Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Graphics and Modelling 22 (2003) 83–92
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

Comparative pharmacophore development for inhibitors of human and rat 5-α-reductase

Jane Faragalla [a], John Bremner [a,*], David Brown [b], Renate Griffith [c], Andrew Heaton [b]

[a] *Department of Chemistry and Institute for Biomolecular Science, University of Wollongong, Wollongong, NSW 2522, Australia*
[b] *Novogen Ltd., 140 Wicks Road, North Ryde, NSW 2113, Australia*
[c] *School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia*

Accepted 17 March 2003

Abstract

There are a number of diseases where the 5-α-reductase (5AR) enzyme is of therapeutic interest as a drug target. Currently the crystal structure for 5-α-reductase is unavailable, thus ligand-based pharmacophore techniques are beneficial in the drug development process. We have developed pharmacophores to aid inhibitor design for both human types I (preliminary) and II 5-α-reductase isozymes and also the rat type II isozyme. To our knowledge, these are the first published pharmacophores for inhibitors of the human type I and rat type II enzymes. A comparison between isozymes and the previously published human type II isozyme pharmacophore is also presented.

Keywords: 5-α-reductase; Inhibitors; Structure–activity relationships; Pharmacophore; Variable weight pharmacophore; Training set randomization

1. Introduction

The 5-α-reductase (5AR) enzyme is bound to the nuclear membrane and converts the endogenous hormone, testosterone (T), to the more potent androgen dihydrotestosterone (DHT), with the involvement of the cofactor NADPH [1]. The enzyme is involved in many conditions and diseases with elevated DHT levels, including benign prostatic hyperplasia, prostate cancer, hirsutism, acne and male patterned baldness [2]. Inhibitors of 5AR are thus of therapeutic interest, [3] particularly for prostate cancer. This type of cancer is the most common non-cutaneous cancer among men in most western countries and the second most deadly cancer [4].

There are two isozymes of 5AR, namely types I and II. Type I is prevalent in hair follicles and subcutaneous glands of the skin while type II is prevalent in the prostate, genital skin, seminal vesicles and epididymis [5]. The occurrence of 5AR has also been noted in the liver and central nervous system [6]. The isoforms have optimal activities at different pH ranges and there are also differences in the enzyme between humans, rats and dogs [7]. By designing different isoform selective inhibitors the likelihood of unwanted side effects should be decreased. For example a selective type 1 inhibitor could treat acne without significantly affecting testosterone metabolism in the prostate, thus allowing sexual function to continue as normal. There is no crystal structure for 5AR, thus the use of ligand-based pharmacophore design is pertinent for inhibitor development.

Various steroidal [8] and non-steroidal [9–16] inhibitors have been synthesized and tested against 5AR. Of these, only Finasteride (PROSCAR®) (**1**) (Fig. 1), a 4-azasteroid, has been used clinically as a type II-selective inhibitor for benign prostatic hyperplasia [8]. However, Finasteride is slow acting [8] and produces side effects affecting sexual function [17] which may be associated with its steroidal structure. Therefore there is a clear need for new non-steroidal inhibitors as therapeutics for diseases involving 5AR for both isozymes.

As part of a program to develop such non-steroidal inhibitors we have investigated ligand-based pharmacophores for human 5AR types I and II, and rat type II enzyme, to inform the design process for new selective inhibitors and to gain a greater understanding of the structure–5AR inhibitory activity relationships between species. Recently, a pharmacophore for human 5AR type II inhibitors was published but included steroidal structures, [16] and in the present paper a comparison of pharmacophores is presented together with

* Corresponding author.
E-mail addresses: jef02@uow.edu.au, john_bremner@uow.edu.au (J. Bremner).

1093-3263/03/$ – see front matter
doi:10.1016/S1093-3263(03)00138-4

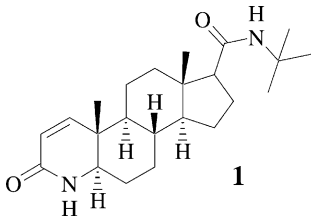

Fig. 1. The chemical structure of the human 5AR type II 4-azasteroidal inhibitor, Finasteride (PROSCAR®) (**1**).

the first pharmacophore (preliminary) for non-steroidal inhibitors of human 5AR type I, and for inhibitors of rat type II enzymes.

2. Methodology

2.1. General methodology

The CATALYST® program, version 4.5, from Accelrys® was used for all pharmacophore development.

2.2. Training set selection and generation

For all pharmacophores, compounds from the literature with a range of 5AR enzyme inhibitory activities, spanning at least four orders of magnitude, were included in the training sets (Table 1). The IC_{50} values were taken from the literature; standard deviations were not given. Training set compounds and references to the literature are given in the supporting material. Only non-steroidal molecules with defined stereochemistry were included, so racemates were excluded. A range of different structural types was also incorporated into the pharmacophores. The human type I isozyme pharmacophore contains biphenyls, tricyclic lactams and tricyclic thiolactams. The human type II pharmacophore includes indoles, biphenyls and benzoquinolinones, while the rat type II isozyme pharmacophore contains biphenyl, indole and arylpiperazine derivatives. CATALYST® does not account for a loss of activity as a result of steric hindrance [18]. Compounds which had a low

Table 1
The number of compounds and their range of activities included in each of the 5AR inhibitor pharmacophores

Pharmacophore	No. of compounds included	Range of activity IC_{50} (nM)
Human type I	15	3.4–1400
Human type II	46	0.29–10000
Rat type II	27	1.1–12000

biological activity for suspected steric reasons, were thus excluded from the training sets. For example, the biphenyl compound **2**, which has a very low activity compared to **4**, was excluded from the human type II pharmacophore as the extra phenyl ring marked by the arc in Fig. 2, was likely to sterically inhibit binding.

