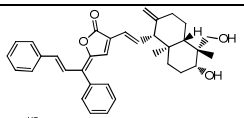
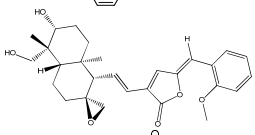
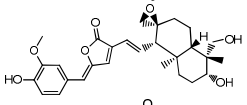
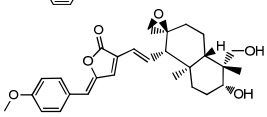
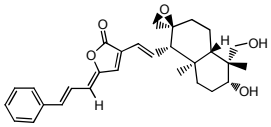
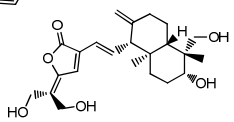
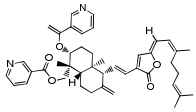
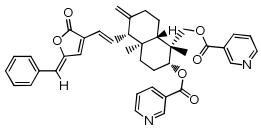
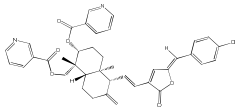
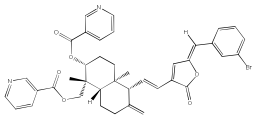
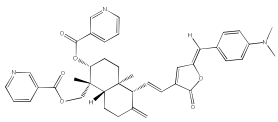
Database Generation and software

The structure of Andrographolide analogues (Table 1) and their activity data in the form of pIC50 were taken from literature [13-16]. All the structures are sketched and converted in 3D format by using Chem draw ultra 8.0 and imported to MASSTRO 9.1 of Schrödinger LCC [17] running over Windows Vista Home basic operating system. Using LigPrep module of Schrödinger with OPLS_2005 force field [18] all

the molecules were prepared for further calculation and model generation. Potential energy was calculated by using Jaguar module of Schrödinger LCC which is a high concert abintio module for both gas and solution phase recreation. Jaguar proceeds faster than the other conventional methods and it makes more possible to carry out more calculations at a single time [19]. P^s 6-31G basic set was used for the calculation using DFT (Density functional Theory) and B3LYP hybrid.

Table 1: Data Set and activity

Ligand Name	STRUCTURE	pIC_{50}	QSAR Set	Predicted Activity
1		3.921	training	3.98
2		3.959	training	3.97
3		3.959	test	3.99
4		3.959	training	3.98
6		3.971	training	4.24
7		3.983	training	4.12
8		3.996	training	4.06
9		4	training	4.12
10		4	training	4.02
11		4	test	4.04
13		4	training	4.02
14		4	test	4.11

15		4.076	training	4.00
16		4.155	training	3.91
17		4.237	test	4.04
18		4.237	training	3.98
19		4.444	training	4.59
20		4.553	training	4.88
21		4.602	training	5.04
22		4.796	test	4.76
23		4.854	test	4.74
24		4.959	training	4.49
25		5.222	training	4.95

Generation of Common Pharmacophore and QSAR model generation

The common pharmacophore hypothesis was carried out by PHASE module of Schrödinger. Conformational space was explored through combination of Monte-Carlo Multiple Minimum (MCMM) / Low Mode (LMOD) with maximum number of conformers 1000 per structure and minimization steps 100 [20-21]. Each minimized conformer was filtered through a relative energy window of 50 kJ/mol and redundancy check of 2Å in the heavy atom positions. Common

pharmacophoric features were then identified from a set of variants-a set of feature types that define a possible pharmacophore-using a tree-based partitioning algorithm with maximum tree depth of five with the requirement that all actives must match. After applying default feature definitions to each ligand, common pharmacophores containing five sites were generated using a terminal box of 1 Å. Scoring of pharmacophore with respect to activity of ligand was conducted using default parameters for site, vector and volume terms (**Table 1**). For QSAR model development, van der Waals models of the aligned training set (70% of total data set) molecules were placed in a regular grid of cubes, with each cube selected zero or more 'bits' to account for the different types of atoms in the training set that occupy the cube. This representation gives rise to binary- valued occupation patterns that can be used as independent variables to create partial least-squares (PLS) QSAR models. Atom-based QSAR models were generated for hypotheses using the 16-member training set using a grid spacing of 0.5 Å. The best QSAR model was validated by predicting activities of the 6 test set compounds.

