



Evaluation of the Utility of The Abiraterone Scaffoldas Lead in CYP17A1 Receptor Modulation for the Management of Prostate Cancer

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ABSTRACT

This project utilised abiraterone as a lead molecule for further iterative design of novel anti-prostate cancer drugs which modulate the CYP17A1 receptor. The protein data bank crystallographic deposition describing the bound co-ordinates of abiraterone and the CYP17A1 enzyme was selected. Abiraterone and the CYP17A1 were examined using structure activity relationship studies; Sybyl[®]-X was used to generate the *apo*-receptor and abiraterone extract. For *in silico* ligand based drug design, ViCi[®] Hamburg screened for molecules similar to abiraterone. A protomol for CYP17A1 was generated, using Sybyl[®]-X, in order to probe areas of instability, within the active site region. Both abiraterone extract and the *apo*-receptor were later imported into X-SCORE[®] to calculate the ligand binding affinity and the ligand binding energy (kcal mol⁻¹). A total of three seeds was generated using Sybyl[®]-X, from which *de novo* molecules were generated, using LigBuilder[®]. Novel structures divided into various families were generated having different pharmacophores and filtered in accordance to Lipinski's rule of five. The protomol and the key site volumes were then compared using UCSF[®] Chimera. 1000 molecules were generated using *in silico* based drug design, of which 756 were Lipinski rule compliant; 99 molecules exhibited a *total score* of 6 or higher, when docked into the protomol. 727 *de novo* molecules were generated; 465 were found to be Lipinski rule compliant and hence further used in the study for pharmacophoric evaluation. Some of the *de novo* molecules exhibit a pKd higher than the baseline value of 7.04, for abiraterone molecule.

Keywords: 3RUK, Abiraterone, CYP17A1, Malta, prostate cancer, protomol.

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INTRODUCTION

Prostate cancer (PCa) is the most common form of solid neoplasia in Europe, amongst men. About 214 men out of 1000 suffer from PCa, outnumbering both colorectal and lung cancer¹. PCa occurrence is more common in elderly men than in their younger counterparts, and in general there is a higher occurrence of cancer in developed (15% occurrence) than in developing countries (4% occurrence)². In the last two decades the incidence rates of PCa have risen by a factor of 4 in Europe. The documented rapid rise in incidence has been attributed to the widespread use of prostate-specific antigen screening (PSA) in asymptomatic patients³. Between 2003 and 2007, 692 new cases of PCa were registered with the Malta National Cancer Registry, with an annual average incidence rate of 60.96 per 100,000 men⁴.

Eurostat prostate cancer statistics show that 72,000 men died from PCa, in 2012, being equivalent to 5.6% of all deaths from cancer and 1.4% of the total number of deaths from any cause, in Europe. In the same year, death rate for PCa was 39.7 per 100,000; the death rate was higher for men aged 65 years and over, many times higher than for younger men. In 2012, the death rate for Maltese males, from PCa, was less than half of the standardised death rates for PCa in the Baltic States and Slovenia (death rates of 65% per 100,000 inhabitants). In 2013, Malta exhibited one of the lowest patient hospital discharge rate, for patients suffering with PCa, with 17 discharges per 100,000 patients. The latter therefore means that the average length of stay for hospital in-patients suffering from PCa in Malta, was very long; the average length of stay was more than 11.8 days for Maltese patients⁵.

Recent studies have shown that although cancers may not respond to traditional androgen inhibitors, they still show androgen-receptor signalling, intra tumoural androgens and overexpression of enzymes; all involved in androgen synthesis^{6,7}. Blocking the CYP17A1 enzyme will interrupt both the oestrogen and androgen biosynthesis. Abiraterone (or pro-drug Abiraterone acetate) is a highly selective and irreversible CYP17A1 inhibitor; it can be administered orally, exhibiting very low toxicity⁸ (refer to figure 2).