The compounds were also chosen according to the type of biological testing in which they were involved. The included compounds had been tested on protein extracts of cells; results obtained from cell-based assays were not included. The source of protein was considered to be critically important. Ideally, training set compounds should be tested on extracts taken from cells containing recombinant enzyme consisting of a pure isozyme. However, compounds tested in this manner are a very limited group in the literature and insufficient for meaningful pharmacophore generation. Thus compounds tested on human scalp protein extracts were used for the human type I isozyme pharmacophore, while for the human type II isozyme pharmacophore, data based on protein extracts from benign hyperplastic prostatic tissue were used. These tissues were chosen as the sources as they contained a dominant amount of the relevant isozyme [19]. For the rat type II enzyme inhibition data from rat prostatic tissue was used.

Conformers of each molecule were generated using the standard 20 kcal/mol limit (relative to the lowest energy conformer found) and the best conformer search option. While the energy limit of 20 kcal/mol of a conformer is high, it is the recommended value for CATALYST®, as it provides a large spread of conformers. CATALYST® accounts for conformers of a high energy during the hypothesis generation process through a penalty system and CATALYST® is in fact optimized for a 20 kcal/mol limit. CATALYST® uses

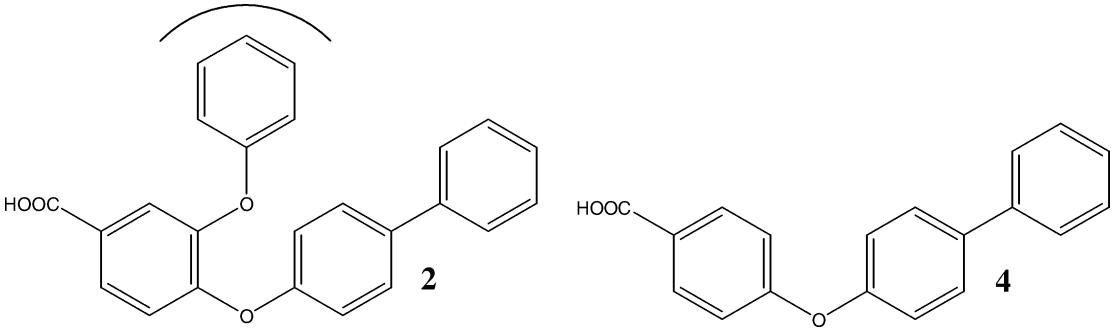

Fig. 2. The biphenyl compound **2**, which was excluded from the 5AR type II pharmacophore training set on steric grounds, compared with **4**.

