

Optimisation of Abiraterone Based Non-Steroidal Lead Molecules

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ABSTRACT

Management of castration resistant prostate cancer is limited by androgen receptor reactivation resulting in loss of remission. A recent study indicated that abiraterone exhibits antagonist activity towards the androgen receptor in addition to CYP17A1 inhibition. Metribolone has demonstrable *in vitro* and *in vivo* high affinity for the AR thus it was established as a benchmark against which the affinity of abiraterone and de novo designed non-steroidal molecules could be compared. Binding affinities of abiraterone manually superimposed onto the steroid scaffold of metribolone (pKd 7.16) and abiraterone that was allowed limited rotation (pKd 7.23) were comparable to metribolone (pKd 7.44). The de novo study generated an 8 analogue molecular series with affinities ranging between 5.26 and 7.23. This study yielded sufficient analogues that may be proposed for further molecular optimisation to yield innovative non-steroidal high affinity molecules with superior side-effect profiles for the management of prostate cancer.

Introduction:

Advanced state prostate cancer initially responds well to medical or surgical castration only to result in a castration-resistant state. Castration resistant prostate cancer (CRPC) refers to the state of the disease whereby the prostate-specific antigen continues to increase which indicates androgen receptor (AR) reactivation resulting in loss of remission followed by the development of disease-related symptoms.¹ Older terminologies used to describe advanced state prostate cancer such as hormone-refractory and androgen-independent are no longer appropriate as evidence has shown that growth may still be dependent on AR signalling. Androgen signalling is mediated through the AR; the inactive form of the AR is initially bound to heat shock proteins but is activated on binding to androgens resulting in the dissociation of heat shock proteins in order to allow transcription pathways for androgen-dependent genes. However, AR mutation may occur in CRPC leading to potential loss in AR specificity to androgens thus resulting in an increased affinity towards oestrogens, progesterone and anti-androgens.²

Studies over the past decade have shown that CRPC may still remain dependent on AR signalling for growth and these insights have led to the development of new agents which specifically target the resistance causing alterations occurring in the signalling pathway of the AR in CRPC.^{1, 3} Prostate cancer accounts for 11% of male cancer deaths⁴ thus drug design at the AR is still of significant importance owing to the fact that current therapy is not curative.

CRPC treatment witnessed significant improvement with the April 2011 Food and Drug Administration (FDA) and the September 2011 European Medicine's Agency (EMA) approval of the CYP17A1 inhibitor abiraterone acetate for use in combination with prednisone for the treatment of metastatic CRPC. Abiraterone acts by inhibiting the CYP17A1 enzyme which plays a critical role in the androgen biosynthetic pathway specifically in the formation of dehydroepiandrosterone (DHEA) and androstenedione, the precursors of testosterone (Ref. Fig. 1).^{5, 6} It is important to note that recent studies indicated that abiraterone exhibits antagonist activity towards the AR in addition to CYP17A1 inhibition. *In vitro* data was indicative of the fact that abiraterone exhibits a dose proportional inhibition of both wild type and mutant AR variants with the most significant inhibitory effect being observed at doses $\leq 10 \mu\text{mol L}^{-1}$, suggesting a dual mode of action for this drug.⁷ It was consequently concluded that abiraterone binds with

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high affinity to CYP17A1 and with lower affinity to the AR.^{7, 8}

The CYP inhibitor abiraterone is a pregnenolone derivative (Ref. Fig. 2). Its key design features include the 3-pyridyl substituent and 16, 17- double bond. The 3-pyridyl moiety appears to have the correct orientation for optimum binding to the haem iron of the CYP17A1. Evidence gathered

from structure activity relationships (SARs) have concluded that the 3-pyridyl substituent results in more potent inhibition when compared to 2-pyridyl substituents, and even more so in comparison to 4-pyridyl substituents. It has also been postulated that the 16, 17- double bond is responsible for the irreversible inhibition of CYP17A1.^{9, 10, 11}

Figure 1: A Schematic Representation of Steroidogenesis.

