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QSAR STUDIES ON HUMAN 5α-REDUCTASE INHIBITORS: UNSATURATED 3-CARBOXYSTEROIDS

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Benign prostatic hyperplasia (BPH), a leading disorder of the elderly male population, is the non-malignant enlargement of the prostate gland. It involves an increase in cell numbers in both the epithelial and stromal elements within the peri-urethal transition zone of the prostate (1). BPH prevalence increases with age affecting around 80% by the age of 80 years causing considerable voiding dysfunction affecting quality of life (2). Due to the circumjacent relationship of the prostate to the urethra, glandular enlargement could result in the compromise of urinary function requiring medical treatment (3). Clinically, BPH causes a constellation of symptoms known as lower urinary tract symptoms (LUTS), which include frequency, hesitancy, urgency, nocturia, slow urinary stream and incomplete emptying (4).

Discovery indicating that growth and maintenance of prostatic tissue requires dihydrotestosterone has led to the development of potent steroidal 5α-reductase inhibitors. They have been found to inhibit the conversion of testosterone (T) to dihydrotestosterone (DHT) (5). Steroid 5α-reductase enzyme (3-oxo-steroid-4-ene dehydrogenase) is a membrane bound NADPH-dependent enzyme responsible for the conversion of testicular T into DHT (Fig. 1) (6). Thus, 5α-reductase dictates the cellular availability of DHT to prostatic epithelial cells and consequently modulates its growth.

Russell and Wilson's molecular cloning studies led to the identification of two genes that encode the two isozymes of 5α-reductase termed as 5α-reductase type I and II (7). Type I isozyme gene is located on the short arm of chromosome 5 and is expressed mainly in the human liver and sebaceous glands. Type II isozyme gene is located on the short arm of chromosome 2 and is expressed mainly in the prostate stromal and basal epithelial cells along with the dermal papilla of beard hair follicles and a deficiency in gene leads to male pseudohermaphroditism (8, 9).

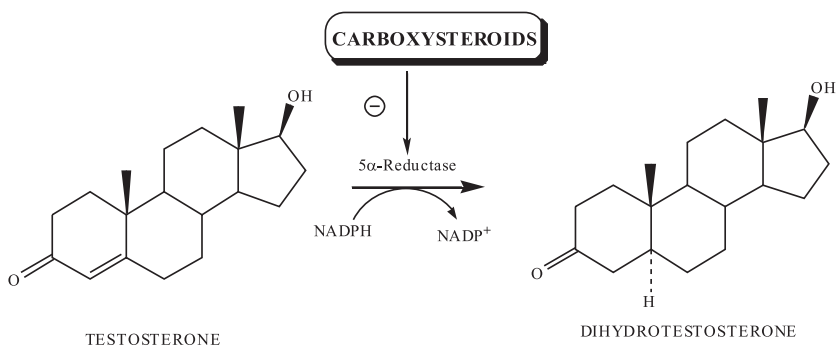

Figure 1. Site of action of 5α-reductase inhibitors

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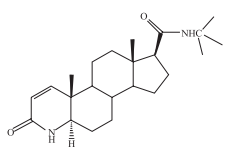
Both isozymes differ remarkably in the enzymatic activities like in pH optima as well as K_m values. Type I isozyme has found to be active at pH 6.5–9 whereas type II is active mainly at pH 5.5. The affinity for the substrate testosterone is higher for isotype II (K_m being 0.4 μM) than for isotype I (K_m around 10 μM). The two isozymes also differ in the constitution of amino acids as well as molecular weight; the type I isozyme is having molecular weight of 29,462 daltons and has 259 amino acids whereas the type II isozyme has molecular weight of 27,000 daltons and has 245 amino acids (10, 11).

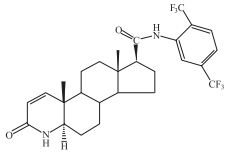
Genome-wide gene expression profile analyses led to the identification of a third type of 5α reductase enzyme (type III) in hormone-refractory prostate cancer cells (HRPC). This enzyme also converts T to DHT in HRPC cells in a similar way to type I enzyme and was found to be active at pH 6.9 (12). Northern blot and real time RT-PCR analyses have identified this enzyme in both androgen and non-androgen target human tissues such as pancreas, brain, prostate cancer cell lines, skin and adipose tissues (13).