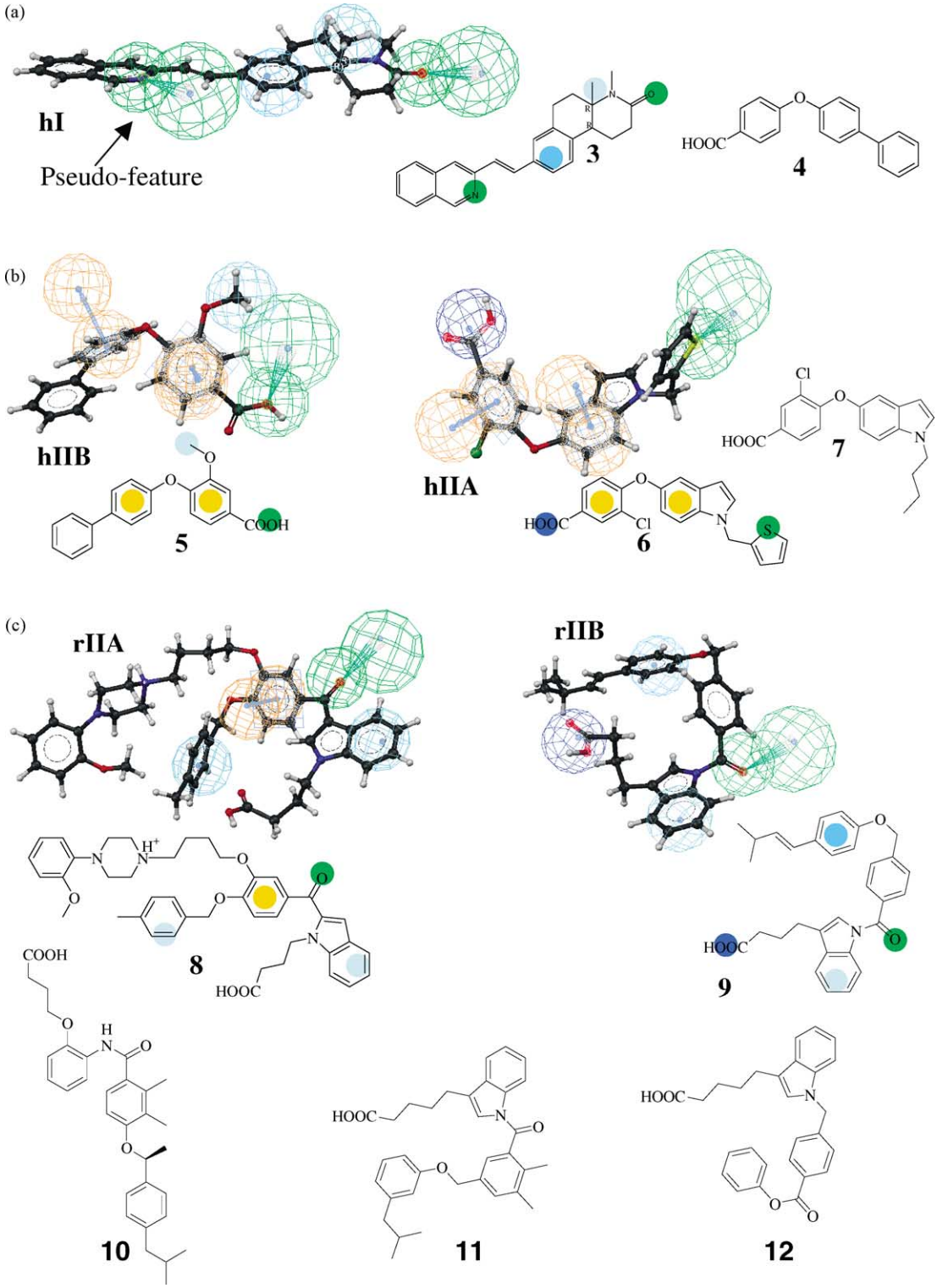

Fig. 3. The compounds which make up the most active group of the corresponding training sets. Hypotheses for inhibition of the rat and human 5AR enzymes ((a) human type I, (b) human type II and (c) rat type II) with the most active compounds **3** (hI), **5** (hIIB), **6** (hIIA), **8** (rIIA), and **9** (rIIB) in the training sets mapped onto them. The wire cage spheres represent the pharmacophore features. For hydrogen bond acceptor (HBA) and ring aromatic features the smaller sphere represents the area where the feature of the molecule lies during the mapping, and the larger sphere represents the interaction point of the enzyme. The coloured circles on the two dimensional chemical structures show where a feature has been mapped onto a compound. Colour coding: dark blue, negative ionisable; blue, hydrophobic aromatic; light blue, hydrophobic; orange, ring aromatic; green, HBA.

the 'poling algorithm' [20–22] to ensure effective sampling of conformational space.

2.3. Generation of hypotheses

CATALYST® generates hypotheses consisting of features. These features are displayed as round mesh balls, which represent an area in space relative to the other features where a characteristic of a molecule should be to induce the desired activity (location constraints).

CATALYST® generates hypotheses with a heavy emphasis on a group of molecules which are the most active of the training set with activities falling into a certain activity range. The upper limit of this activity range is defined by CATALYST® to be the product of the uncertainty value multiplied by the activity of the most active compound in the training set. The lower limit of the activity range is the activity belonging to the most active molecule. It is very important that the hypotheses generated are not biased by any one chemical structure type, so it is necessary to ensure that compounds of more than one structural type are included in the most active group in the training set.

Compound **3** and the structurally different biphenyl **4** (Fig. 3) are considered by CATALYST® to be the most active compounds in the human 5AR type I training set, and they therefore heavily biased the pharmacophore generation. The most active compound in the human type II training set was the biphenyl derivative **5** (Fig. 3), with an IC_{50} of 0.29 nM. As the uncertainty of the training set was three (standard value), the compounds which were considered to be the most active had an activity up to 0.87 nM. For the human type II inhibitor training set, this group thus consisted of three compounds, namely compound **5** and the indoles **6** and **7** (Fig. 3). The CATALYST® program again heavily biases pharmacophores on this most active set of compounds. As the biphenyl compound is quite different structurally from the indoles, it is reasonable to assume that the pharmacophore generated is not biased toward one type of compound. The most active compound in the rat 5AR type II training set is **8** which has an IC_{50} of 1.5 nM, which indicates that compounds **8**–**12** are in the most active group of the rat 5AR type II training set.

In this study, hypotheses were generated using default parameters including a minimum number of features of three. The allowed set of features were hydrophobic, hydrophobic (aromatic), ring aromatic, hydrogen bond acceptor and negative ionisable groups. A ring aromatic feature is defined as a feature which maps 5- and 6-membered aromatic rings (which may be next to a charge) and the negative ionisable feature is defined as a feature which maps atoms or groups of atoms that are likely to be deprotonated at physiological pH [23]. Hydrogen bond donor (HBD) groups were trialled, although CATALYST® never included any HBD feature in the generated pharmacophores and they were thus left out. The negative ionisable feature was included in the allowed set as a number of the inhibitors had carboxylic acid groups.

The CATALYST® default mode generates hypotheses where each feature relatively has equal importance or weight within the hypothesis. However, it is possible to generate a hypothesis with different relative weights for features, thus allowing different features to have ranked importance. This variable weight option was considered for each pharmacophore generated. For the human type II pharmacophores the variable weight option was not utilized, since when this option was trailled, the configurational cost (see next section for a discussion of hypotheses costs) was too high and the same pharmacophores were generated without this option. However, for the human 5AR type I and rat type II pharmacophores, the variable weight option was utilized as the correlations increased (from 0.76 to 0.79 and 0.85 to 0.94, respectively; see next section for a discussion of hypothesis costs) making the sacrifice of a high configurational cost (22 and 32, respectively) worthwhile.

3. Results and discussion

CATALYST® generates hypotheses by assigning chemical features to the training set molecules, then arranging the features so that the molecules map with a ranking which correlates with their activity. The hypothesis generation places a greater importance on the molecules contained in the most active group of the training set.

CATALYST® produces 10 hypotheses (sometimes 9) which are arranged in a hierarchical manner according to the CATALYST® cost analysis, which takes into account many factors including the size of the training set. The most influential parameter contributing to this cost analysis is the correlation between the hypothesis estimated activity values and the real activity values. The correlation value can lie between zero and one, with one being a perfect correlation. CATALYST® cost analysis also takes into account a configurational parameter which describes the complexity of the problem (this parameter should be under 17, otherwise not all possibilities for patterns will be searched exhaustively). CATALYST® cost analysis also employs the principle of Occam's razor [24] whereby a hypothesis should be as simple as possible. This cost analysis allows one to assess the validity of the produced hypotheses. This involves the use of three cost parameters:

1. The null hypothesis cost parameter assumes that all training set molecules have the same activity, so that there is no statistically significant structure in the training set. This cost parameter has the highest numerical value of all the cost parameters.
2. The ideal hypothesis cost parameter assumes all training set molecules fit the simplest possible hypothesis perfectly. This cost parameter has the smallest numerical value of all the hypotheses cost parameters.
3. The individual hypothesis cost, which results in a ranking of generated hypotheses. This parameter takes into

account the correlation of the training set molecules' tested activities with the activity estimated by the hypothesis.