The CYP17A1 enzyme

The CYP17A1 gene is classified as a cytochrome P450, family 17, subfamily A, poly peptide 1; the whole enzyme consists of four repeating subunits (refer to figure 1). The CYP17A1 gene codes for the steroid 17-alpha-hydroxylase, also referred to as the steroid 17-alpha-monooxygenase, which mediates both 17-alpha-hydroxylase and 17, 20-lyase activity. The latter

activities allow the adrenal glands and the gonads to be able to synthesize 17-alpha-hydroxylated glucocorticoids and sex steroids (via 17, 20-lyase activity) respectively⁹¹⁰¹¹. The 17-alpha-hydroxylation and 17, 20-lyase catalyses, involve the conversion of 21-carbon steroids to 19-carbon precursors of sex steroids¹² (refer to figure 2).

The abiraterone molecule

Abiraterone is an irreversible inhibitor of CYP17A1 enzyme through 17 alpha-hydroxylase and C17, 20-lyase. The drug is taken orally and acts by blocking testosterone production in the testis, adrenal glands and prostate gland¹³. Abiraterone can be administered orally to patients in its ester form, abiraterone acetate, which is a pro-drug of abiraterone¹⁴. By irreversibly inhibiting the enzyme CYP17A1, abiraterone prevents the formation of testosterone due to lack of metabolism of the molecules dehydroepiandrosterone and androstenedione¹⁵ (refer to figure 2).

Aims and objectives

Computational drug design is an interesting and ever-growing field in which many techniques can be utilised to discovery new molecular entities from template structures, with the aim of developing novel molecules or drugs that both research studies or the clinical scenario can benefit from. In this project the drug abiraterone was utilised as a lead molecule for further iterative design of molecular structures capable of targeting and inhibiting the enzyme CYP17A1. Both *in silico* and *de novo* drug design methods were used with the aim of targeting and inhibiting the CYP17A1 enzyme, which in turn lowers the rate at which the prostate cancer develops, in the prostate gland. A protomol was also generated for CYP17A1, in order to probe the entire area of instability, within the active site region of CYP17A1.



Figure 1: 3-Dimensional structure of CYP17A1 enzyme, highlighting the four repeating subunits using four different colours. Structure rendered using protein workshop® 4.1.0¹⁶ using protein data bank identification 3RUK¹⁷.

