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Human Androgen Receptor Inhibitors: Computational 3D QSAR Studies to Design Lead Compounds for Treatment of Prostate Cancer

[İnsan Androjen Reseptör İnhibitörleri: Prostat Kanseri Tedavisinde Kurşun Bileşikleri Tasarımında Sayısal 3D QSAR Çalışmalar]*

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ABSTRACT

Objectives: The inhibition of Androgen receptors have been vigorously pursued as a promising target for the treatment of prostate cancer. A set of 40 training set compounds and 20 test set compounds reported as Androgen receptors inhibitors were analyzed by employing the molecular field analysis (MFA) and Receptor surface analysis (RSA) techniques to investigate the structural requirements for various analogues to inhibit Androgen receptors and to derive a highly predictive model used for the designing of novel Androgen receptors inhibitors.

Material and Methods: Pharmacophore generation and 3D-QSAR studies have been performed for developing novel Androgen Receptors inhibitors using Cerius2 and Catalyst programs. QSAR equations have been generated for 40 Androgen Receptors inhibitors employing Molecular Field Analysis (MFA) as well as Receptor surface Analysis (RSA) using Genetic function approximation (GFA) as regression method.

Results: The best equations with training set produced r^2 value of 0.856 and r^2cv value of 0.739 in 2D-mode, r^2 value of 0.839 and r^2cv value of 0.793 in MFA-model and r^2 value of 0.910 and r^2cv of 0.856 in the RSA-model. For the 20 test set molecules predicted activities, had a correlation of 0.840 and 0.856 for MFA and RSA with observed activities.

Conclusion: The 3D–QSAR models exhibited good correlation and predictive ability. The model showed that steric (CH_3) and electrostatic (H^+) interactions play an important role in the inhibition of Androgen receptor by the analogues. The model generated could be exploited for further structural modification in order to improve Androgen receptor inhibition activity.

Key Words: 3D QSAR, molecular field analysis (MFA), receptor surface analysis (RSA), androgen receptor, prostate cancer

Conflict of Interest: The author declares that no conflict of interest exists.

ÖZET

Amaç: Androjen reseptörlerin inhibisyonu, prostate kanseri tedavisinde en çok takip edilen ve umut vaat eden yollardan birisidir. Prostate kanseri tedavisinde kullanılmak üzere Androjen reseptörlerinin ekspresyonunu ve/veya fonksiyonu inhibe edebilecek kapasiteye sahip olan yeni bir inhibitor modeli türetmek ve androjen reseptörlerin inhibisyonu için çeşitli analogların yapısal gerekliliklerini araştırmak için Moleküler alan analizi (MFA) ve Reseptör yüzey analizleri (RSA) ile analiz edilmiş olan 40 alıştırma seti ve 20 test seti bileşikleri Androjen reseptör inhibitörleri olarak rapor edilmiştir.

Gereç ve Yöntemler: Yeni Androjen reseptör inhibitörlerinin geliştirilmesinde, Cerius2 program suite kullanılarak Pharmacophore jenerasyon ve 3D-QSAR çalışmaları yapılmıştır. Regresyon metodu Genetik fonksiyon tahmini (GFA) kullanılarak, Moleküler Alan Analizi (MFA) ve Reseptör yüzey analizleri çalışılmış ve 40 Androjen reseptör inhibitörleri için QSAR denklemler çıkarılmıştır.

Bulgular: Denemelerde üretilen en iyi denklemelerin r^2 değerleri, için 2D modunda, MFA modellemesinde ve RSA modellemesinde sırasıyla 0.856, 0.739 ve 0.839, 0.793 olarak bulunmuştur.20 test seti için tahmin edilen aktivitelerine karşı, MFA ve RSA için gözlemlenen aktivitelerin korelasyonu sırasıyla 0.840 and 0.856 olarak bulunmuştur.

Sonuçlar: 3D–QSAR modelleme iyi bir korelasyon ve öngörü düzeyi yüksek bir özellik göstermiştir. Modelleme göstermiştir ki sentezlenen analogların Androjen reseptörlerini inhibe edişlerinde sterik (CH_3) ve elektrostatik (H^+) interaksiyonlar önemli rol oynamaktadır. Androjen reseptör inhibisyon aktivitesini artırmak amacıyla, üretilen modeler üzerinde daha fazla yapısal değişiklik için yapılabilinmesi için oluşturulan modellemeler kullanılabilinmektedir.

Anahtar Kelimeler: 3D QSAR, moleküler alan analizi (MFA), reseptör yüzey analizi (RSA), katalizör 4.11, androjen reseptör, prostat kanseri

Çıkar Çatışması: Yazarlar çıkar çatışması olmadığını beyan ederler.

Introduction

Quantitative structure-activity relationships (QSAR) have been applied for decades in the development of new drugs. Although a QSAR does not completely eliminate the trial and error factor involved in the development of a new drug, it certainly decreases the number of compounds synthesized by facilitating the selection of the most promising examples. The success of QSAR has tempted scientists, particularly in the pharmaceutical arena, to investigate relationships of molecular parameters with properties other than activity [1]. Quantitative structure–activity relationships (QSAR) have been applied for decades in the development of relationships between physicochemical properties of chemical substances and their biological activities to obtain a reliable statistical model for prediction of the activities of new chemical entities. The fundamental principle underlying the formalism is that the difference in structural properties is responsible for the variations in biological activities of the compounds. In the classical QSAR studies, affinities of ligands to their binding sites, inhibition constants, rate constants, and other biological end points, with atomic, group or molecular properties such as lipophilicity, polarizability, electronic and steric properties (Hansch analysis) or with certain structural features (Free-Wilson analysis) have been correlated. However such an approach has only a limited utility for designing a new molecule due to the lack of consideration of the 3D structure of the molecules. 3D-QSAR has emerged as a natural extension to the classical Hansch and Free-Wilson approaches, which exploits the three-dimensional properties of the ligands to predict their biological activities. It has served as a valuable predictive tool in the design of pharmaceuticals and agrochemicals. Although the trial and error factor involved in the development of a new drug cannot be ignored completely, QSAR certainly decreases the number of compounds to be synthesized by facilitating the selection of the most promising candidates. Several success stories of QSAR have attracted the medicinal chemists to investigate the relationships of structural properties with biological activity [2]. QSAR is a data exploration and productivity tool that can provide insight into structure-activity relationships. A QSAR (*quantitative structure-activity relationship*) is a multivariate, mathematical relationship between a set of 2D and 3D physicochemical properties (*descriptors*) and a biological activity. The QSAR relationship is expressed as a mathematical equation. The analysis of the statistical relationships between molecular structure and various properties provided by QSAR+ facilitates the understanding of how chemical structure and biological activity relate. The following methodology was followed as described in Accelrys QSAR module [3].