To determine whether the pharmacophores produced are statistically significant, the individual hypothesis costs should be subtracted from the null hypothesis cost, giving rise to the resultant cost. If the resultant cost is over 60 bits then the hypothesis is deemed statistically significant (over 90% chance that the hypothesis is a true correlation), while a resultant cost of 40–60 bits translates into a 75–90% statistical probability and for a value below 40 bits the probability drops to below 50% [23,25].

4. Hypothesis analysis

4.1. Human type I inhibitor pharmacophore

Hypothesis generation for the human 5AR type 1 training set produced nine pharmacophores. The pharmacophore which was scored as the sixth highest hypothesis (hI) was chosen as the most accurate pharmacophore. The highest scoring hypothesis was blank and the next four were rejected on the basis that they contained a 'pseudo-feature' which meant the pharmacophores consisted of less than the required three feature minimum. A pseudo-feature is defined as a feature which only maps the most active compound and none of the other compounds in the training set. Although the hypothesis generation was limited to a minimum of three features the five top scoring hypotheses each essentially contained two features only.

Pharmacophore hI consists of essentially three features and one pseudo-feature: a HBA, a hydrophobic, a hydrophobic–aromatic feature and a HBA pseudo-feature (Table 2). The pseudo-HBA feature was not considered as an accurate feature and will not be discussed. The distances and angles between features are shown in Table 3. Although the pharmacophore generation was set up for variable weights, the hI pharmacophore features had similar weightings of around 2. The resultant cost of the sixth scoring hypothesis is −14 (Table 2) and a correlation of 0.72. The resultant cost is obviously not large enough to deem the pharmacophores statistically significant and therefore it was considered as a qualitative and not quantitative pharmacophore.

Compound **3** and the structurally different biphenyl **4** (Fig. 3) are considered by CATALYST® to be the most active compounds in the training set, and they therefore heavily biased the pharmacophore generation. Fig. 3a depicts the mapping of compound **3** onto hypothesis hI.

4.2. Human type II inhibitor pharmacophore

The training set for human 5AR type II produced two different types of hypotheses. The highest scoring hypothesis for each type is presented (Fig. 3), these being the top scoring hypothesis (IIA) and the fourth scoring hypothesis (IIB). The difference between the cost of the null hypothesis and these

Table 2
Summary of pharmacophores discussed, including their features, resultant costs, and feature weightings

Pharmacophore name	Enzyme type	Features	Resultant cost (null-hypothesis)	Feature weighting
hI	Human type I	HBA Hydrophobic Hydrophobic–aromatic	−14	1.96120 1.96120 2.54956
hIIA	Human type II	HBA Negative ionisable Ring aromatic Ring aromatic	100	Equal
hIIB	Human type II	HBA Hydrophobic Ring aromatic Ring aromatic	96	Equal
rIIA	Rat type II	HBA Hydrophobic1 Hydrophobic2 Ring aromatic	32	2.57685 2.57685 1.61053 2.57685
rIIB	Rat type II	HBA Negative ionisable Hydrophobic Hydrophobic–aromatic	30	2.50054 2.50054 1.34644 2.50054
Chen et al. [16]	Human type II	HBA1 HBA2 Hydrophobic Hydrophobic Hydrophobic	22	Equal

Table 3
Distances and angles of the 5AR inhibitors pharmacophores from this work and the pharmacophore of Chen et al. [16]

Pharmcophore	Distance ranges (Å) and angles between features[a]
h1	HBA-H, 3.4–5.4; HBA-HA, 6.2–8.2; H-HBA-HA, 24.2–34.2°
hIIA	HBA-NI, 9.3–11.3; HBA-RA1, 8.5–10.5; HBA-RA2, 5.5–7.5; RA1-NI-RA2, 25.5–35.5°
hIIB	HBA-H, 3.9–5.9; HBA-RA1, 7.0–9.0; HBA-RA2, 2.9–4.9; RA1-H-RA2, 31.8–41.8°
rIIA	HBA-H1, 7.6–9.6; HBA-H2, 3.3–5.3; HBA-RA, 2.8–4.8; H1-RA-H2, 104.7–114.7°
rIIB	HBA-HA, 6.8–8.8; HBA-NI, 7.4–9.4; HBA-H, 3.7–5.7; HA-NI-H, 87.5–97.5°
Chen et al. [16]	HBA1-H1, 2.2–2.4; HBA1-HP2, 6.1–8.1; HBA1-HBA2, 9.8–11.8; HBA1-H3, 10.8–12.8; H1-H2, 3.2–5.2; H1-HBA2, 7.2–9.2; H1-H3, 8.9–10.9; H2-HBA2, 3.3–5.3; H2-H3, 5.6–7.6; HBA2-H3, 2.3–4.3

[a] Abbreviations used for features: HBA, hydrogen bond acceptor; RA1, ring aromatic; NI, negative ionisable; H, hydrophobic; HA, hydrophobic–aromatic.

hypotheses are 100 and 96, respectively (Table 2), indicating both are statistically valid. The configurational cost for this set of hypotheses is 16.5, thus all pharmacophore options were considered by CATALYST®.

Hypothesis IIA consisted of a hydrogen bond acceptor, negative ionisable group and two ring aromatic features (Tables 2 and 3). This pharmacophore maps all the features for the most active compound of the training set. However, the other two compounds in the most active group of the training set map all but the hydrogen bond acceptor feature.

Hypothesis IIB consists of a hydrogen bond acceptor, hydrophobic and two ring aromatic features (Tables 2 and 3). While this hypothesis may be considered for deletion due to its high non-specific feature content, if the importance of the hydrophobic nature of 5AR inhibitors is considered, [15] then the hypothesis should be deemed valid. Further validation of this hypothesis was forthcoming from the fact that all compounds that constituted the most active group in the training set plus the next most active compound, mapped all four features of the hypothesis, while all other compounds in the training set missed at least one feature when they were mapped, which explained their lower activities.