During last two decades, various steroidal and non-steroidal inhibitors ranging from classical, reversible, irreversible inhibitors and transition state analogues to mechanism-based analogues have been reported. Finasteride [17β-(*N-tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one (MK-906)] (**A**), a competitive inhibitor of 5α-reductase type II with 10-fold high affinity than type I, due to formation of stable complex with enzyme, was the first 5α-reductase inhibitor approved in U.S. for the treatment of benign prostatic hyperplasia (14). Merck demonstrated that finasteride and related analogs are slow offset, essentially irreversible inhibitors due to the alkylation of the enolate formed on 1,4-reduction of the Δ^1 A-ring of the finasteride skeleton (15).

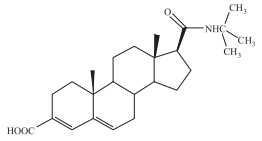
U.S. FDA in 2002 approved dutasteride (**B**) for the symptomatic treatment of BPH (16). Epristeride (**C**) is a novel uncompetitive inhibitor of 5α-reductase II and is an interesting analogue in the treatment of BPH (17). It lacks lactam in ring A and belongs to the category of unsaturated carboxysteroids. It has shown uncompetitive action against both testosterone and NADPH (18). It also attenuates the growth rate of some androgen responsive prostate cancers.



Finasteride (A)



Dutasteride (B)



Epristeride (C)

Holt et al. designed number of 3-androsten-3-carboxylic acids (**1**–**23**) because of presumably favorable electrostatic interaction between the carboxylate and the positively charged oxidized cofactor. The carboxylic group was found to preferentially bind in a ternary complex with enzyme and $NADP^+$, leading to the observed uncompetitive kinetic mechanism (19).

As the crystal structure of the target enzyme i.e., human 5α-reductase is not available, we have recently reported ligand based 3D-QSAR technique using Self Organizing Molecular Field Analysis (SOMFA) to design new inhibitors of 5α-reductase (20–22) To further optimize the molecular architecture, we applied the linear free energy related (LFER) approach of Hansch (23) on a series of unsaturated 3-carboxysteroid derivatives as epristeride (**C**), an important molecule in clinical trials, belongs to this group. It will further help in rationalizing the physicochemical properties required in a molecule to develop new and effective inhibitors.

COMPUTATIONAL METHOD

Data sets and biological activity

Data set

A data set of 23 molecules belonging to unsaturated 3-carboxysteroid derivatives as human 5α-reductase inhibitors were taken from the literature and used for QSAR study (19). Different QSAR models were generated for this series. The unsaturated 3-carboxysteroid derivatives used in the present QSAR study along with their observed and predicted biological activities are presented in Table 1.

Biological activity

The negative logarithm of the measured K_i (nM) against human 5α-reductase enzyme as pK_i (log $1/K_i$) was used as dependent variable (24), thus correlating the data linear to the free energy change. Only those compounds which showed significant activity/ inhibition were included in the present QSAR study.

Molecular modeling

The three-dimensional structures of the unsaturated 3-carboxysteroid derivatives were constructed using Chemdraw Ultra 8.0 running on an Intel

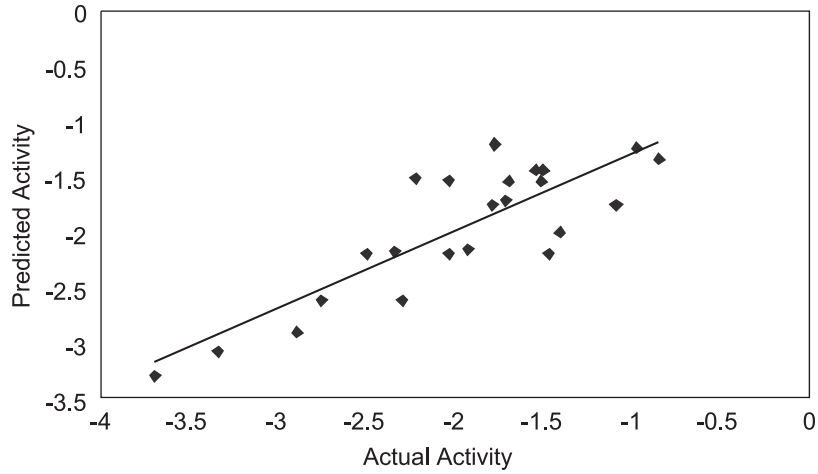


Figure 2. Plot of observed and predicted activities obtained using the best model 1