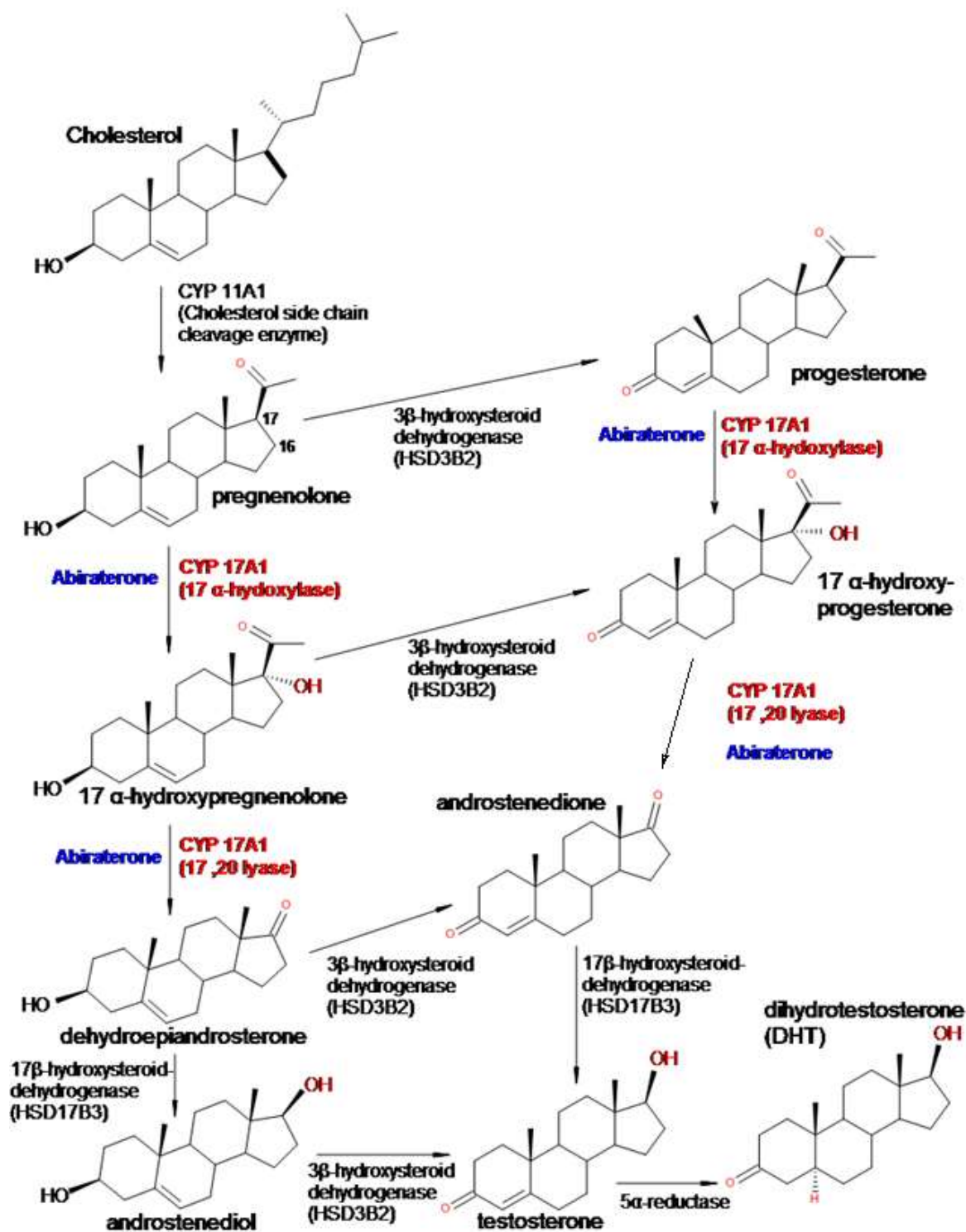


Figure 2: The biosynthetic processes involving the faith of cholesterol for the formation of

testosterone and also the involvement of the CYP17A1 (red text) and abiraterone (blue text). Diagram rendered in Accelrys[®] Draw 4.1¹⁸; adapted from Devore and Scott, 2012¹⁷.

MATERIALS AND METHOD

The study was divided into two sections, each representing different methods of computational medicinal chemistry, with the aim of optimizing the lead molecule abiraterone. Both *in silico* and *de novo* drug design methods were implemented in order to maximize the study positive impact outcome.

Virtual screening and *in silico* drug design

In the first section of the study, a 3-dimensional (3D) X-ray crystallographic deposition of the target receptor CYP17A1 was selected using the protein data bank (PDB)¹⁹. The PDB identification (ID) 3RUK¹⁷ was identified, describing the coordinates between ligand molecule abiraterone bound to its target receptor CYP17A1. The PDB file was then used as a template for the study being very ideal since it presented with a resolution value of 2.60 angstroms (Å), denoting a good crystallographic data for the structure.

Sybyl[®]-X v1.1^{20,21} was then used to extract the abiraterone molecule from the CYP17A1 receptor of 3RUK, producing the *apo*-structure for CYP17A1 and the abiraterone extract. During the extraction process it was ensured that the abiraterone molecule binding coordinates were preserved. Both the *apo*-receptor and the abiraterone extract files were saved in *.pdb* and *mol2* format respectively for further structural analysis and computational use.

ViCi[®] Hamburg²², an online molecule database, containing 8 million compounds was used for *in-silico* based drug design. For this step, the abiraterone.mol2 molecule was submitted to ViCi[®] Hamburg online database, and was rapidly screened for molecules which were predicted to have a similar shape and electrostatic composition to that of abiraterone. All the molecules extracted from ViCi[®] Hamburg, presented as single *.mol2* files, were collated into a single file using MONA^{®23} and then opened in a Sybyl[®]-X v1.1^{20, 21} spreadsheet and analysed for Lipinski rule of five²⁴ compliance. Some of the molecules were found to have a Log P higher than 5 and therefore excluded from the list due to Lipinski rule of five non-compliance.


