



I will cover two methods for the design of steroid analogues a more traditional approach, involving the stepwise modification of steroid cores followed by a computational method which can generate visual map as to how to try designing analogues.

Intro

Despite the availability of a large number of chemotherapeutic antineoplastic agents, the medical need remains still largely unmet. The main reasons behind the failure of chemotherapy include (i) the lack of selectivity of conventional drugs, (ii) the metastatic spreading of initial tumours, (iii) the heterogeneity of the disease, and (iv) multidrug resistance. These drawbacks prompt medicinal chemists to design and develop safer, target specific, and effective antineoplastic agents.

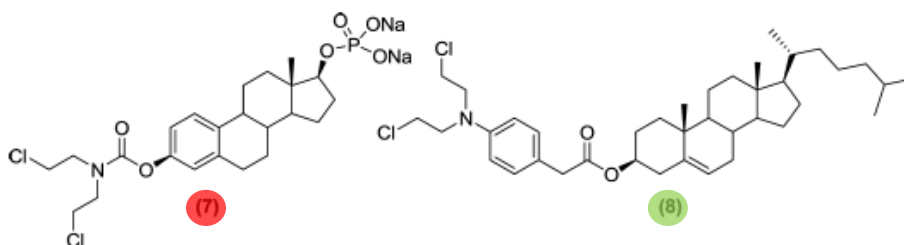
Steroids have massively versatile uses as therapeutics. One of these uses is in the treatment of cancer. A major advantage of steroids as cancer therapeutics is that the simplest modification of a steroid core can result in substantial changes in the activity and biological target.^{21,22} The growth of malignant tumours of the breast, prostate are often dependent on the hormonal balance of the body, steroids can affect these levels bringing out remission from cancer.²⁸⁻³⁰ The majority of approved drugs by the FDA over the last twenty years are compounds where new therapeutic uses have been found or analogues of existing drugs.²⁴

Steroid Conjugates

Steroidal alkylating agent hybrids have been shown to be very potent in the treatment of cancer,³⁹⁻⁴⁴ Steroid could deliver the alkylating agent to a specific target tissue more easily, reducing systemic toxicity due to reduced dose, increasing the bioavailability, and improving the therapeutic efficacy. Advantages of linking steroids and alkylating agents are as follows

- (i) tissue-selective cytotoxicity due to transportation by steroid
- (ii) reduced toxicity to healthy cells due to selectivity in the cancerous cells
- (iii) steroid moiety confers enhanced activity compared to the alkylating agent alone
- (iv) alkylating agent

Here we are going to focus extensively on the effect of attaching N,N-bis(2-chloroethyl)amine moiety (nitrogen mustard, nitrosourea, and cyclophosphamide) to steroidal skeletons, and see how slight modifications affect biological activity.



Estramustine **(7)** was designed with a carbamate linker between the steroid and nitrogen mustard. Compound **(7)** has no cytotoxic activity due to the unprecedented stability of the carbamate linker, if this linker is switched to an aromatic carbonate as seen in **(8)** cytotoxicity is turned on. The bridge is now able to be cleaved in the body to release the alkylating agent.



Compound **(8)** does not just act as just an alkylating agent, but by a different mode of action. Cytotoxicity was independent of any hormonal or alkylating activities these chemohormonal agents act in a manner different from that of both alkylating agent and the steroid. They are also superior to mixtures of unlinked alkylating agents and hormones.⁵⁶⁻⁶⁰

Steroid–Nitrogen Mustard Conjugates with a Rigid N–C Linkage

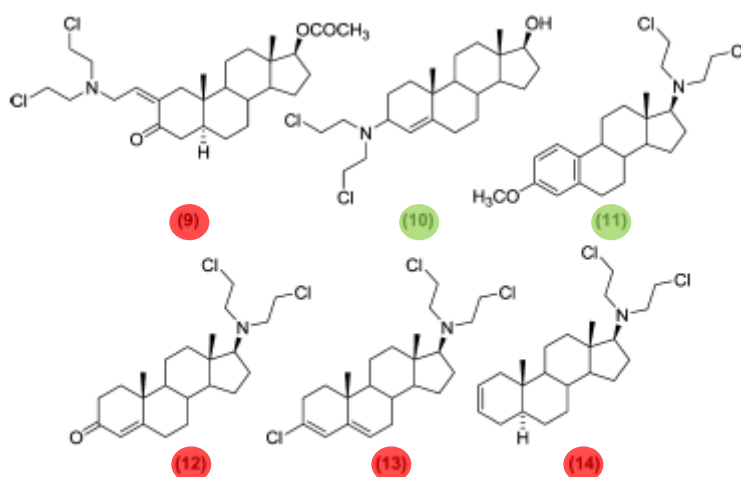


Figure 3. Steroidal nitrogen mustard conjugates with a rigid N–C linkage.