Scoring Pharmacophores according to actives and inactives

The pharmacophore hypotheses were scored pertaining to the active ligands. To ensure that no inappropriate pharmacophore is inside the survived pharmacophore models least squares site-to-site alignment is considered. Now the scoring of the pharmacophore hypotheses was done in relation to the information from the active ligands considering various geometric and heuristic factors .The alignment to a reference pharmacophore is considered according to RMSD of the site points and the average cosine of the vectors keeping their tolerance 1.2 Å and 0.5 respectively was set. To preferentially get the reference ligand from the most active set the ones scoring the upper 10 % was considered for score calculation. For further refinement volume scoring is also done in order to measure quantitatively of how each non-reference ligand is superimposing with the reference ligand, in account of Vander Waals models of the structures and taking into account all heavy atoms of the active ligands. Here the cutoff for volume scoring was kept at 1.00 for the non reference pharmacophores. To ensure the lowest energy ligands for better binding to be incorporated in the best pharmacophore the relative conformational energy of the reference ligand was constrained to 0.000. Thus we generated the survival active scores for the pharmacophore hypotheses. A ligand can be inactive due to number of ways but for successful implementation of only those characters which are important for good binding we need to incorporate the knowledge why the inactive molecules are inactive. This would make our pharmacophore models a better one having ability to distinguish between an active and an inactive molecule. This score inactive is calculated with the help of fitness score which is assesed with the same constraints as that of score active. A good hypothesis has a low fitness score multiplied by a user adjustable factor which was set to default mode.

3D-QSAR model generation

We generated atom based 3D QSAR model of pharmacophore hypotheses. We choose to build the ligand based 3D QSAR model as our data set ligands showed quite very good alignment as it consisted of a large variety of derivatives of a parent molecule. It's also true that atom based 3D QSAR model provides more chemical significance than pharmacophore based 3D QSAR model can give which only depends upon pharmacophore sites for alignment to the hypothesis selected. This is because of the fact that atom based 3D QSAR takes the total molecule and facts like probable steric hindrance with the receptor site can be taken into account while building a model with this atom based approach. The PHASE algorithm uses a very versatile approach for the development of 3D QSAR model. It considers a rectangular grid of 1 Å grid distance in a 3D space. Thus it creates cubes of said dimension in the 3D space. The atoms of the molecules which are considered as overlapping Vander Waal spheres fall inside these cubes depending on the volume of the atomic spheres. These occupied cube spaces are termed as volume bits. A volume bit is allocated for each different class of atom that occupies a cube. There are six atom classes two hydrogen bond acceptor (A), one positively ionizable (P) and two aromatic ring (R) used for classifying the atom characteristics. The total number of volume bits consigned to a specified cube is based on how many training set molecules

occupy that cube. A single cube may represent the occupation by one or various atoms or sites, and even those from the same molecule or may be from unlike molecules of the training set. Thus A molecule may be represented by a binary string concurrent to the occupied cubes, and also the various types of atomic sites that exist in those cubes. To create an Atom based QSAR model, these volume bits which encodes the geometrics and chemical characteristics of the molecule are regarded as independent variables in PLS (Partial Least square) regression analysis. For generating a predictive QSAR model we have to select 3 number of PLS factor. The maximum PLS factor that can be taken is N/5 where N is the number of ligands present in the training set.

Result and Discussion:

The aim of the study is to elucidate the 3D structural features of clinically significant anti-tubercular agents crucial for effective therapy against M/XDR TB, by generating 3D pharmacophore and to quantify the structural features of Andrographolide derivatives essential in biological activity by generating atom based 3D QSAR model. For the pharmacophore modeling and QSAR studies we have used Phase module of Schrodinger suite. Hence we have used conformation that suggested by the hypothesis for generating 3D QSAR model that identifies overall aspects of molecular structure that govern the activity**.** For the generation of pharmacophore model we have considered 25 compounds. We used minimum sites 4 and maximum sites 5 to have optimum combination of sites or features common to the active compounds. The molecules were classed out into actives and in-actives based on activity threshold for identifying the pharmacophore features (**Figure-1**) considering the highest active molecule.The pharmacophore Hypotheses were developed. On the basis of scoring and re-scoring of active and inactive molecules AHHRR.16 hypothesis was selected for aligning the molecules. The scoring algorithm includes contributions from the alignment of site points and vectors, volume overlap, selectivity, number of ligands matched, relative conformational energy, and activity. However these pharmacophore models should also discriminate between active and inactive molecules. It is true that the hypothesis incomplete if it lacks either a critical site that explains the binding or information on what prevents inactive from binding. To identify the pharmacophore models with more active and less inactive features among these models, they were mapped to inactive compounds and scored. If inactive score well, the hypothesis could be invalid because it does not discriminate between actives and inactive. Therefore adjusted survival score was calculated by subtracting the inactive score from survival score of these pharmacophores

All the molecules showed good alignment with good fitness score ranging from 3.00 (for highest active) to 1.84 (for lowest active). The three dimensional QSAR analysis was carried out based on the pharmacophore alignment. The scoring algorithm includes contributions from the alignment of site points and vectors, volume overlap, selectivity, number of ligands matched, relative conformational energy, and activity.