Lastly a protomol was generated using Sybyl[®]-X v1.1^{20, 21} Surflex-Dock feature²⁵. The aim of generating a protomol was to create a computational representation of the intended binding site for CYP17A1, to which Lipinski rule²⁴ compliant molecules obtained from ViCi[®] Hamburg²² were docked and analysed for binding efficacy. An *apo* structure for CYP17A1 receptor was

created within the Surflex-Dock feature²⁵, also retaining water molecules since these are required for structure activity relationship (SAR) purposes as specified by Devore and Scott, 2012¹⁷. In addition to the Lipinski rule compliant molecules obtained from ViCi[®] Hamburg²², an additional 12 dummy molecules were also docked into the protomol, for comparison purposes. A result table was generated, containing all the information for each and every docked molecule, including a *total score* value; the higher and more positive the *total score* value was, the more favourable the binding of the molecule to CYP17A1. The result table obtained was saved in *.tbl* Sybyl[®]-X v1.1^{20, 21} and Microsoft[®] spreadsheet format.

***de novo* drug design**

In the second part of the study, the ligand binding affinity (pKd) and the ligand binding energy (kcal mol⁻¹) for abiraterone were calculated using X-SCORE[®] v1.3²⁶. The abiraterone extract and the *apo*-receptor structure of CYP17A1, obtained in section one were utilised in order to carry out the latter calculations.

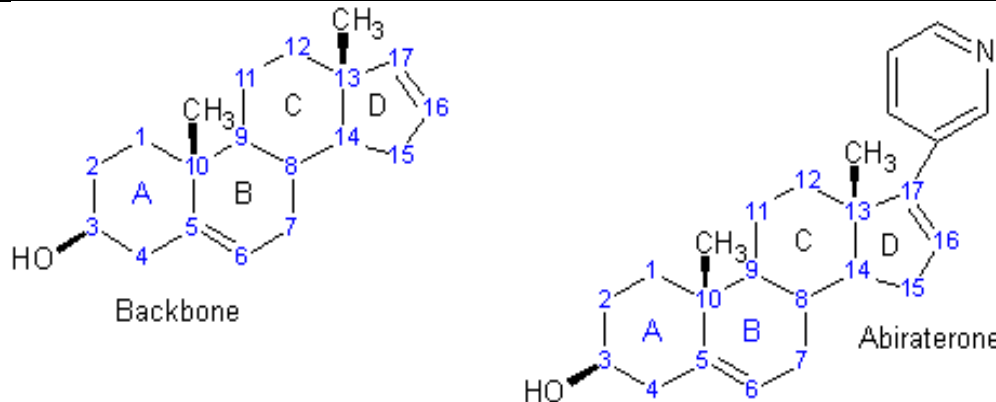
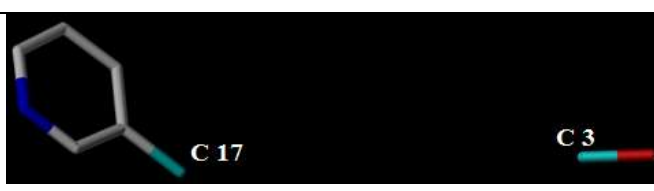
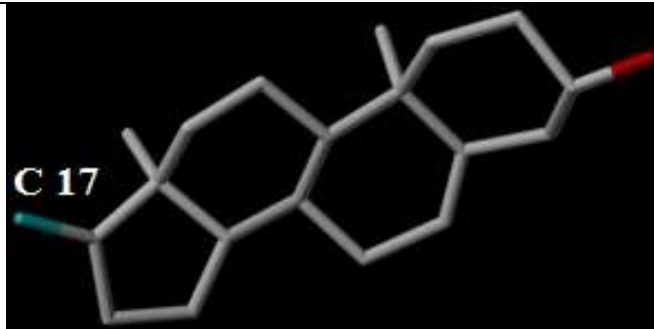
The abiraterone extract with the saved bound coordinates in *.mol2* and the *apo*-structure of CYP17A1, were also used to generate a 3D structure of the ligand binding pocket (LBP), including a 'key site' and 'pharmacophore' structure. These structures were generated using the POCKET module of LigBuilder[®] v1.2²⁷.