A distinguishing feature between the hypotheses is the error of the training set compound **13** (Fig. 4), when mapped onto the hypotheses. For hypothesis IIA, **13** had an error of +68, however, in the hypothesis IIB **13** had an error of 5.4; this may be due to the nitro functionality mimicking the carboxylate ion. Thus we conclude that hypothesis IIB is more appropriate for this isozyme.

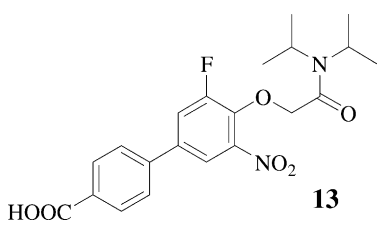

Fig. 4. The training set compound **13** that distinguishes between the two types of 5AR type II hypotheses, IIA and IIB.

4.3. Rat type II inhibitor pharmacophore

The training set of the 5AR rat inhibitors was trialled with and without variable weights. These two trials produced similar results in terms of the cost analysis, however, the correlations improved from 0.8 to 0.9 with the introduction of variable weights.

The pharmacophores produced can be categorized into one of two groups: pharmacophores which contain one HBA or negative ionizable feature and pharmacophores which contain two HBA or negative ionizable features. The top scoring pharmacophore of each group is presented in Fig. 3. The pharmacophores with pseudo-features are not included.

The two top scoring pharmacophores contain pseudo-features. Therefore, the third scoring hypothesis (rIIA) is presented as it was the top scoring pharmacophore with one HBA group (and no negative ionizable group) which does not contain a pseudo-feature. The highest scoring pharmacophore containing a HBA and a negative ionisable group with no pseudo-features, is the ninth scoring pharmacophore (rIIB).

Pharmacophore rIIA contains one HBA group, a ring aromatic feature and two hydrophobic features of unequal weighting (Table 2). The distances and angles between the features are shown in Table 3. The resultant cost is 32 bits which is interpreted as a hypothesis of moderate statistical validation.

The pharmacophore rIIB contains a HBA feature, a negative ionisable feature, a hydrophobic feature and a hydrophobic–aromatic feature also of unequal weighting (Table 2). The distances and angles between the features are shown in Table 3. The resultant cost is 30 bits which is interpreted in the same way as rIIA.

The resultant costs of the pharmacophores rIIA and rIIB do not reach the 60 bit statistical validation, thus an additional statistical analysis was performed. This additional analysis is done by generating hypotheses with the same training set, however the activities were randomly mixed, and the process was repeated eight times. These generations produced hypotheses with smaller resultant cost and correlation values than those of the hypotheses which are

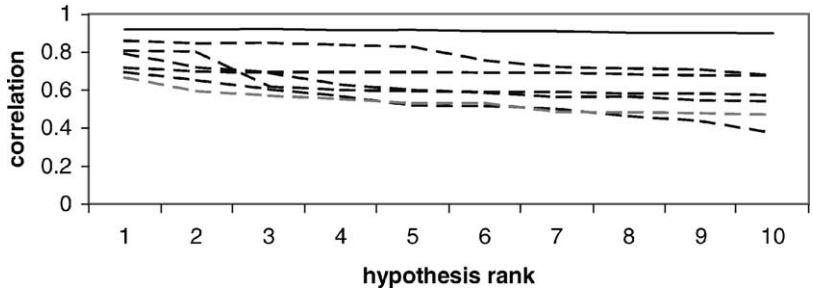

Fig. 5. The correlation trends of the rat 5AR type II non-randomized and randomized hypotheses. The solid line represents correlations from the unrandomized rat 5AR type II training set and the broken lines represent correlations of hypotheses generated using the randomized training sets.

attributed to the true training set. This gives an additional statistical validation to the rIIA and rIIB hypotheses. It was also observed that the decline in the correlations was much more marked in the randomized hypotheses than in the true hypotheses (Fig. 5).

The isoflavonoid rat 5AR type II inhibitors used in a paper by Chen et al. [16] were mapped onto both rat type II pharmacophores. These inhibitors have IC_{50}'s larger than the IC_{50}'s of rat type II inhibitors found in the training set. The rat type II pharmacophores predicted these isoflavanoid rat 5AR type II inhibitors to be poor inhibitors, thus giving additional validation to the pharmacophores.

4.4. Comparisons between training sets and pharmacophores of human 5AR type II inhibitors and previously published work

Chen et al.'s recently published pharmacophore [16] for inhibitors of human 5AR type II has points of similarity and difference between the human 5AR type II pharmacophores which are presented in this paper. The major difference in the training sets is that Chen et al. used both steroidal and non-steroidal compounds, while we have only used non-steroidal compounds. As it is not known whether steroidal structures bind in the same way as non-steroidal structures (and to eventually develop new inhibitors free of steroidal side effects), we decided to restrict our training set to non-steroidal compounds. The inclusion of steroidal training set molecules may bias their pharmacophore. In fact, their most active compound Finasteride **1** (IC_{50}, 0.18 nM) is an aza-steroidal derivative. Chen et al. also used standard parameters in the generation of their pharmacophore. Presumably, this included an uncertainly of three, which meant that only compounds with an IC_{50} of 0.54 nM or below were included in their most active compound group. This criterion is only met by Finasteride **1** in their training set, thus giving their pharmacophore a bias toward this compound. Another difference is in the number of compounds used in the training set, with 46 in this study and 15 in that of Chen et al. This variation is reflected in the resultant cost of Chen et al.'s and our hypotheses (22 and ∼100, respectively). The main similarity between the two training sets is the use of similar indole compounds **14**–**18** which exhibit a high degree of similarity (Fig. 6).

Chen et al. presented their top scoring human 5AR type II pharmacophore, which consists of two hydrogen bond acceptor and three hydrophobic features (Table 2). The main difference between this pharmacophore and our pharmacophores is that the last two only contain four features while the first contains five. Since they only allowed hydrophobic and HBA features for pharmacophore generation, their pharmacophore can also be thought of as two negatively polarized features and three non-polar features.