QSAR:- which generates quantitative structure-activity relationship models in both basic default and customizable modes. It calculates 2D and 3D spatial, electronic, fragment, topological, thermodynamic, conformational, and shape properties (descriptors), and statistically analyzes relationships between molecular structures and the descriptors to provide correlations for predicting biological activity. More than 100 relevant descriptors are included, and new descriptors can be added.

Molecular Field Analysis (MFA):- which quantifies the interaction energy between a probe molecule and a set of aligned target molecules in a QSAR Interaction energies measured and analyzed for a set of 3D structures can be useful in establishing QSARs.

Genetic Function Approximation (GFA):- which is a statistical analysis method that generates multiple QSAR models. Usually, this population of models contains many models comparable or superior to the single model generated with standard regression analysis. The multiple models are created by evolving random initial models using a genetic algorithm. The default is to build linear models, but other options, including higher order polynomials, splines, or other non-linear functions, also can be built. A method that combines Genetic Function Approximation and Partial Least Squares, G/PLS, is also available.

Molecular Shape Analysis (MSA):- which extends QSAR operations for performing 3D QSAR studies. This technique generates quantitative measurements of molecular shape properties as part of QSAR analysis.

Diversity:- which provides tools to build combinatorial libraries based on scaffold-plus-R-groups methods, and to optimize and visualize the diversity of combinatorial libraries.

Alignment:- which provides tools to superimpose molecules to satisfy various alignment conditions. These tools permit alignment of molecules using least square fitting with atom equivalencies specified either by automatic atom matching algorithms or by manual atom matching. In addition to rigid body super positioning, the module provides tools for flexibly aligning one molecule over another using a fit optimizer algorithm. Interfaces for Catalyst ConFirm and Catalyst HipHop, which access Catalyst applications that provide tools to generate pharmacophoric hypotheses. The hypotheses are generated by first generating conformations for a set of study molecules and then using the conformations to find and align chemically important functional groups common to the molecules in the study set [3**].**

Androgen Receptor in Prostate Cancer

The androgen receptor (AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is a type of nuclear receptor which is activated by binding of either of the androgenic hormones testosterone or dihydrotestosterone in the cytoplasm and then translocating into the nucleus. The normal development and maintenance of the prostate is dependent on

androgen acting through the androgen receptor (AR). AR remains important in the development and progression of prostate cancer. AR expression is maintained throughout prostate cancer progression, and the majority of androgen-independent or hormone refractory prostate cancers express AR. Mutation of AR, especially mutations that result in a relaxation of AR ligand specificity, may contribute to the progression of prostate cancer and the failure of endocrine therapy by allowing AR transcriptional activation in response to antiandrogens or other endogenous hormones. Similarly, alterations in the relative expression of AR coregulators have been found to occur with prostate cancer progression and may contribute to differences in AR ligand specificity or transcriptional activity. Prostate cancer progression is also associated with increased growth factor production and an altered response to growth factors by prostate cancer cells. The kinase signal transduction cascades initiated by mitogenic growth factors modulate the transcriptional activity of AR and the interaction between AR and AR coactivators. The inhibition of AR activity through mechanisms in addition to androgen ablation, such as modulation of signal transduction pathways, may delay prostate cancer progression.

Despite earlier detection and recent advances in surgery and radiation, prostate cancer is second only to lung cancer in male cancer deaths in the United States. Hormone therapy in the form of medical or surgical castration remains the mainstay of systemic treatment in prostate cancer. Over the last 15 years with the clinical use of prostate specific antigen (PSA), there has been a shift to using hormone therapy earlier in the disease course and for longer duration. Despite initial favorable response to hormone therapy, over a period of time these tumors will develop androgen-independence that results in death. The androgen receptor (AR) is central to the initiation and growth of prostate cancer and to its response to hormone therapy. Analyses have shown that AR continues to be expressed in androgen-independent tumors and AR signaling remains intact as demonstrated by the expression of the AR regulated gene, PSA. Androgen-independent prostate cancers have demonstrated a variety of AR alterations that are either not found in hormone naïve tumors or found at lower frequency. These changes include AR amplification, AR point mutation, and changes in expression of AR co-regulatory proteins. These AR changes result in a "super AR" that can respond to lower concentrations of androgens or to a wider variety of agonistic ligands. There is also mounting evidence that AR can be activated in a ligand independent fashion by compounds such as growth factors or cytokines working independently or in combination. These growth factors working through receptor tyrosine kinase pathways may promote AR activation and growth in low androgen environments. The clinical significance of these AR alterations in the development and progression of androgen-independent prostate cancer remains to be determined [4].

Prostate cancer (PCA) is the most common type of cancer found in American men, and androgen deprivation is the main therapy currently in use for both primary and advanced PCA. This treatment exerts its effect on target tissue by either blocking androgen (testosterone (T) and dihydrotestosterone (DHT)) synthesis or preventing binding of androgens to the androgen receptor (AR). The consequence of both strategies is interference with androgenic effects responsible for stimulation of prostate cancer cell growth. However, even the highly androgen dependent cases of PCA that are initially responsive to androgen deprivation therapy eventually develop resistance due to selection or adaptation of androgen-independent clones. For these patients, no therapy has been shown to be effective and new therapeutic strategies are urgently needed. The androgen receptor (AR) is central to growth signaling in prostate cancer cells and experimental data suggest that the AR remains functional and active in androgen-independent/ refractory prostate cancer through a variety of mechanisms aimed at increasing the growth response to lower levels of a wide variety of compounds. In the castrate environment, prostate cancer cells develop a growth advantage by amplifying or mutating the AR, altering AR co-regulatory molecules and developing ligand-independent AR activation pathways. Indeed, the AR is expressed in all histological types and stages of PCA, including hormone refractory tumors. With this knowledge, it is reasonable to suggest that effective strategies (investigational new drugs) that lead to AR down-regulation and/or AR modulation may be useful for preventing the development, progression and treatment of PCA [5].