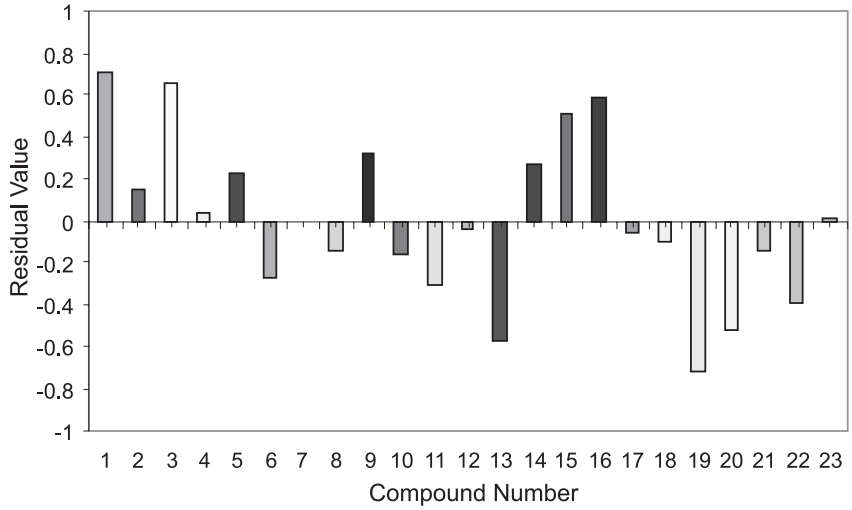


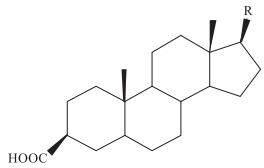
Figure 3. Plot of residual values of various compounds obtained using the best model 1

Pentium IV 2.80 GHz Processor / Microsoft Win XP Home Edition platform. All molecules were built using Chemdraw module and were subjected to energy minimization using molecular mechanics (MM2). The minimization is continued until the root mean square (RMS) gradient value reaches a value smaller than 0.001 kcal/mol Å. The lowest energy structure of the compounds in the series was used to calculate physicochemical properties using the 'Analyze' option of the Chem3D (25).

The physicochemical properties calculated include thermodynamic, steric and electronic descriptors: molar refractivity, log P, Connolly accessible area (CAA), Connolly molecular area (CMA), Connolly solvent excluded volume (CSEV), molecular weight, principal moments of inertia–x component (PMI–X), principal moments of inertia–y component (PMI–Y), and principal moments of inertia–z component (PMI–Z), dipole moment (DM) , Balaban index, total dipole moment, the highest occupied molecular orbital energy (HOMO) and lowest unoccupied molecular orbital energy (LUMO).

Multiple linear regression analysis (26) method was used to generate different QSAR models employing VALSTAT software. To check predictive power of the models, cross validation was done by leave one out procedure. The following statistical

Table 1. General structure of unsaturated 3-carboxysteroid derivatives and their observed activities as pK_i (nM) as well as predicted and residual activities using model 1



Compd. number	Unsaturation	Substitution	R	Observed Activity	Predicted Activity	Residual Activity
1	3-4	-	-CON(iPr)$_2$	–1.477	–2.184	0.707
2	3-4	-	-CONH(tBu)	–2.041	–2.187	0.146
3	3-4,5-6	-	-CON(iPr)$_2$	–1.097	–1.751	0.654
4	3-4, 5-6	-	-CONH(tBu)	–1.519	–1.549	0.030
5	2-3	-	-CON(iPr)$_2$	–1.929	–2.160	0.231
6	-	(3β)	-CON(iPr)$_2$	–3.342	–3.068	–0.274
7	2-3, 4-5	-	-CON(iPr)$_2$	–1.716	–1.716	0.000
8	4-5	(3α)	-CON(iPr)$_2$	–2.756	–2.615	–0.141
9	4-5	(3β)	-CON(iPr)$_2$	–2.301	–2.615	0.314
10	4-5	(3β)-OH	-CON(iPr)$_2$	–2.342	–2.179	–0.163
11	4-5	(3α)-OH	-CON(iPr)$_2$	–2.505	–2.193	–0.312
12	1-2, 3-4	-	-CON(iPr)$_2$	–1.792	–1.750	–0.042
13	1-2, 3-4, 5-6	-	-CON(iPr)$_2$	–1.778	–1.199	–0.579
14	2-3, 4-5, 6-7	-	-CON(iPr)$_2$	–0.978	–1.247	0.269
15	3-4, 5-6, 11-12	-	-CON(iPr)$_2$	–0.845	–1.350	0.505
16	3-4	4-F	-CON(iPr)$_2$	–1.415	–2.002	0.587
17	3-4, 5-6	6-F	-CON(iPr)$_2$	–1.505	–1.45	–0.055
18	3-4, 5-6	4-CH_3	-CON(iPr)$_2$	–1.544	–1.439	–0.105
19	3-4, 5-6	6-CH_3	-CON(iPr)$_2$	–2.23	–1.511	–0.719
20	3-4, 5-10	19-nor	-CON(iPr)$_2$	–2.041	–1.521	–0.520
21	3-4, 5-6	19-nor	-CON(iPr)$_2$	–1.699	–1.554	–0.145
22	3-4	-	20(*S*)CH_3CHCH_2OH	–3.699	–3.300	–0.399
23	3-4, 5-6	-	-CN	–2.898	–2.910	0.012