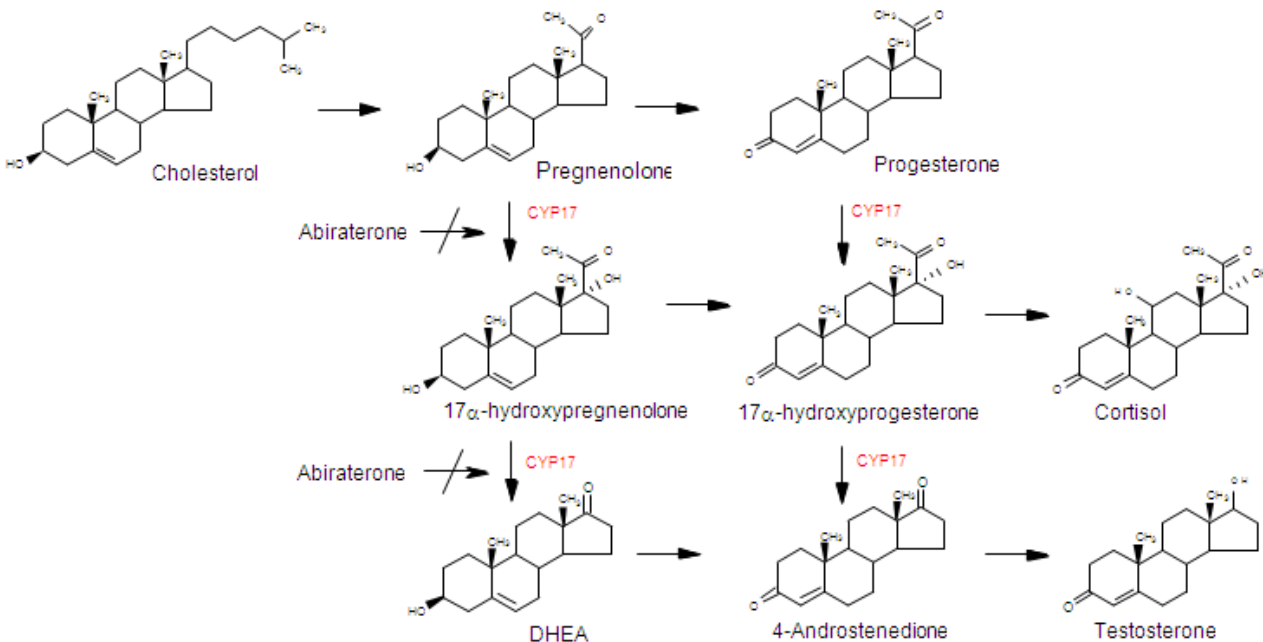
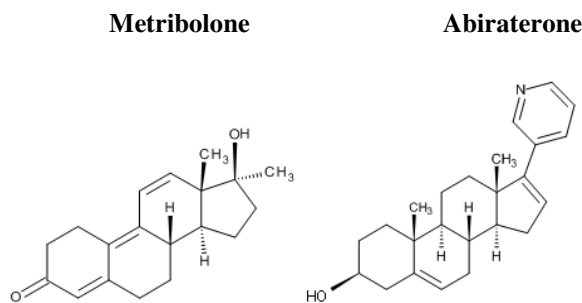


Figure 2: The 2D structures of Metribolone and Abiraterone



Through its CYP17A1 inhibitory effect, abiraterone inhibits the enzymes 17 α -hydroxylase and C_{17,20} lyase; which play a critical role in the production of cortisol and androgen synthesis. 17 α -hydroxylase converts progesterone into 17 α -hydroxyprogesterone and pregnenolone into 17 α -hydroxyprogesterone; while C_{17,20} lyase converts 17 α -hydroxyprogesterone and 17 α -hydroxyprogesterone into androstenedione and DHEA (Ref. Fig. 1). Adrenocorticotrophic hormone (ACTH) induces the

conversion of cholesterol into pregnenolone, and is kept in check via negative feedback triggered by an increased cortisol level. By blocking the activity of 17 α -hydroxylase, cortisol production is diminished and the negative feedback effect on ACTH is removed, leading to an accumulation of ACTH. Thus abiraterone must be given with a low dose of a glucocorticoid in order to avoid side effects that would otherwise result from an accumulation of ACTH including fluid retention, hypokalaemia, and hypertension.^{2, 7, 9}

Through this study we report the use of abiraterone as a lead molecule in the *de novo* design of novel non-steroidal AR antagonists suitable for the long term management of prostate cancer.