The three dimensional quantitative structure activity relationships (3D-QSAR) may be useful in drug discovery and design [6]. As the most popular QSAR methods, Comparative Molecular Field Analysis (CoMFA) [7] and Comparative Similarity Indices Analysis (CoMSIA) [8] studies incorporate 3D information for the ligands by searching for sites on molecules capable of being modified into better specific ligands. As a useful methodology for studying the interaction mechanism, receptor based molecular docking analysis can offer vivid interaction on picture between a ligand and an acceptor [9]. Combined 3D-QSAR and docking study could offer more information to understand the structural features of bonding site of protein and the detail of protein–ligand interactions for purposive directing the design of new potential molecules [10].

Materials and Methods

Molecular modeling

Molecular modeling analysis was performed using Cerius2 software of Accelrys. The structures of the compounds

were built using molecular sketcher facilities provided in the modeling environment of Cerius2. Geometric optimization was carried using DREIDING force field. Partial atomic charges were calculated using the Gasteiger method. Multiple conformations of each molecule were generated using the Boltzmann Jump as a conformational search method to obtain lowest energy conformation. All molecules were initially energy minimized with smart minimizer option in Cerius2 software. Further geometric optimization of each molecule was carried out with MOPAC 6 package using the semi–empirical AM1 (Austin Model) Hamiltonian [11, 12].

Experimental section

Biological Data and Molecular Structure Generation

The activity data and two-dimensional structures for analogs were taken from the literature**.** Inhibitory constant values (IC50) reported for the compounds were converted to their corresponding pIC50 values, using a simple transformation (-log IC50) where pIC50 represents the value in nanomolar (nM) concentration. All the molecules were initially modeled using 3D Sketcher module of Cerius2 software. Partial atomic charges were assigned using the Gasteiger method. Initial geometries of the molecules were minimized using the smart minimizer and further geometric optimizations were performed in MOPAC using AM1 method. The dataset compounds were divided into two sets, namely training set of 40 molecules and test set consisting of 20 molecules [13].

Alignment of 3D QSAR

Alignment was performed using the align module of Cerius2. Core Substructure Search (CSS) alignment was carried out keeping the align strategy as Consensus as seen in figure 1.

3D QSAR Studies

Three dimensional quantitative structure activity relationship (3D-QSAR) models were developed using Molecular Field Analysis (MFA) and Receptor Surface Analysis (RSA) methods implemented in Cerius 2.

Molecular Field Analysis

Molecular field values were generated on a rectangular grid for all the aligned molecules using CH3 (steric) and H+ (electrostatic) probes. Only 10% from the total variables, with the highest variance were considered as independent variables(Y). The biological activities of all the molecules in the training set were used as dependent variables (Table 1). Genetic function algorithm (GFA) combined with partial least square (PLS) approach was used for variable selection and fitting. MFA study was carried out using G/PLS method consisting of 5,000 crossover generations on a population of 100 parent equations as seen in figure 2. The equation length was set to 10 terms including a constant [14, 15].

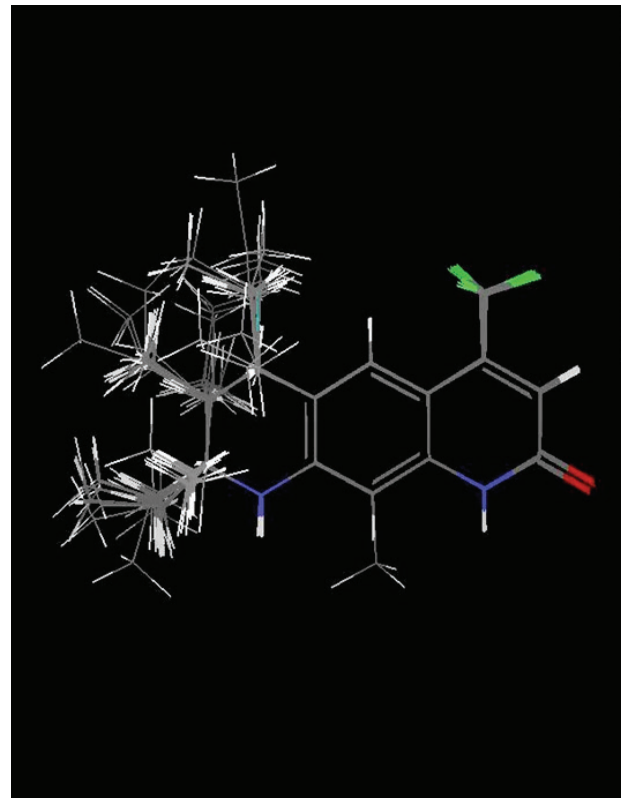


Figure 1. Alignment of study molecules

Receptor Surface Analysis

The RSA was used to construct a hypothetical model of the receptor site that embodies essential information about the receptor in terms of hydrophobicity, charge, electrostatics (ELE) potential as seen in figures 3, 4 and 5. The receptor surface was generated, using *van der Waals* field function, with weights proportional to the biological activity. RSA analysis was carried out using G/PLS method consisting of 5,000 crossover generations on a population of 100 parent equations. The equation length was set to 10 terms including a constant [16, 17].

The present study aimed at elucidating the structural features required for Androgen receptor inhibition and to obtain predictive 3D–QSAR model, which may guide the rational synthesis of novel inhibitors. The 3D–QSAR model was generated using the popular computational methods, molecular field analysis (MFA) and Receptor Surface analysis (RSA). This MFA and RSA model would give insight to the influence of various interactive fields on the activity thereby aiding in designing and forecasting the Androgen receptor inhibitory activity of novel molecules. The knowledge of pharmacophore hypothesis for Androgen receptor inhibitors can be very useful for virtual screening to design more potent lead moieties for the treatment of prostate cancer [18].