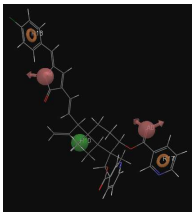


Figure1: Developed Pharmacophore

However these pharmacophore models should also discriminate between active and inactive molecules. It is true that the hypothesis incomplete if it lacks either a critical site that explains the binding or information on what prevents inactive from binding. To identify the pharmacophore models with more active and less inactive features among these models, they were mapped to inactive compounds and scored. Pharmacophore based QSAR do not consider ligand features beyond the pharmacophore model, such as possible steric clashes with the receptor. This requires consideration of the entire molecular structure, so an atom-based QSAR model is more useful in explaining structure activity relationship. In atom-based QSAR, a molecule is treated as a set of overlapping van der waals spheres. Each atom (and hence each sphere) is placed into one of six categories according to a simple set of rules: hydrogens attached to polar atoms are classified as hydrogen bond donors (D); carbons, halogens, and C–H hydrogens are classified as hydrophobic/non-polar (H); atoms with an explicit negative ionic charge are classified as negative ionic (N); atoms with an explicit positive ionic charge are classified as positive ionic (P); non-ionic atoms are classified as

electron-withdrawing (W); and all other types of atoms are classified as miscellaneous (X).

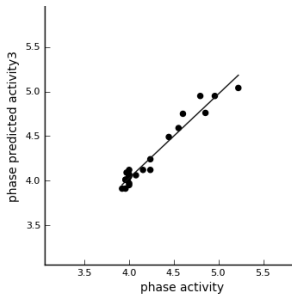


Figure 2 Actual Vs Predicted activity

The variants with a site score 0.92, vector score 0.98, and volume score of 0.90 was chosen to be the common pharmacophore hypothesis. The pharmacophore hypothesis AHRRRR with all active molecules aligned. All the molecules in the active set/modeled molecules matched with the hypothesis **AHHRR.16**. This pharmacophore hypothesis was then used for the generation of QSAR model.

For the QSAR model generation, non modeled (inactive or moderately active) molecules in the dataset were then aligned based on the matching with at least three of the pharmacophoric features. The dataset was randomly divided into a training set of 55 compounds and 08 in the test set with a bias given to the structural diversity in both the training and test set so as to form the standard 4:1 training set to test set ratio for a QSAR study. The PHASE statistical analysis for each of the test set selection methods is summarized in Table 2. The validity of each of the models was predicted from the calculated correlation coefficient for the randomly chosen test set comprising of diverse structures Figure 3. The squared correlation for the test set (random selection (R^2_{pred}=0.98)) confirms the good predictability of the final QSAR model for the test set.

Analysis of PHASE 3D-QSAR Model

Figure 3 shows the volume occlusion maps for the atom-based PHASE 3D-QSAR model (donor, hydrophobic, and electronegative) represented by color codes.

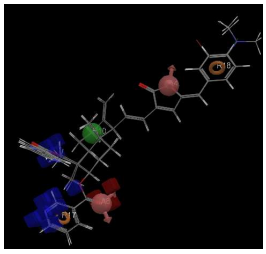


Figure 3: Counter Map

These maps represent the regions of favorable and unfavorable interactions. The volume occlusion maps of hydrogen bond donor describe the spatial arrangement of favorable hydrogen bonding interactions to acceptor groups of the target protein. Hydrophobic volume occlusion maps from PHASE 3-D QSAR model. The map showed a big red colored region indicating that an increase in the hydrophobicity in this region is expected to improve the activity of the Andrographolide based molecules. A blue color contour opposite to that of black disfavors the placement of hydrophobic groups.

Conclusion

3- D QSAR study was performed on the series of Andrographolide derivatives as a alpha glucosidase inhibitor by generating volume occupied maps, which demonstrated that the activity may be increased by substituting the donor groups the with the binding site of the receptor. Placement of the hydrophobic groups at the particular position of phenyl ring of the Andrographolide may results in increase in the activity. Also demonstration of the pharmacophore hypothesis AHHRR.16 used for QSAR model to that of the binding mode of the Andrographolide which validates the pharmacophore hypothesis. This study can be further use for the synthesis of newer and may be more potent derivatives for developing newer therapy against diabetes.

Acknowledgment

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Table 2: QSAR MODEL

ID	Factors	SD	R^2	F	P	Stability	RMSE	Q^2	Pearson-R
AAHRR.377	1	0.1811	0.8052	62	1.05E-06	0.9638	0.1652	0.785	0.9448
	2	0.1424	0.8875	55.2	2.28E-07	0.9401	0.1032	0.9162	0.971
	3	0.0885	0.9597	103.2	2.57E-09	0.8229	0.0937	0.9309	0.9781

"Cite this article"

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