Material and Methods:

The pdb crystallographic deposition 1E3G was selected as a template for this study.¹² 1E3G describes the bound coordinates of the human AR, crystallised as a monomer and bound to the small agonist molecule metribolone. The 3D coordinates of this deposition were read into SYBYL¹³ ensuring preservation of the bound coordinates of the complex and consequently its suitability to be utilised as a template for this study. Molecular simplification of the AR through the removal of co-crystallised water molecules was based on the premise that literature indicates the absence of water molecules critical to ligand binding.¹² This process was executed in SYBYL in order to reduce computer intensiveness in subsequent calculations. The metribolone molecule was subsequently extracted from its LBP and saved in *mol2* format with the now apo AR being saved in pdb format (Ref. Fig. 3). These files were exported to Xscore¹⁴, which through its static algorithm quantified a predicted *in silico* affinity (pKd) of metribolone for its cognate AR_LBP. Given that metribolone has demonstrable *in vitro* and *in vivo* high affinity for the AR, this computed figure was established as a benchmark against which the affinity of abiraterone and of the *de novo* designed molecules could be compared.

Figure 3 (A): 1E3G depicting the AR bound to the ligand metribolone

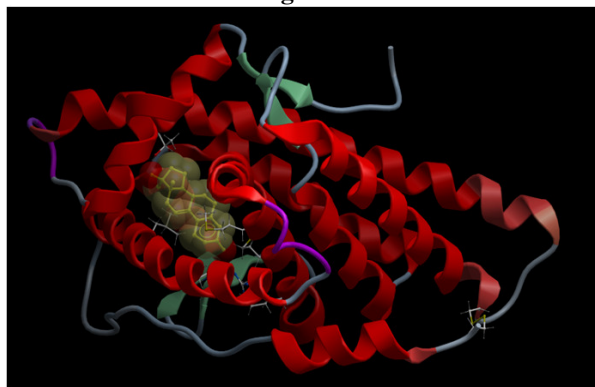


Figure 3(B): AR void of ligand & water molecules

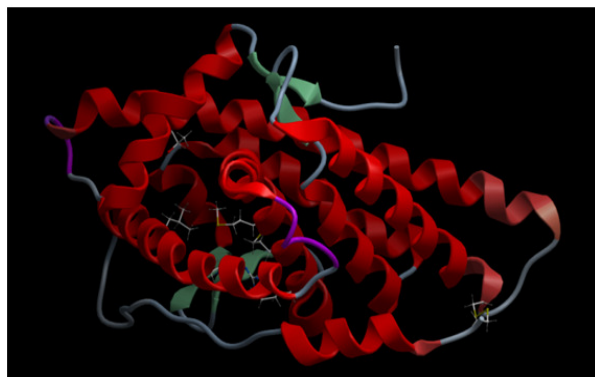
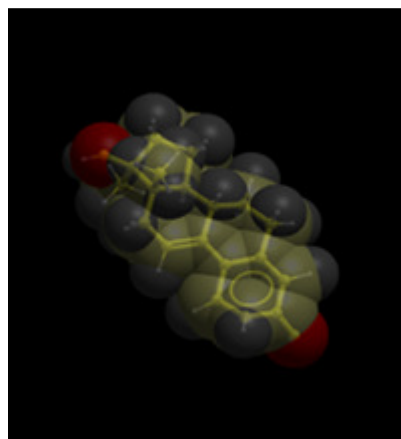


Figure 3 (c): Depicts the extracted ligand metribolone using Pymol®



De novo in silico design was carried out using LigBuilder v1.2.¹⁵ The extracted metribolone molecule was used to probe the AR_LBP such that a 3D image of the LBP and a proposed pharmacophoric structure (Ref. Fig.5) based on the bound coordinates of metribolone were generated.

The abiraterone molecule was constructed and optimised in SYBYL. Two methodologies were considered for the estimation of the LBA of abiraterone for the AR_LBP. In the first approach an assumption that the steroidal scaffolds of metribolone and abiraterone could occupy identical positions within the AR_LBP was made. Consequently, the steroidal scaffold of abiraterone (Ref. Fig. 2) was manually superimposed onto that of metribolone. The new coordinates of abiraterone were saved in *mol2* format and exported together with the apoAR into Xscore for *in silico* quantification of predicted affinity for the AR in a process that was analogous to that carried out previously for metribolone.