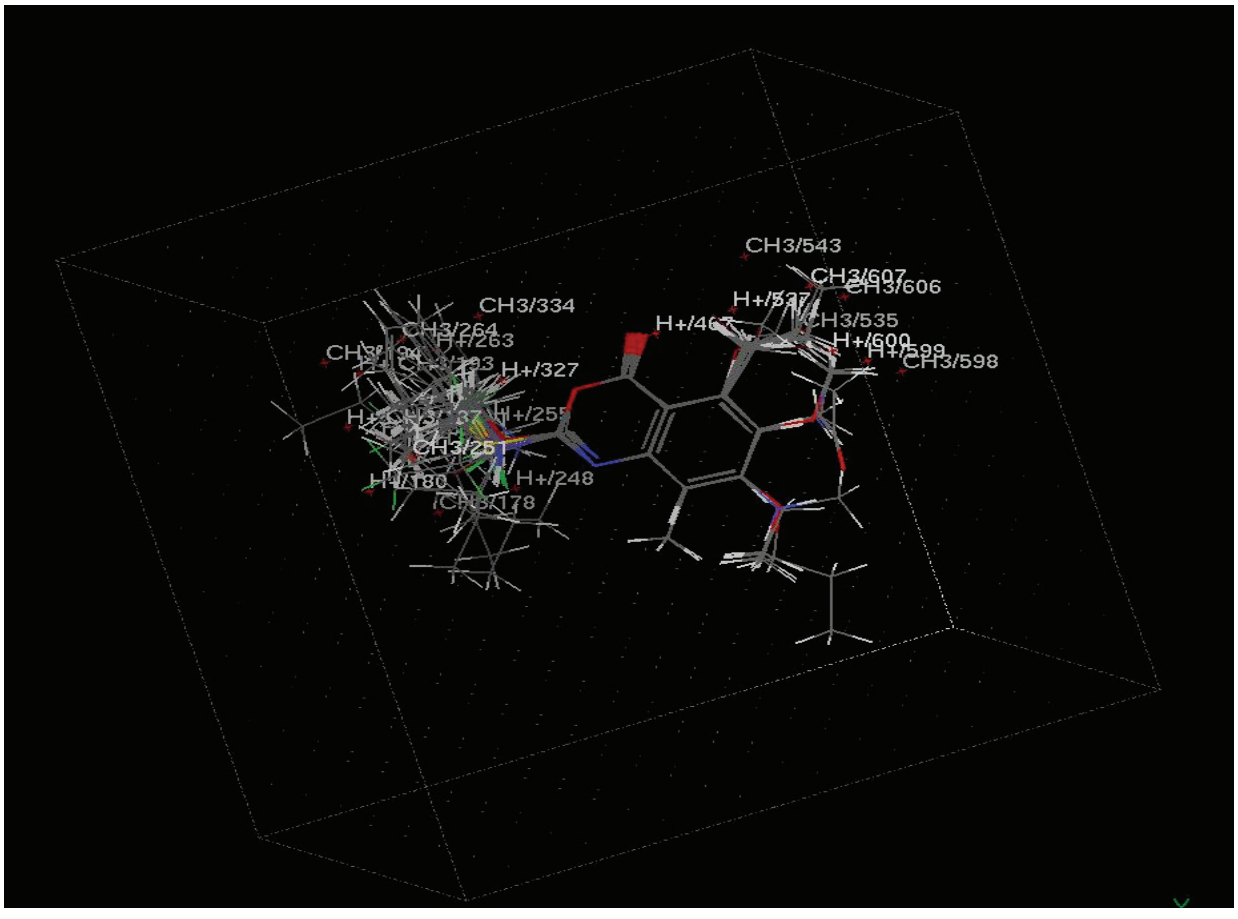


Figure 2. Stereo view of rectangular molecular field surrounding aligned molecules. Some of the field descriptors involved in the equation, are indicated. correlation of MFA (0.867)

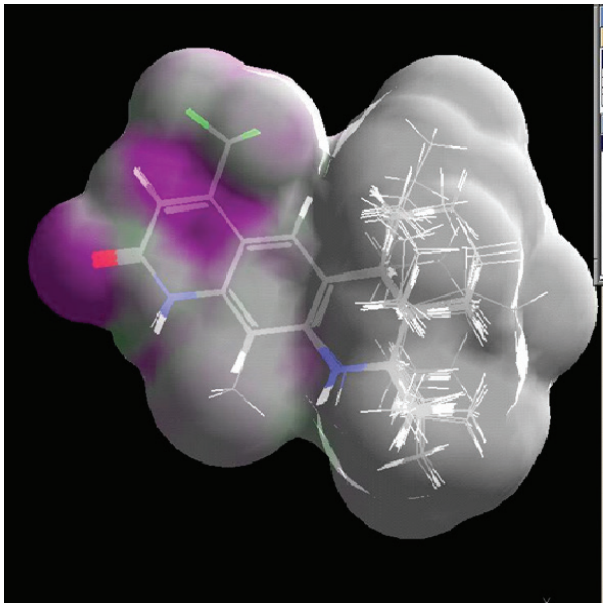


Figure 3. RSA model with charge surface receptor mapped onto it.