When considering the two types of pharmacophores generated from our human type II training set, the Chen et al. pharmacophore is most similar to the IIA pharmacophore, which includes a HBA and a negative ionisable feature. This can also be thought of as two negatively polarized functionalities, therefore equating with Chen et al.'s HBA features. Strong similarities are evident, however, in the distance ranges and angles between these features in each pharmacophore, as can be seen in Table 3. The distances between two of Chen et al.'s hydrophobic features and their distances to the HBA features correlate with the distances between IIA's ring aromatic features and the corresponding HBA or negative ionisable group (Table 3).

Pharmacophore IIA further resembles the Chen et al. hypothesis in that it contains two ring aromatic features and a hydrophobic region, which are three non-polar features, thus correlating with their three hydrophobic features.

4.5. Comparisons between human types I and II training sets and pharmacophores

There is a large degree of difference between training sets of the human 5AR types I and II pharamcophores presented in this paper, the main difference being the number of compounds in the training sets. This difference is reflected in the resultant costs of the pharmacophores (−14 and ∼100, respectively). This large variation in resultant costs is also a reflection of the difference in correlations between rIIA (0.88) and rIIB (0.86), and hI (0.73). However, a similarity between the training sets is the presence of similar biphenyls **4** and **5** in their most active compound groups.

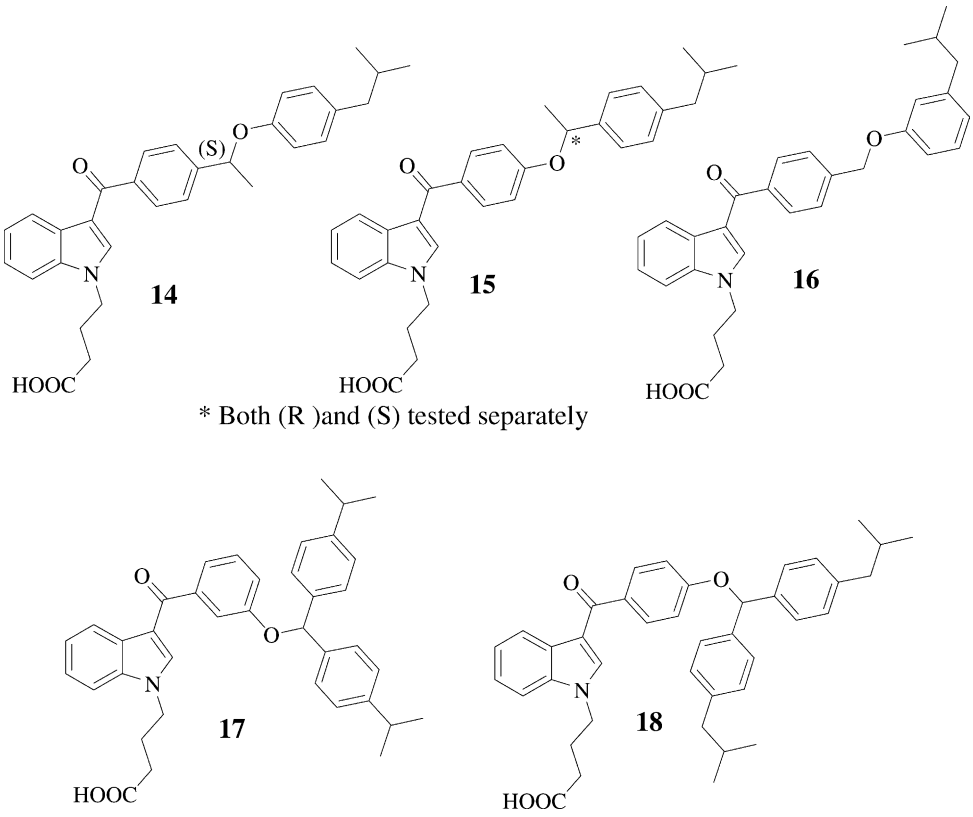

Fig. 6. The similar compounds used in the human type II (this work) and Chen et al. [16] training sets. Compounds **16** and **18** are from our human 5AR type II training set, the others from Chen et al. [16].

A large difference between the actual pharmacophores is that type I only has three features, plus one pseudo-feature, while type II pharmacophores contain four.

The hI pharmacophore is more similar to pharmacophore IIB than IIA (overlays in Fig. 7), with both hI and IIB having a HBA feature and no negative ionisable feature, while hypothesis hIIA for the type II isozyme contains both a HBA and a negative ionsable feature. The distances between the HBA and hydrophobic features in the hI and IIB pharmacophores are similar (within 0.5 Å; Fig. 7). Also

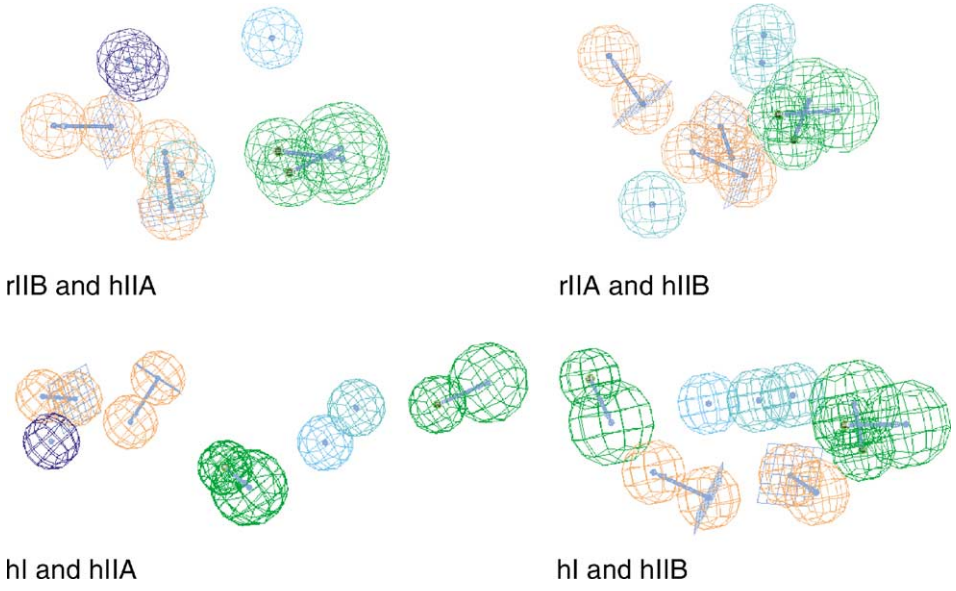

Fig. 7. Overlays of the rat and human 5AR type II pharmacophores, and the human 5AR types I and II pharmacophores; see Fig. 3 for details.