Based on the premise that ligand:protein complexes are essentially dynamic, a second approach was employed in order to attempt quantification of the LBA of abiraterone for the AR_LBP. This was carried out utilising the similarity suite embedded in SYBYL which essentially directs a test ligand (in this case abiraterone) into a target LBP (in this case the AR_LBP) based on the bound coordinates of a small molecule of established affinity for the target (in this case metribolone for the AR_LBP). The advantage of this method is that the introduced molecule (in this case abiraterone), is allowed conformational freedom within the target AR_LBP. This process resulted in the identification of the 21 conformations with highest affinity for the AR_LBP, where affinity was subsequently quantified (predicted pKd) in XScore.

The conformation of abiraterone with the highest affinity for the AR_LBP as generated through similarity suite analysis was recruited as a template for the *de novo* design exercise. Through this process, molecular fragments were modelled in SYBYL which could then be planted and allowed growth within the AR_LBP using the GROW algorithm in LigBuilder v1.2.

Seed structure creation was approached judiciously. SAR analysis of abiraterone was indicative of the fact that its 3-pyridyl substituent was responsible for the inhibitory effect of abiraterone at the CYP17A1 enzyme.⁹ It was consequently decided to retain this moiety, and to then allow freedom of growth within the AR_LBP stemming from the terminus of the 3-pyridyl group. Molecular modelling was carried out in SYBYL and involved the removal of all moieties extraneous to the 3-pyridyl group. Furthermore, a change in atom type from a sp³ carbon to an *H.spc* was also carried out in order to establish growing sites for the GROW module in LigBuilder v1.2. This process effectively represented an attempt to eliminate the steroidal backbone inherent to abiraterone with consequent elimination of the steroidal side effects associated with their long-term use.

Results:

The *in silico* predicted pK_d for metribolone post extraction from its cognate receptor (PDB 1E3G) was 7.44. The predicted LBA (pK_d) of abiraterone after manual superimposition of its steroidal nucleus over that of

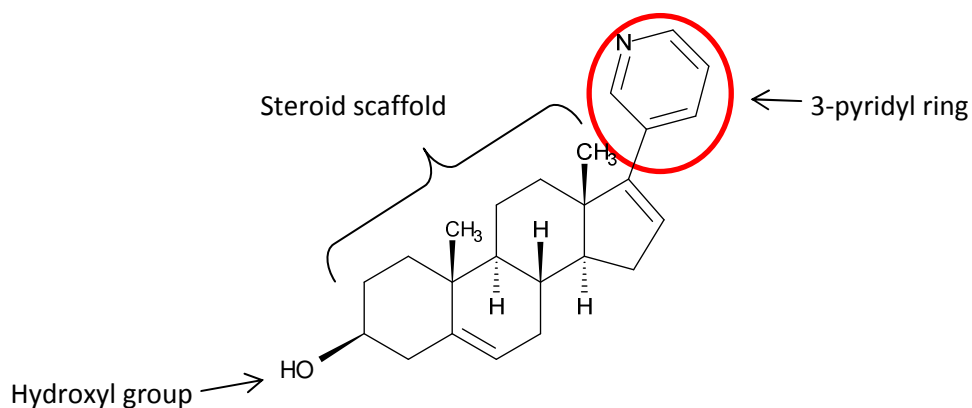
metribolone was estimated to be 7.16. When abiraterone was allowed computational freedom within the metribolone bound conformation of the AR, 21 high affinity conformers were generated with predicted *in silico* LBA (pK_d) that ranged between 6.50 and 7.23 (Ref. Table 1). The binding energies (kcal mol⁻¹) were also quantified in SYBYL for each of the 21 conformations. These ranged from -8.87 to -9.86 kcal mol⁻¹ and are shown, plotted together with predicted *in silico* LBA (pK_d) for each of the 21 generated high affinity conformations of abiraterone in Figure 5.

The 3D image of the mapped AR_LBP and a proposed pharmacophore based on the bound co-ordinates of metribolone are shown in Figure 6.