parameters were considered to compare the generated QSAR models: correlation coefficient *r*, r^2, standard deviation (*S*), F–test and internal predictive power by cross validated coefficient (r^2_{cv}).

RESULTS AND DISCUSSION

The correlation between calculated descriptors as independent variable and 5α-reductase inhibitory activity as response variable was calculated using multiple linear regression analysis. Only those parameters having intercorrelation below 0.5 were considered to select the best model. The different models generated for better correlation by statistical analysis have been given in Table 2.

The best model given in equation (Model 1) exhibits good internal predictivity as established by the cross validation r^2_{cv} value (0.586) of the model and also good external predictivity indicated by r^2 (0.707). The absence of any serious multicollinearity between the descriptors present in the model was confirmed by the calculation of correlation matrix, which shows that the descriptors: molar refractivity, Balaban index, log P, total dipole moment, H-bond donor, and LUMO were not intercorrelated. The plot of observed *vs.* predicted activities and residual values have been given in Figs. 2 and 3, respectively. The descriptors in the best model indicate effect of

Table 2: Various model obtained by multilinear regression analysis and statistical parameters indicating significance of models.

S. No.	Equation	r	r^2	$r^2_{cv}(q^2)$	S	F-test
Model 1	pK_i = + 0.2246 H-bond donor + 0.0736 MR – 4.5648 Balaban Index – 0.6257 LUMO + 0.0015	0.841	0.707	0.586	0.429	10.897
Model 2	pK_i = – 0.1795 Log P + 0.0695 MR – 3.9089 Balaban Index – 0.5586 LUMO + 0.0014	0.835	0.698	0.573	0.436	10.392
Model 3	pK_i = + 0.0738 Total Dipole + 0.0641 MR – 4.0882 Balaban Index – 0.5281 LUMO + 0.0022	0.835	0.698	0.567	0.426	10.381

molar refractivity, Balaban index, H-bond donor and LUMO on the biological activity. It is also evident from the equation that molar refractivity is positively correlated with biological activity that means, the higher is the molar refractivity, the higher will be inhibitory activity against human 5α-reductase. It has also been observed that in all models contribution of molar refractivity to inhibitory activity is positive.

Balaban index is negatively correlated, that means the higher is the Balaban index the lower will be inhibitory activity against human 5α-reductase. It has been observed in all models that contribution of Balaban index to inhibitory activity is negative. LUMO, an electronic descriptor, is also negatively correlated in all three models. Total dipole moment and H-bond donor are also contributed positively, whereas other parameters like log P are negatively correlated with the biological activity.

CONCLUSIONS

We have developed predictive QSAR models for unsaturated 3-carboxysteroid derivatives having human 5α-reductase inhibitory activity. The results obtained for the present series of unsaturated 3-carboxysteroid derivatives showed good correlation as evident from the best model 1 ($r = 0.841$; $r^2 = 0.707$, $F = 10.897$, $S = 0.429$) with human 5α-reductase inhibitory activity. The prediction power of the QSAR model was tested by LOO method, which gives a good internal predictivity with r^2_{CV} (0.586). The results of the QSAR study indicate characteristic influence of various physicochemical parameters, which mainly includes molar refractivity, total dipole moment, H-bond donor (positive) while Balaban index, log P, and LUMO (negative) of unsaturated 3-carboxysteroid derivatives against human 5α-reductase. These results, along with our previously reported study (20), will be helpful in providing structural insights for designing new specific inhibitors of human 5α-reductase and as agents for the treatment of BPH.

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