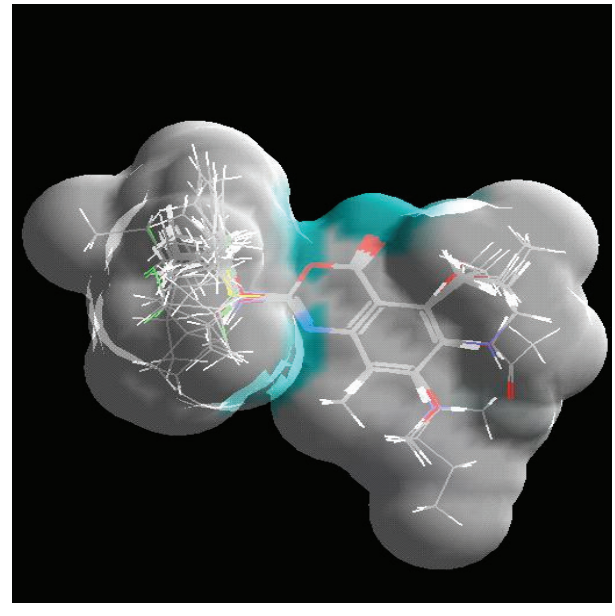


Figure 4. RSA model with hydrogen bonding mapped onto it

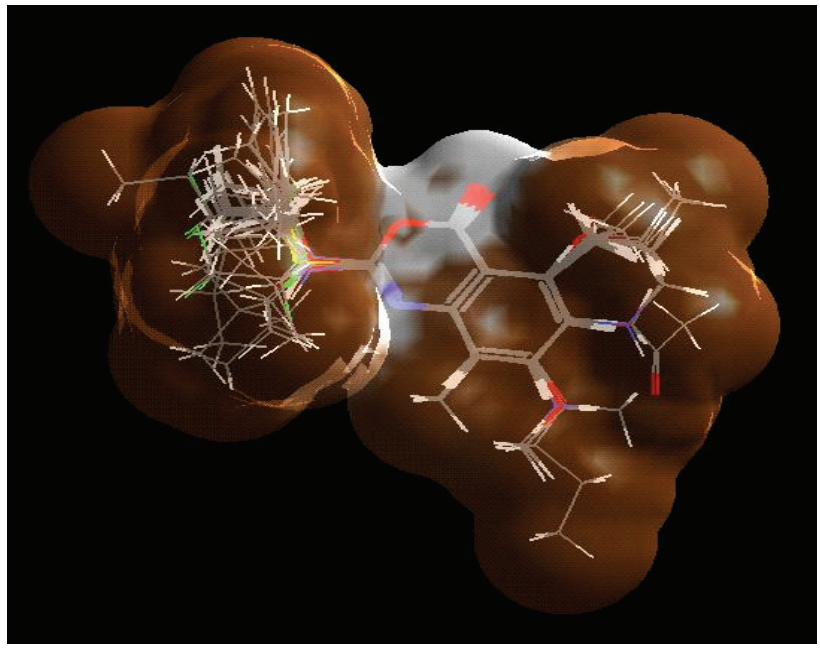


Figure 5. RSA model with hydrophobic property mapped onto it

Training set

A set of 40 molecules are taken in the training set and all the observational preferences are set to the desired effect so as to predict to the maximum extent. A diversified set of molecules with much molecular dissimilarity and diversified biological activity are chosen. The statistical method G/PLS is taken to analyze the statistical results. The numbers of components taken in PLS are 8 and the numbers of crossovers taken are 20000. According to the descriptors added to the different types (MFA, RSA, and 2D QSAR) the independent variables are taken with more than 90% variance and activity is set to dependent variable. The prediction in three of the different analysis gave satisfactory results. The highly active compounds are predicted to highly active compounds and inactive compounds are regulated by the mode of predicted function and are eliminated from the set of molecules so as to improve the equation [19, 20]. Finally the mode of prediction is good for all the molecules present in the training set according to the equation produced- Refer Training Set with Experimental and Predicted Activity (Table 1).

Test set

The purpose of QSAR is not only to predict the biological activity of the training set but also to predict the values of the test set molecules. From the above equations obtained from the training set molecules of known or unknown activity are introduced to study table so as to predict the biological activity. A series of molecules are introduced to study table which are known as test set molecules [21]. After the prediction of activities of test set molecules the activity of prediction crosses over 80% and 2 molecules which are inactive are trying to show as predictive Refer Test Set with Experimental and Predicted Activity (Table. 2).

Statistical details of 2D, MFA, & RSA analysis

MFA: Molecular Field Analysis, RSA: Receptor surface Analysis, R^2: Regression Analysis, XVR^2: Cross validated R^2, PRESS: Predicted sum of squared residuals (Table 3).

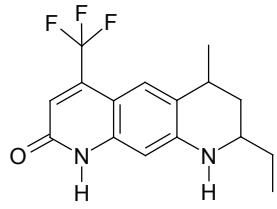
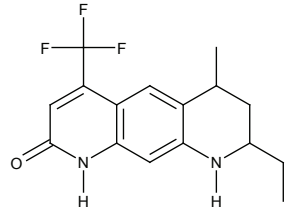
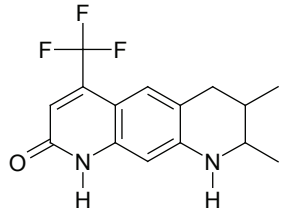
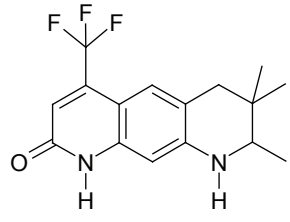
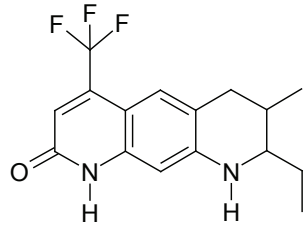
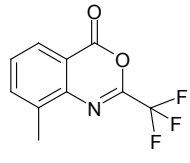
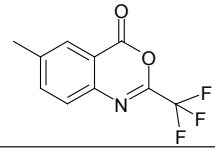
The result generated from QSAR equation the values observed for r^2, xvr^2, PRESS and for others mentioned above, are in a specific range and there is a good correlation between experimental and G/PLS predicted activity (Table 4).

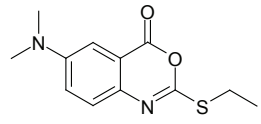
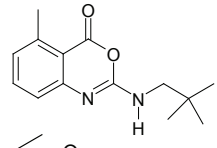
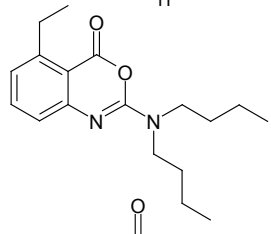
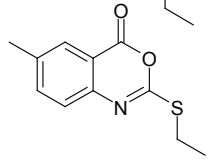
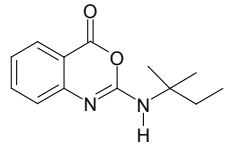
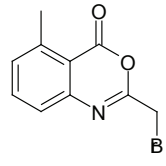
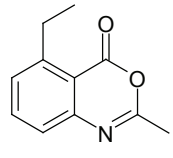
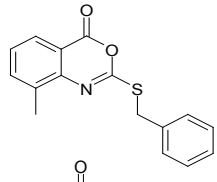
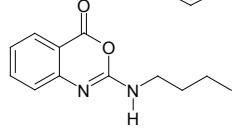
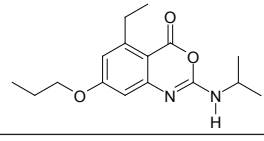
The regression analysis on training set molecules produced a QSAR model as shown in equation above for MFA, RSA and 2D QSAR respectively. From the QSAR results generated from all the methods like from Descriptors, MFA and RSA the observations that can be made on the Biological activities for all the molecules are: 1) The good correlation is observed between the experimental IC50 and computationally predicted IC50 values from all the methodologies; 2) All the molecules are proved to have the best biological activities experimentally; the Predicted biological activities similar to that of the experimental values are achieved by this computational study.