Implementation of the *de novo* design process on the prepared seed structure (Ref. Fig. 4) according to the GROW algorithm resulted in the elaboration of a total of 83 molecules which subscribed to 8 families. The best conformer from each family was selected as a potential non-steroidal lead compound based on molecular weight, log P and LBA (Ref. Table 2).

Figure 4 Abiraterone and the seed structure

Abiraterone:



Seed Structure:

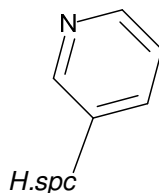


Figure 5: Graph of Predicted LBA (pKd)/Binding Energy (kcalmol⁻¹) for the 21 structural conformations of Abirateronefor the AR

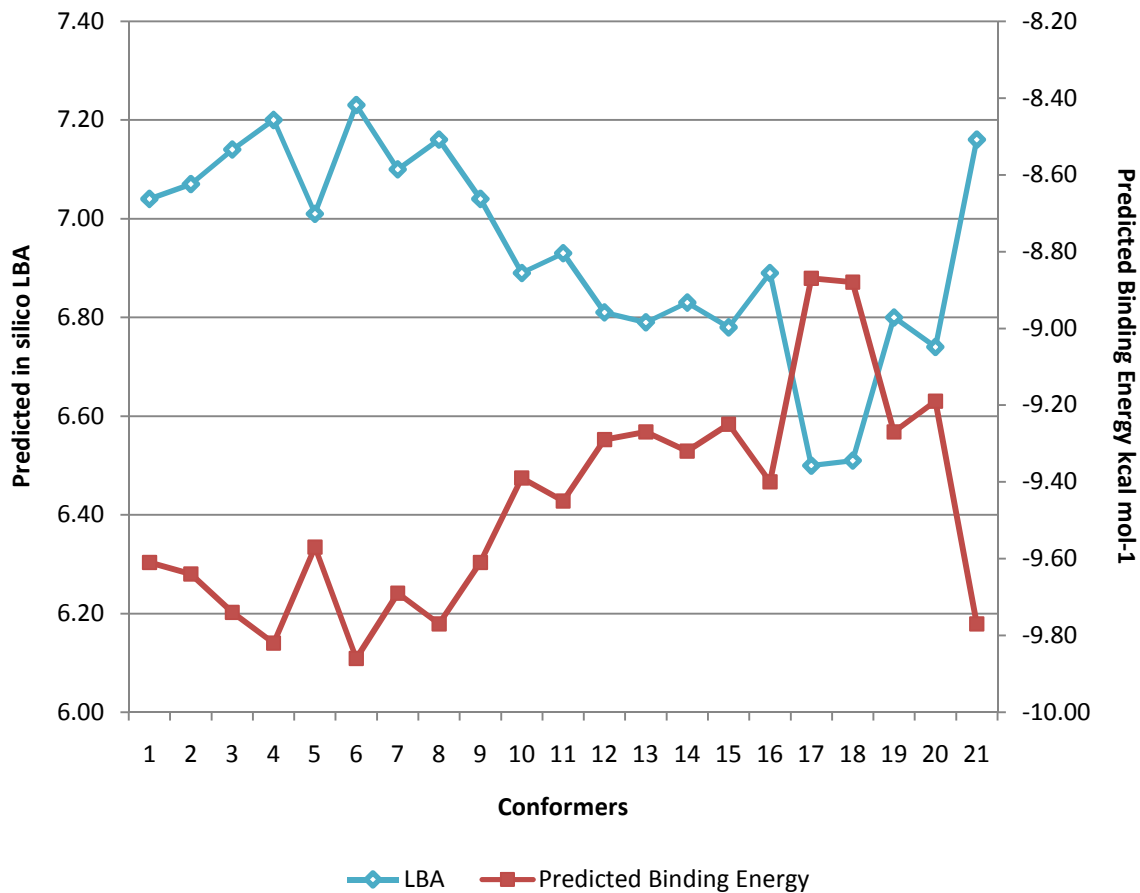


Figure 6: LBP map of metribolone in its cognate crystallographic deposition 1E3G shown in (a) and the respective pharmacophoric structure is shown in (b). Hydrogen bond donor grids can be seen in blue, hydrogen bond acceptor grids are shown in red, while hydrophobic grids are shown in cyan. Images generated using VMD.