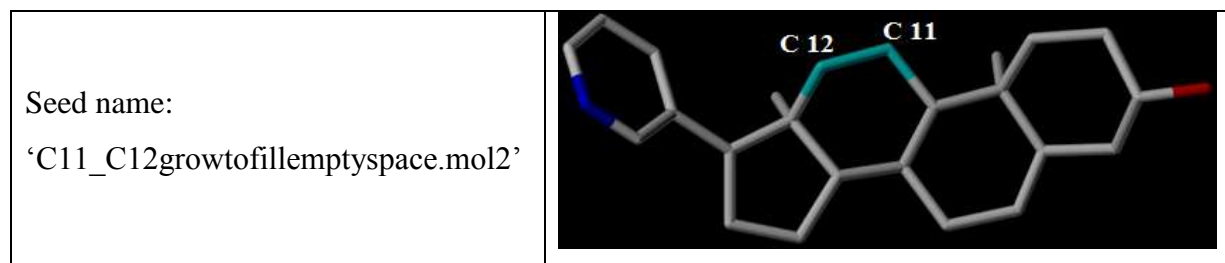
Seed structures were created using the abiraterone extract molecule in Sybyl[®]-X v1.1^{20,21}, the seed structures were created in order to generate novel molecules via *de novo* drug design using LigBuilder[®] v1.2²⁷. A total of three seed structures were created on the basis of SAR information obtained from literature^{17,28,39,30}. For each and every seed structure *H.spc* areas were created, this included two *H.spc* areas for the removal of the steroidal backbone for seed 'Abr_Hspc_OH_Pyr.mol2', one *H.spc* area for the removal of the pyridyl moiety for seed 'non-pyridine.mol2' and two *H.spc* areas for seed 'C11_C12growtofillemptyspace.mol2'. For all three seed structures, the 3 -OH moiety was retained, owing to the fact that this has been identified as important, contributing to hydrogen bonding within the active site and also with bridging water molecules (Devore and Scott, 2012)¹⁷. The *H.spc* areas in seed 'non-pyridine.mol2' and 'C11_C12growtofillemptyspace.mol2' were recognised by the GROW algorithm whilst the *H.spc* areas in seed 'Abr_Hspc_OH_Pyr.mol2' were recognised by the LINK algorithm in LigBuilder[®] v1.2²⁷ (refer to table 1).

de novo molecules were generated using the LINK and GROW algorithms in LigBuilder[®] v1.2²⁷; for this process the 'link_index' and 'grow_index' files were used as templates in order to specify the structural parameters of the novel molecules. Once the console terminal command

lines were inputted and executed, *population.lig* and *ligands .lig* files were generated for each seed. Both files were processed via the PROCESS console in order to generate the *results.mdb* folder, containing all the novel molecules. All novel molecules obtained for each seed were analysed for Lipinski's rule of five²⁴ and grouped into a spreadsheet divided into a number of families. Non-Lipinski rule compliant molecules were removed from the study, whilst each family containing a different number of molecules was used for pharmacophoric evaluation, followed by a structural comparison of the highest ranking molecules from each family, using the baseline LBA score for abiraterone.

Table 1: Creation of the three seed structures with *H.spc* areas marked in cyan colour.

 <p>Abiraterone structural backbone and full molecular structure.</p>	
Seed name: 'Abr_Hspc_OH_Pyr.mol2'	
Seed name: 'non-pyridine.mol2'	



The last phase for section two included measuring the 'key site' and protomol volume generated, using UCSF Chimera^{®31}. The volume occupied by both structures was measured using angstrom cubed units (\AA^3), via the surface/binding analysis feature in UCSF Chimera[®].

RESULTS AND DISCUSSION

From *in silico* drug design a total of 1000 molecules were obtained using ViCi[®] Hamburg²², out of the 1000 molecules, 756 molecules were found to be Lipinski rule²⁴ compliant. All the 756 molecules were collated into a single *.mol2* file and loaded into the protomol using Sybyl[®]-X v1.1^{20,21}, the *total score* values obtained for the 756 molecules ranged from -7.94 to 7.97; negative values are of no concern to the study since only the positive values show promising ligand binding potential. The result outcome also presented all the possible 30 conformations that can be attained by each molecule when docked into the active site of CYP17A1; molecule Chemdiv_J006-1410 produced the highest scoring value of 7.97 (refer to figure 3).