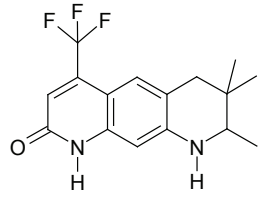
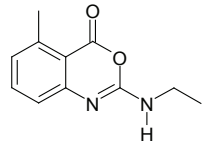
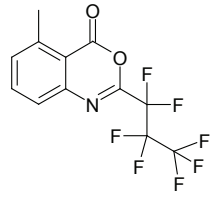
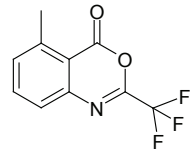
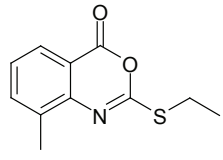
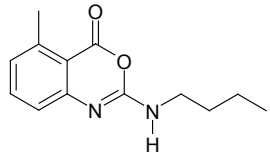
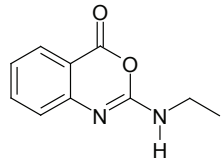
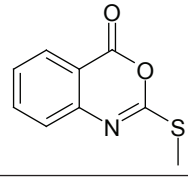
Molecular Field Analysis (MFA)

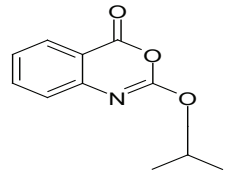
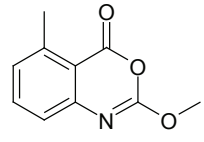
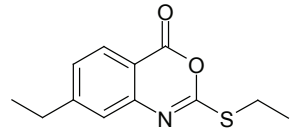
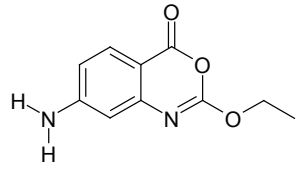
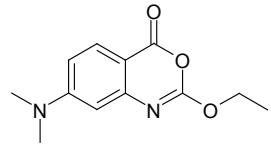
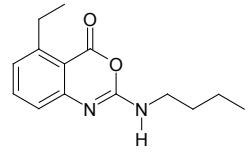
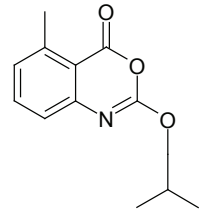
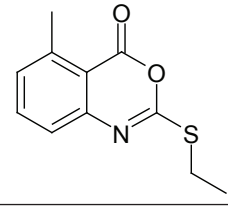
This method is for quantifying the interaction energy between a probe molecule and a set of aligned target molecules in QSAR. Interaction energies measured

Table 1 : Training Set with Experimental and Predicted Activity

Compound No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
1		4.954	5.559	4.909	4.133
2		5.322	5.661	5.564	5.609
3		5.342	5.371.	5.572	5.969
4		4.913	5.371	5.572	5.971
5		1.041	6.403	6.667	6.872
6		5.920	5.668	5.992	5.692
7		6.000	6.221	6.115	7.076

Compo-und No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
8		6.270	6.423	5.983	6.627
9		6.290	6.358	6.310	6.321
10		6.470	7.542	5.992	6.560
11		6.62	5.931	6.116	7.135
12		6.750	6.790	7.15	6.916
13		6.770	7.681	6.777	6.777
14		6.810	7.651	7.70015	7.640
15		6.820	6.5213	6.34516	7.158
16		6.920	7.623	6.63817	7.181
17		6.960	6.951	6.323	7.203

Compo-und No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
18		7.080	7.678	7.609	7.436
19		7.280	7.403	7.618	6.744
20		7.360	7.973	6.349	6.247
21		7.470	7.898	8.062	7.322
22		7.510	6.290	7.952	6.796
23		7.540	6.358	7.358	7.671
24		7.550	8.432	7.618	6.991
25		7.640	7.725	8.005	7.508

Compo-und No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
26		7.670	6.615	8.265	7.915
27		7.680	7.222	7.618	6.950
28		7.940	8.158	7.609	8.085
29		8.000	7.549	8.115	7.103
30		8.090	7.894	7.618	7.846
31		8.110	7.056	7.610	7.559
32		8.240	7.956	7.618	8.005
33		8.310	8.260	7.619	8.015

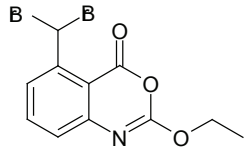
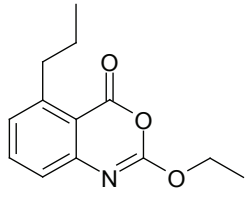
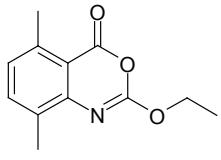
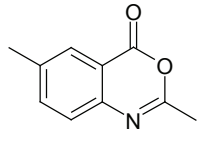
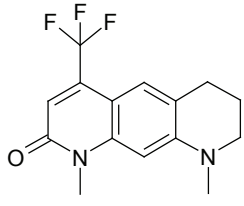
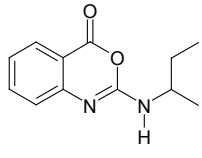
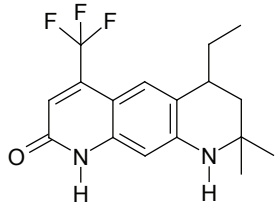
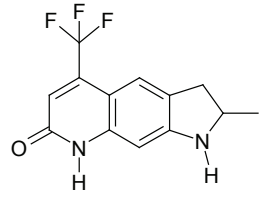
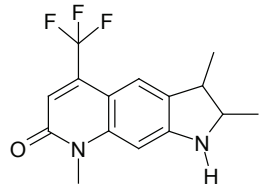
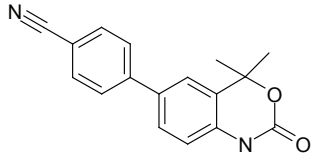
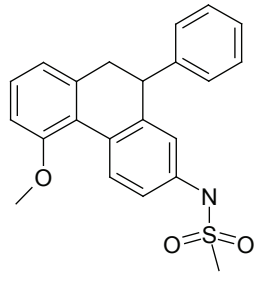
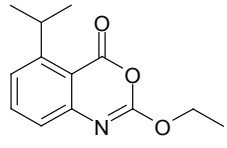
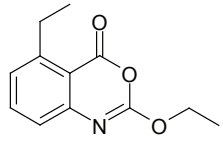
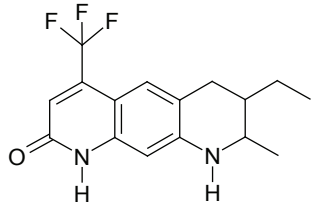
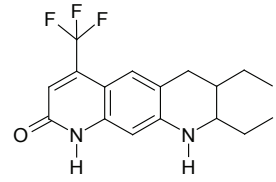
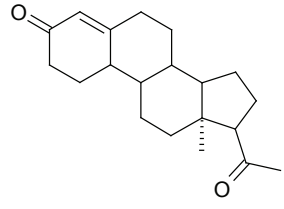
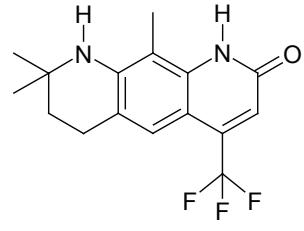
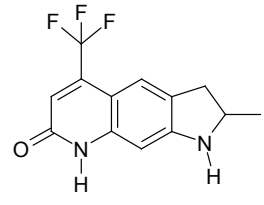
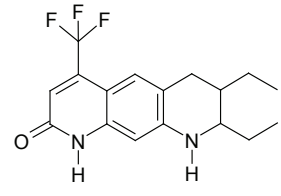
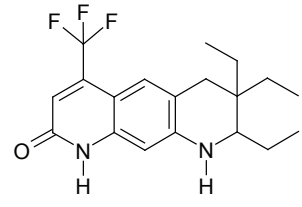
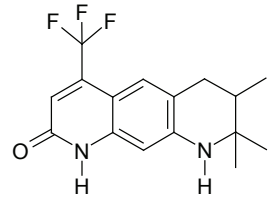
Compo-und No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
34		8.380	8.229	8.540	8.323
35		8.880	8.260	7.928	9.208
36		9.0300	8.390	9.008	8.425
37		9.330	9.868	8.443	9.646
38		9.6420	9.790	8.540	9.748
39		10.020	9.103	9.052	9.572
40		10.170	10.014	10.845	10.405