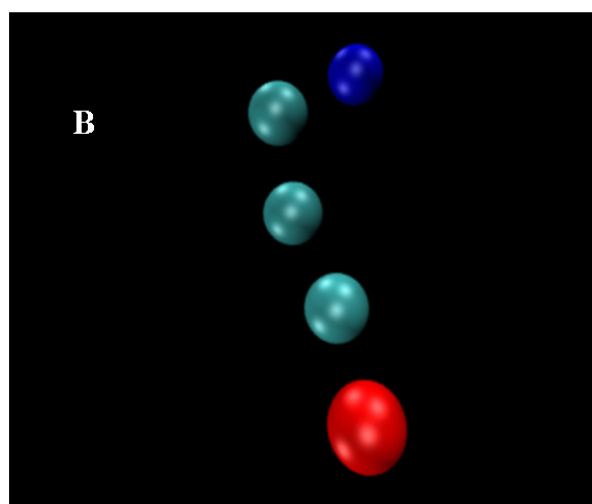
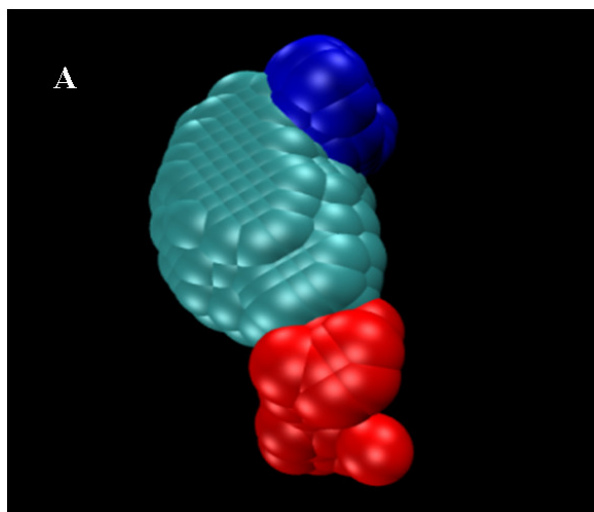


Table 1: Metribolone, superimposed abiraterone and the best binding conformer of abiraterone for the AR_LBP. Structural images generated using Pymol®

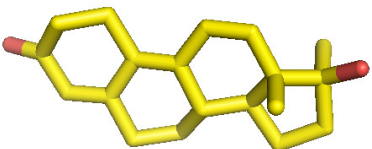
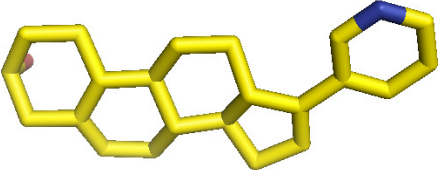
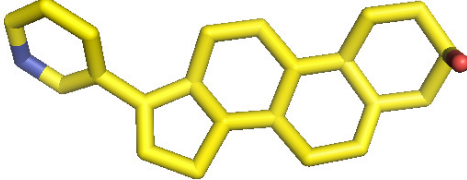
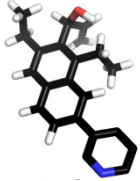
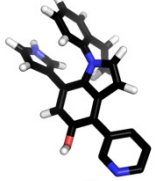
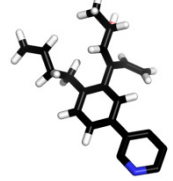
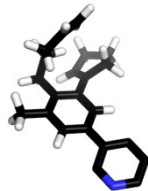
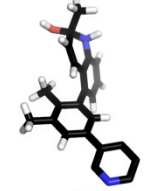
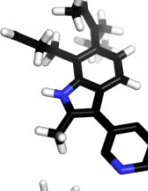
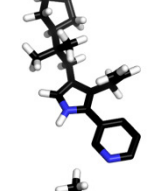
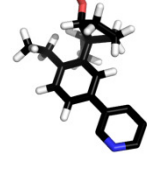
	Structure	Predicted LBA -log(pKd)	Predicted binding energy (kcal/mol)
Metribolone		7.44	-10.15
Abiraterone superimposed onto the bound coordinates of metribolone		7.16	-9.77
Best binding conformation of Abiraterone		7.23	-9.86

Table 2: The ligands with the highest LBA from each generated family are shown. Structural images were generated using Pymol®