the distances between the HBA and hydrophobic–aromatic feature in hI are similar to the distances between the HBA and ring aromatic 1 in hIIB (within 0.8 Å), however this is not apparent in the overlay shown in Fig. 7 (see Table 3 for actual distances). The distances between the negative ionisable feature and the two ring aromatic features of hIIA also are similar to the distances between the HBA feature and the hydrophobic and hydrophobic aromatic features in hI. Furthermore the angle RA1-NI-RA2 in hIIA is very similar to the angle H-HBA-HA in the hI pharmacophore (within 1.3°). However, the angle H-HBA-RA2 in the hIIB pharmacophore is nearly double the angle HA-HBA-H in the hI pharmacophore.

4.6. Rat type II and human type II pharmacophore comparison

The training sets for these two sets of pharmacophores were quite different in terms of the number of compounds included (Table 1), with the human type II training set being much larger. This is reflected in the resultant cost, with the human pharmacophores having a large resultant cost (∼100) and the rat pharmacophores possessing a modest cost (∼32). The differences in resultant costs are not due to differences in the correlations of the pharmacophores, with all pharmacophores having correlations of about 0.9 (rIIA, 0.91; rIIB, 0.90; hIIA 0.88; hIIB, 0.86). The most active compounds of the training sets are generally quite different, however, they both include indole compounds, specifically **6** and **7** for human type II and **8**, **9**, **11** and **12** for rat type II (Fig. 3).

Two categories of pharmacophores were produced, one of which had two HBA/negative ionisable features, while the other category had only one HBA feature and no negative ionisable feature. The corresponding rat and human type II pharmacophores have been compared within these catagories.

Pharmacophores hIIA and rIIB comprise the category of pharmacophores which have two HBA /negative ionisable features, and they contain a HBA and a negative ionisable feature. Although similar overall, the distance between the negative ionisable group and the HBA group of rIIB is smaller than that of hIIA (Table 3); and there is a good overlap between these two features (Fig. 7). Furthermore they both contain two additional non-polar features. For hIIA these features are ring aromatic features and for rIIB they are a hydrophobic feature and a hydrophobic–aromatic feature (Table 2). From the overlay of the two pharmacophores in Fig. 7 it can be seen that the orientations of these features are quite different in the rat and human pharmacophores. It can also be seen that the distances between the two non-polar groups from the negative ionisable group in rIIB and hIIA are very similar, but the angle enclosed by the non-polar-negative ionisable/HBA-non-polar features is about four times smaller in the hIIA pharmacophore than in the rIIB pharmacophore. The two non-polarized (ring aromatic) features in hIIA are a lot closer together (4.0–6.0 Å) than the non-polarized features in rIIB (9.1–11.1 Å).

Pharmacophores hIIB and rIIA are the pharmacophores which comprise the category of rat and human 5AR type II pharmacophores containing only one HBA feature. An additional similarity between these pharmacophores is that they both contain three non-polarized features with some similarities in orientation (Fig. 7). The ring aromatic feature of rIIA and the ring aromatic feature 2 of hIIB are the same distance away from the HBA feature in their respective pharmacophores. The ring aromatic feature 1 in pharmacophore hIIB is also the same distance away from the HBA feature in pharmacophore rIIA, however, as can be seen in Fig. 7, the orientations of the two groups are different.

5. Conclusion

The human 5AR type I non-steroidal inhibitor pharmacophore consists of three features, a HBA, a hydrophobic and a hydrophobic–aromatic feature. This is only a preliminary qualitative model, because of the small number of published test results for this isozyme. The rat and human 5AR type II pharmacophores returned two similar categories of pharmacophores, both with a total of four features. One of these categories is defined by a HBA and a negative ionisable feature (plus for the human enzyme inhibitors, two ring aromatic features or, for the rat enzyme inhibitors, hydrophobic and hydrophobic–aromatic features), while the other category is defined by a HBA feature and no negative ionisable feature (plus, for the human enzyme inhibitors, a hydrophobic or for the rat, two hydrophobic and a ring aromatic feature).

These inhibitor pharmacophore findings reinforce the presence of, and highlight the differences between, human and rat 5AR enzymes, as well as the differences between the human type I and human type II isozymes. The rat and human 5AR type II pharmacophores show some similarities in the type of features they incorporate, but the arrangement of the non-polar groups is different. This highlights the desirability of testing potential leads for medicinal agents on human tissue. One of our human type II pharmacophores (hIIA) is surprisingly similar to the previously published pharmacophore for this isozyme given the differences in the training sets. In our calculations, however, a different type of hypothesis (hIIB) is more likely to be a better design base for inhibitors of this isozyme, at least for non-steroidal inhibitors.

The human type I pharmacophore and one of the type II pharmacophores show some similarities, but again the features are orientated differently, which is an encouraging result in terms of the future development of potentially selective type II compounds as anti-prostatic cancer agents with limited side effects.

Supplementary material available via www.elsevier.com/locate/JMGM.

Acknowledgements

Support from the University of Wollongong and Novogen Ltd. is gratefully acknowledged.