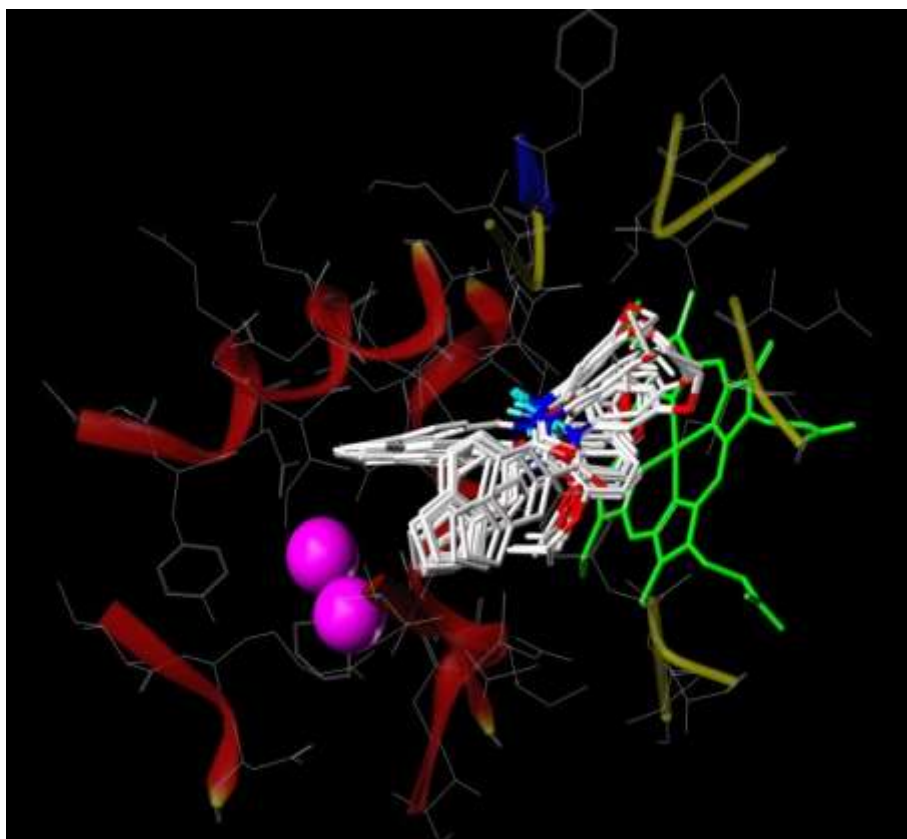


Figure 3: All 30 conformations of Chemdiv_J006-1410, shown docked into the active site of CYP17A1, via docking into the protomol. Rendered using Sybyl[®]-X v1.1^{20,21}.

The X-SCORE[®] v1.3²⁶ results obtained for ligand binding energy and the ligand binding affinity were $-9.60 \text{ kcal mol}^{-1}$ and 7.04 respectively. The lower the binding energy the greater the feasibility for a ligand-receptor complex.

A total of 727 novel molecules were obtained from *de novo* drug design using LigBuilder[®] v1.2²⁷, of which 465 were found to be Lipinski rule²⁴ compliant (refer to table 3). Seed ‘non-pyridine.mol2’ generated the highest number of molecules (total of 140) with all pKd values higher than the baseline value for that of abiraterone, this was followed by seed ‘Abr_Hspc_OH_Pyr.mol2’ (total of 67) and lastly, seed ‘C11_C12growtofillemptyspace.mol2’ (total of 7). The highest scoring molecule for seed ‘Abr_Hspc_OH_Pyr.mol2’ was result_094.mol2 (family 2) with a pKd value of 8.54, the highest scoring molecules for seed ‘non-pyridine.mol2’, included four novel molecules. All four molecules exhibited a pKd value of 10, this included result_017.mol2 (family 2), result_018 (family 2), result_051.mol2 (family 3) and result_196.mol2 (family 4). For seed ‘C11_C12growtofillemptyspace.mol2’ the highest scoring molecule was result_001.mol2 (family 1) with a pKd value of 7.87 (refer to table 3).

The results generated using UCSF Chimera^{®31}, indicated that the volume of the surface occupied by the protomol is higher than that for the ‘key site’ (see figure 3). The volume obtained for the protomol was that of 946.5 \AA^3 whilst the volume obtained for the ‘key site’ was that of 686.7 \AA^3 . When the values were subtracted, this resulted in a volume difference of 259.8 \AA^3 .