Molecule No.	Family No.	Chemical formula	Molecular Weight	LogP	Average score pKd	3D structure
1	<1>	C ₂₂ H ₂₁ NO	315	5.45	7.23	
48	<2>	C ₂₅ H ₁₇ N ₃ O	375	5.71	7.10	
54	<3>	C ₂₁ H ₁₉ NO	301	5.56	6.87	

75	<4>	C ₂₂ H ₂₃ N	301	5.96	6.84	
77	<5>	C ₂₃ H ₁₉ N ₂ O	339	5.10	6.38	
80	<6>	C ₂₃ H ₂₄ N ₂ O	344	5.88	6.08	
81	<7>	C ₂₁ H ₂₄ N ₂	304	5.63	5.60	
83	<8>	C ₂₁ H ₂₃ NO	305	5.02	5.26	

Discussion:

The value of this study stems from the fact that the abiraterone moiety associated with CYP17A1 inhibition was retained in *ade novo* drug design exercise that aimed to identify novel non-steroidal structures bearing a similarity to abiraterone, but which also showed high affinity for the AR. The implication of this is that these identified novel structures would have the potential to simultaneously inhibit CYP17A1, hence mimicking the identified mode of action of abiraterone, as well as the AR- consequently retaining the traditional approach to prostate cancer management. The non-steroidal nature of these molecules also implies the absence of the steroidal adverse effect profile associated with their long term use. The evident predicted *in silico* LBA (pKd) of abiraterone for the AR_LBP must be discussed, especially in light of the proposed mode of action of abiraterone which has CYP17A1 inhibition and not the AR as its *in vitro* target. Abiraterone had a high *in silico* predicted binding affinity for the AR, which high affinity was recorded irrespective of the approach adopted in this study. In fact, when the steroidal nucleus of abiraterone was manually superimposed over that of metribolone the LBA (pKd) of abiraterone for the AR_LBP, the LBA (pKd) was 7.16 (*versus* 7.44 for metribolone). When abiraterone was allowed limited motion (single bond rotation) within the AR_LBP the predicted *in silico* LBA (pKd) ranged

between 6.50 and 7.23 for the 21 highest affinity conformations which again compare well to the LBA estimated for metribolone (pKd 7.44).

These results support the *in vitro* data of Soifer⁸ and Richards⁷ and point towards a possible scenario of dual affinity of abiraterone for the AR and the CYP17A1. From an *in silico* perspective it must be pointed out that this study adopted a rigid model. The superimposition of abiraterone onto metribolone essentially fixed the steroidal backbone of abiraterone onto the bioactive conformation of metribolone as described in the crystallographic deposition 1E3G. This model consequently assumed a rigid ligand and receptor binding pocket, in contrast to the second approach in which, although the receptor binding pocket was kept rigid, the ligand was allowed limited motion within the confines of the AR_LBP. Both these approaches consequently require further corroboration through dynamic studies, a scenario for which this study already paved the way through the molecular simplification processes carried out prior to estimation of *in silico* LBA. More clearly, the elimination of all moieties and water molecules considered superfluous to binding was important in ensuring that only motion associated with binding would be calculated in a process that is both computationally and time intensive.

The *de novo* drug design exercise yielded 83 non-steroidal ligands subscribing to 8 families with predicted *in silico* LBAs (pKd) ranging from 5.26 to 7.23. Generation of these non-steroidal ligands was based on the premise that the AR binds with high affinity to a general pharmacophoric structure. The generated pharmacophore comprises of 2 polar termini; a hydrogen bond donor and a hydrogen bond receptor grid, and a hydrophobic core (Ref. Fig. 5). Thus through the retention of the 3-pyridyl ring which binds to the hydrogen bond donating grid on the AR_LBP, LigBuilder v1.2 was able to generate non-steroidal ligands possessing a hydrophobic core and a hydrogen bond donor moiety at the other hydrogen bond accepting terminus of the AR_LBP. The major outcome of this study is the identification of a series of novel non-steroidal molecules with demonstrable binding affinity for the AR and with the potential for dual activity at the CYP17A1 and the AR. These molecules should consequently be considered as lead molecules for further iterative optimisation and also for inclusion in molecular databases that may be used in High Throughput Screening for the identification of antagonists for the AR.

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