References

[1] Y. Jin, T.M. Penning, Steroid 5-α-reductases and 3α-hydroxysteroid dehydrogenases: key enzymes in androgen metabolism, Best Pract. Res. Clin. Endocrinol. Metabol. 15 (2001) 79–94.
[2] F. Machetti, A. Guarna, Novel inhibitors of 5-α-reductase, Expert Opin. Therap. Patents 12 (2002) 201–215.
[3] A. Cilotti, G. Danza, M. Serio, Clinical application of 5-α-reductase inhibitors, J. Endocrinol. Invest. 24 (2001) 199–203.
[4] A.G. Papatsoris, A.G. Papavassiliou, Prostate cancer: horizons in the development of novel anti-cancer strategies, Curr. Med. Chem. 1 (2001) 47–70.
[5] A.E. Thigpen, R.I. Silver, J.M. Guileyardo, M.L. Casey, J.D. Mc Connell, D.W. Russell, Tissue distribution and ontogeny of steroid 5-alpha-reductase isozyme expression, J. Clin. Invest. 92 (1993) 903–910.
[6] D.W. Russell, J.D. Wilson, D. Jean, Steroid 5-alpha reductase: two genes/two enzymes, Annu. Rev. Biochem. 63 (1994) 25–61.
[7] T. Liang, M.A. Cascieri, A.H. Cheung, G.F. Reynolds, G.H. Rasmusson, Species differences in prostatic steroid 5-α-reductase of rat, dog, and human, Endocrinology 117 (1985) 571–579.
[8] A. Kurup, R. Garg, C. Hansch, Comparative QSAR analysis of 5-α-reductase inhibitors, Chem. Rev. 100 (2000) 909–924.
[9] A.D. Abell, M.J. Prince, A.M. Mc Nulty, B.L. Neubauer, Simple Bi and tricyclic inhibitors of human steroid 5-α-reductase, Bioorg. Med. Chem. Lett. 10 (2000) 1909–1911.
[10] A. Guarna, F. Machetti, E.G. Occhiato, D. Scarpi, A. Comerci, G. Danza, R. Mancina, M. Serio, K. Hardy, Benz[c]quinolizin-3-ones: a novel class of potent and selective non-steroidal inhibitors of human steroid 5-α-reductase1, J. Med. Chem. 43 (2000) 3718–3735.
[11] F. Picard, T. Schulz, R.W. Hartmann, 5-phenyl substituted 1-methyl-2-pyridones and 4′-substituted biphenyl-4-carboxylic. Acids synthesis and evaluation as inhibitors of steroid-5-α-reductase type 1 and 2, Bioorg. Med. Chem. Lett. 10 (2002) 437–448.
[12] H. Takami, H. Koshimura, N. Kishibayashi, A. Ishii, H. Nonaka, S. Aoyama, H. Kase, T. Kumazawa, Indole derivatives as a new class of steroid 5 α-reducatse inhibitors, J. Med. Chem. 39 (1996) 5047–5052.
[13] K. Sawada, H. Hirai, P. Golden, S. Okada, Y. Sawada, M. Hashimoto, H. Tanaka, (1-Benzylindole-3-yl)alkanoic acids; novel non-steroidal inhibitors of steroidal 5-α-reductase (I), Chem. Pharm. Bull. 46 (1998) 1683–1687.
[14] K. Sawada, P. Golden, S. Okada, N. Kayakiri, Y. Sawada, M. Hashimoto, H. Tanaka, 4-(1-Benzoyl(indole-3-yl)butyric acids and FK 143: novel non-steroidal inhibitors of steroid 5-α-reductase (II), Chem. Pharm. Bull. 47 (1999) 481–491.
[15] S. Igarashi, H. Inami, H. Hara, M. Fujii, H. Koutoku, H. Oritani, T. Mase, A novel class of inhibitors for human steroid 5-α-reductase: synthesis and biological evaluation of indole derivatives (II), Chem. Pharm. Bull. 48 (2000) 382–388.
[16] G.S. Chen, C-S. Chang, W.M. Kan, C.-L. Chang, K.C. Wang, J.-W. Chern, Novel lead generation through hypothetical pharmacophore three-dimensional database searching: discovery of isoflavonoids as non-steroidal inhibitors of rat 5-α-reductase, J. Med Chem. 44 (2001) 3759–3763.
[17] S.L. Sudduth, M.J. Koronkowski, Finasteride: the first 5-alpha-reductase inhibitor, Pharmacotherapy 13 (1993) 309–325.
[18] H. Li, J. Sutter, R. Hoffman, Hypogen: an automated system for generating 3D predictive pharmacophore models in pharmacophore perception, development, and use, in: O.F. Guner (Ed.), Drug Design, vol. 175, International University Line, La Jolla, CA, 2000 (Chapter 10).
[19] P.N. Span, A.G.H. Smals, C.G.J. Sweep, T.J. Benraad, Rat steroid 5-α-reductase kinetic characteristics: extreme pH-dependancy of the type II isozyme in prostate and epididymis homogenates, J. Steroid Biochem. Metabol. 53 (1995) 185–192.
[20] A. Smellie, S.L. Teig, P. Towbin, Poling: promoting conformational variation, J. Comp. Chem. 16 (1995) 171–187.
[21] A. Smellie, S.D. Kahn, S.L. Teig, Analysis of conformational coverage. 1. Validation and estimation of coverage, J. Chem. Inf. Comp. Sci. 35 (1995) 285–294.
[22] A. Smellie, S.D. Kahn, S.L. Teig, Analysis of conformational coverage. 2. Applications of conformational models, J. Chem. Inf. Comp. Sci. 35 (1995) 295–304.
[23] CATALYST® Tutorials Release 4.5, Molecular Simulations Inc., San Diego, 1999, pp. 256–262.
[24] http://www.accelrys.com/catalyst/cathypo.html.
[25] H.J. Bohm, S. Fischer, *De novo* ligand design, in: P.V.R. Schleyer, N.L. Allinger, T. Clark, J Gasteiger, P.A. Kollman, H.F. Schaefer III, P.R. Schreiner (Eds.), Encycylopedia of Computational Chemistry, Wiley, Chichester, UK, 1998, pp. 657–663.