Since many molecules were generated using ViCi[®] Hamburg²², with more than 99 molecules exhibiting a positive *total score* value, these will make ideal candidates for molecular databases which can be made accessible to other research projects. Having said that, these molecules will be ideal candidates for lead molecule search or even to be used for further improvising anti-prostate cancer drugs. Since the molecules were docked into a protomol and not the ‘key_site’ generated using Ligbuilder[®] v1.2²⁷ this offered a greater advantage since the molecules were docked into a much more explored space within the active site. This is in fact evident from the surface volume explored within the active site whilst generating the protomol, which is much higher than that exhibited by the ‘key_site’.

The *de novo* molecules generated from each seed, were analysed for pharmacophoric evaluation. During the latter evaluation, it was critical to identify the newly added moieties or atoms in order to identify the key structural differences when compared with abiraterone and also aid in

identifying key interaction areas within the active site of CYP17A1. The SAR features for abiraterone were established using Discovery Studio[®] v4.1³², indicating that abiraterone exhibited extensive hydrophobic interactions and also haem moiety interaction with the pyridine moiety, as established by Devore and Scott, 2012¹⁷.

For seed 'Abr_Hspc_OH_Pyr.mol2' the steroid backbone was removed and two *H.spc* areas were created to GROW new moieties between the pyridine and 3 β -OH structures. Both the pyridine moiety and the 3 β -OH are vital due to the important interactions formed within the active site of CYP17A1, especially the pyridine moiety which interacts with the protoporphyrin XI (haem) structure. Overall it is interesting to note that the novel molecules generated from this seed structure exhibited the formation of ring structures and also carbon side chains that interact via hydrophobic interactions which mimic similar characteristics to the steroid backbone in abiraterone. Additionally, the side chains also helped fill the empty areas surrounding the 'steroidal' backbone and pyridine moiety, within the active site. This helped fulfil a query that was described by Devore and Scott's study in 2012¹⁷, which limited medicinal chemists in designing molecules like abiraterone by only targeting the protoporphyrin XI (haem) structure within the active site of CYP17A1. All compared molecules exhibited hydrophobic interactions with the protoporphyrin ring XI structure together with some hydrogen bonding which was present in less number than hydrophobic interactions.

Although the pyridine moiety was vital for abiraterone to bind within the active site of CYP17A1, by creating an *H.spc* GROW site for seed 'non-pyridine.mol2', it was tested out whether new moieties would help improve the binding of abiraterone instead of the pyridine moiety. It was very interesting to note that many novel molecules from this seed exhibited either the addition of a ring moiety or chain structures instead of the pyridine ring structure. These observations indicated that although the pyridine moiety was being replaced, in some novel molecules, it was so by including a ring moiety, suggesting that in this area of molecular interaction it may be still required. Additionally, it was also noted that a lot of Nitrogen atoms and/or oxygen atoms were included in the GROW process, suggesting the formation of new interactions. Since the steroid backbone was also retained for this seed, the hydrophobic interactions exhibited by abiraterone, were also observed in these novel molecules, with the additional predominant quantity of hydrophobic interactions exhibited by the newly grown moieties. The novel molecules generated from this seed exhibit a very high level of hydrophobic interactions, which made them contribute to the high pKd results.

For the last seed 'C11_C12growtofillemptyspace.mol2', creating the GROW sites on carbon 12 and carbon 11, was done in order to try and grow new moieties which can possibly fill the empty areas surrounding the pyridine moiety, as described by Devore and Scott, 2012¹⁷. Although not many novel molecules were generated, it was noted that many hydrophobic interactions were involved, by the newly generated moieties, suggesting that like the other seeds, hydrophobic interactions were predominant contributors together with the presence of some hydrogen bonds.

CONCLUSION

Although some structural information is still not available for the CYP17A1 protein structure³³, the *in silico* and *de novo* drug design computational work carried out in this study, helped identify moieties and also structural molecules that are highly contributive towards developing newer and effective molecules for the treatment of prostate cancer. The SAR interactions analysed also helped identify characteristic features of atoms and moieties that may be required in the future development of new drugs, with the aim of having a better drug profile and efficacy, over and above that exhibited by abiraterone. All the molecules generated can also be made available to other research projects or online molecular databases, from which researchers can sift through or select as reference molecules with the aim of developing newer molecules or leads, through the process of drug design. The next step following computational medicinal chemistry work, would be to finalize drug design and embark on validation process by conducting *in vitro* and *in vivo* assays.

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