Table 2: Test Set with Experimental and Predicted Activity

Compound No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
1		5.670	6.181	5.991	6.037
2		6.380	7.803	5.816	6.446
3		6.660	6.049	6.133	6.417
4		6.770	6.004	5.979	6.495
5		7.0340	6.074	5.879	6.957
6		7.040	7.191	7.730	6.331
7		7.406	7.169	6.444	6.891

Compo-und No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
8		7.420	7.622	7.832	7.481
9		7.680	7.898	7.618	7.139
10		8.000	8.365	9.026	9.407
11		8.190	8.176	8.726	8.114
12		8.850	7.906	9.012	8.164
13		9.40	9.824	9.055	9.057
14		9.720	9.898	9.374	9.547

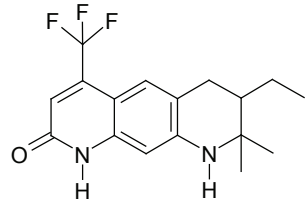
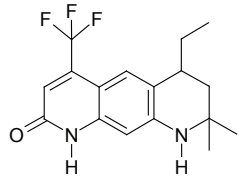
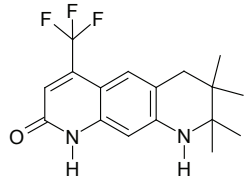
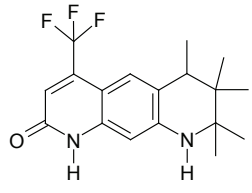
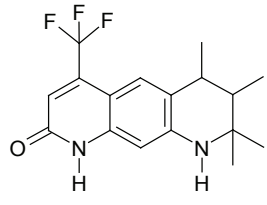
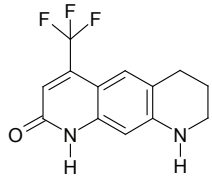
Compo-und No.	Compound Structure	Activity IC50 μM	Experimental Activity pIC50	Predicted activity in MFA	Predicted Activity in RSA
15		9.94	8.673	9.061	9.441
16		9.663	10.014	9.663	9.516
17		10.370	9.680	9.488	9.654
18		10.567	9.567	9.534	9.076
19		10.957	9.632	9.432	9.498
20		10.587	9.890	9.124	9.568

Table 3. Statistical parameters and MFA, RSA, 2D QSAR values

Statistical parameters	MFA	RSA	2D QSAR
R	0.856	0.943	0.867
R^2	0.839	0.910	0.856
XVR^2	0.793	0.839	0.739
Outliers	2	2	2
BSR^2	0.845	0.795	0.798
BSR^2Error	0.002	0.0168	0.013
PRESS	10.56	6.26	9.26

and analyzed for a set of 3D structures can be useful in establishing structure activity relationships. To generate an energy field (also known as a probe map), a probe molecule is placed at a random location, then moved about a target molecule within a defined 3D grid. At each defined point in the grid, an energy calculation is performed, measuring the interaction energy between the probe and the target molecule. Atoms in the target molecule are fixed, so that the intra molecular energy in the target is ignored. When in the target set, energy values for each point in the grid are reported in the columns added to the study table [22, 23].

Creating A Field: The process of generating energy fields around a set of study molecules involves selecting the molecules to use as a target, selecting one or more probes, and then running the calculations. In the present study MFA fields are created with default a probe which generates two fields for each model. One with a proton probe (H+) and the second one with an uncharged methyl probe. Each calculation uses a cubic grid with 2-Angstroms spacing. Energy calculations are made between -30 and 30 kcal. For each map, point values are added to the study table, one value per column. Each column is labeled using the probe name and probe number. A typical map contains several hundred points. Each new column of the probe points is labeled as an independent (X) variable and the activity as dependent (Y) variable. The shape of the field (geometry) can be selected as either rectangular or spherical. A step size increment given to the grid xyz -axes to suit the aligned set of molecules in the grid. The energy values for each field point added to the study table when the fields Calculations are completed. For each field, point values are added as columns, one value per column [24, 25].

Results

The Molecular Field Analysis (MFA) using probes have given good results; this field could not predict the activity, which is closely matched with the experimental biological activities. GFA residual values confirm that there is a variable difference in experimental activities and predicted activities. Hence the QSAR equation generated by MFA is labeled, to generate analogues by Analog Builder. The best equations with training set consisting 40 molecules, produced r^2 value of 0.856 and r^2cv value of 0.739 in 2D-model and r^2 value of 0.839 and r^2cv value of 0.793 in MFA-model and r^2 value of 0.910 & r^2cv of 0.856 in the RSA-model. For the 20 test set molecules predicted activities have correlation of 0.840 and 0.856 for MFA and RSA with observed activities. These results are suggestive of a statically robust and predictive model. The developed 3D-QSAR model could provide crucial information about the field descriptors that could be used for the design of potential inhibitors of Androgen receptor.

Discussion

The 2D descriptors from individual families have been systematically approached to find the predicted activities

Table 4. MFA, RSA and 2D QSAR equations

MFA Equation	Activity - 0.046748 * "CH3/334" + 0.03696 * "H+/467" + 0.04704 * "CH3/543" - 0.027816 * "H+/600" + 0.042922 * "CH3/193" + 0.064957 * "CH3/187" - 0.025432 * "CH3/606" .
RSA equation	Activity -0.90135 - 34.8009 * "TOT/1380" + 36.9685 * "TOT/1104" - 1.6223 * "TOT/2615" - 1.73236 * "TOT/1618" - 4.48241 * "TOT/1974" + 9.67656 * "TOT/2150" - 4.50458 * "TOT/2344"
2D QSAR equation	Activity 0.868874 + 0.071403 "CH3/77" + 0.011895 * "CH3/156" -0.071637 * "CH3/127" + 0.013645 * "CH3/141" + 0.07524 * "H+/171"

for test molecules in QSAR. These 2D descriptors have given better results, when compared with other descriptors, which can match with the experimental biological activities [26, 27]. GFA residual values confirm that there is a slight difference in experimental activities and predicted activities. So these 2D descriptors could predict the activity of the antagonism of Androgen receptor. In these two 2D descriptors, SC-3_P of Topological Descriptors and Jurs_WNSA-1 from Spatial descriptors contributed to predict the activity [28, 29]. RSA has also generated satisfactory results in predicting activities. The Molecular Field Analysis (MFA) using probes have given good results; this field could not predict the activity, which is closely matched with the experimental biological activities. GFA residual values confirm that there is a variable difference in experimental activities and predicted activities [30, 31]. Hence the QSAR equation generated by MFA is labeled, to generate analogues by Analog Builder. A more accurate and Quantitative Structure Activity Relationship considerations have been made by means of a pharmacophore model for Androgen antagonists. The best hypothesis resulted in some findings, which suggest that, the orientation and geometry of the molecule besides the bioactivity of molecules [32, 33]. The direction of any pharmacophoric chemical feature is important for interaction with the receptor and estimated activity depends on how well the features are mapped on hypothesis, wherein partial mapping of a pharmacophoric feature results in low estimated activity. Further approach in these studies will generate more number of analogues with the specified and desired active substituents on the pharmacophore that may have better activities than the leads. The descriptors from individual families and the selected descriptors have been systematically approached to find the predicted activities for test molecules in QSAR [34, 35]. These descriptors could not predict the activity, which can match with the experimental biological activities. A GFA residual value confirms that there is a lot of difference in experimental activities and predicted activities. So these descriptors could not predict the activity of the Androgen receptor Inhibitors. However two descriptors, chiral centers of Structural descriptors and Apol from Electronic descriptors contributed to certain extent to predict the activity. The Molecular Field Analysis (MFA) using probes have given better results, when compared with other descriptors including receptor descriptors. Hence the QSAR equation generated by MFA is labeled to generate analogues by Analog Builder. These analogs have shown better predictive activities when compared with set of molecules taken from Androgen receptor Inhibitors. The hydrophobic component of a model increases the ability of the drug to pass through cell membranes. The QSAR equation generated for the inhibition of Androgen receptor with new analogs possesses steric groups has shown better predictive activities. These steric groups may enhance the hydrophobic property of the molecule [36, 37].

On comparing the results derived from the journal "The Three Dimensional Quantitative Structure Activity Relationships (3D-QSAR) and Docking Studies of Curcumin Derivatives as Androgen Receptor Antagonists" authored by Xu G, Chu Y, Jiang N, Yang J, Li F., the constructed Comparative Molecular Field Analysis (CoMFA) and Comparative Similarity Indices Analysis (CoMSIA) models produced statistically significant results with the cross-validated correlation coefficients q2 of 0.658 and 0.567, non-cross-validated correlation coefficients r2 of 0.988 and 0.978, and predicted correction coefficients r2 pred of 0.715 and 0.793, respectively. These results ensure the CoMFA and CoMSIA models as a tool to guide the design of novel potent AR antagonists [38]. This on comparison with the results obtained from my present research work for 3D QSAR model generation the best equations with training set consisting of 40 molecules produced r^2 value of 0.856 and r^2cv value of 0.739 in 2D-model and r^2 value of 0.839 and r^2cv value of 0.793 in MFA-model and r^2 value of 0.910 & r^2cv of 0.856 in the RSA-model. For the 20 test set molecules predicted activities have correlation of 0.840 and 0.856 for MFA and RSA with observed activities.

Conclusion

These results are suggestive of a statically robust and predictive model. The developed 3D-QSAR models provided crucial information about the field descriptors that could be used for the design of potential inhibitors of Androgen receptors. The results from these QSAR analyses provide a useful insight into the structural and electrostatic requirements for binding of a ligand to the Androgen receptors. 2D, MFA and RSA analysis have provided useful information for developing extremely potent ligands leading to potential Androgen receptor inhibitors. This study also shows how chemical features for a set of compounds along with their activities ranging over several orders of magnitudes can be used to generate QSAR equation that can successfully predict the activity. These models were not only predictive within the same series of training compounds but also for diversified test set compounds. The equation identified for the Androgen receptor can be used to evaluate how well the newly designed compound shows its biological activity before undertaking any further study including synthesis. The knowledge derived from this four-feature pharmacophore hypothesis for Androgen receptor inhibitors can be very useful for virtual screening to design more potent lead moieties for the treatment of prostate cancer. This computational study may also help in identifying or designing compounds for further biological evaluation and optimization to suggest effective strategies (investigational new drugs) that lead to AR down-regulation and/or AR modulation

which may be useful for preventing the development, progression and treatment of Prostate cancer.

Experimental

All molecular modeling works were performed on a Silicon Graphics Octane R12000 computer running Linux 6.5.12 (SGI,1600 Amphitheatre Parkway, Mountain View, CA 94043) Cerius2 of Accelrys was used for 3D QSAR studies and Accelrys Catalyst 4.11 software was used to generate Pharmacophore models.

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