9 = no toxicity but no anti-tumour activity⁸⁰

10 = anti-tumour activity⁸¹

11 = anti-tumour activity⁸²

12 = no anti-tumour activity⁸³

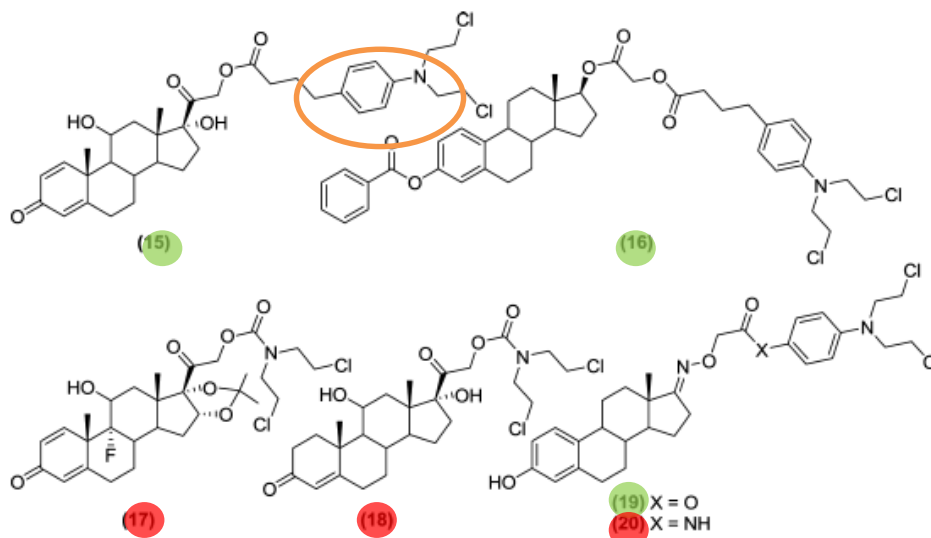
13 = no anti-tumour activity⁸¹

14 = only moderate anti-tumour activity⁸¹

As seen above between **(7)** and **(8)** direct attachment of the alkylating agent to the steroid core is undesired and aromatic mustards are better than. Choice of appropriate steroidal backbone results in effective transport and may also impart selectivity in action. Cytotoxic activity increases if the mustard–steroid link is readily cleavable by hydrolysis or other in vivo processes.⁸³



Steroid–Nitrogen Mustard Conjugates with a Labile Ester Linkage.



Structures of steroidal alkylators with nitrogen mustards conjugated to C₁₇ position of steroid.

15 = anti-tumour activity⁸⁹⁻⁹¹

18 = no activity⁹⁷

16 = anti-tumour activity, selective⁹⁶

19 = anti-tumour activity⁹⁸

17 = no activity⁹⁷

20 = no anti-tumour activity⁹⁸

Increased activity when the alkylating agent is attached to an aromatic group.

Nitrogen Mustard Conjugated to C3 of Steroid

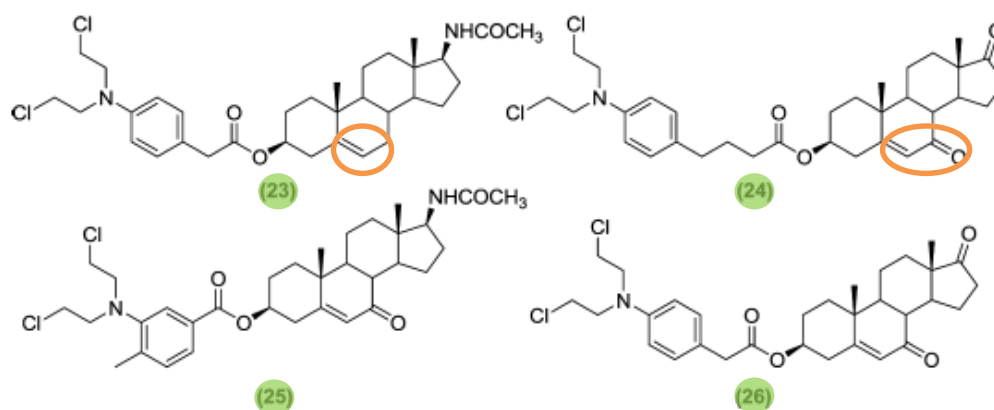


Figure 6. Steroidal alkylating agents with nitrogen mustards conjugated to C₃ of androstane nucleus.

23 = anti-tumour activity, L1210 and P388 leukemias, Lewis lung cancer, Ehrlich ascites tumours⁹⁷

24 = anti-tumour activity, boosts activity¹⁰⁸

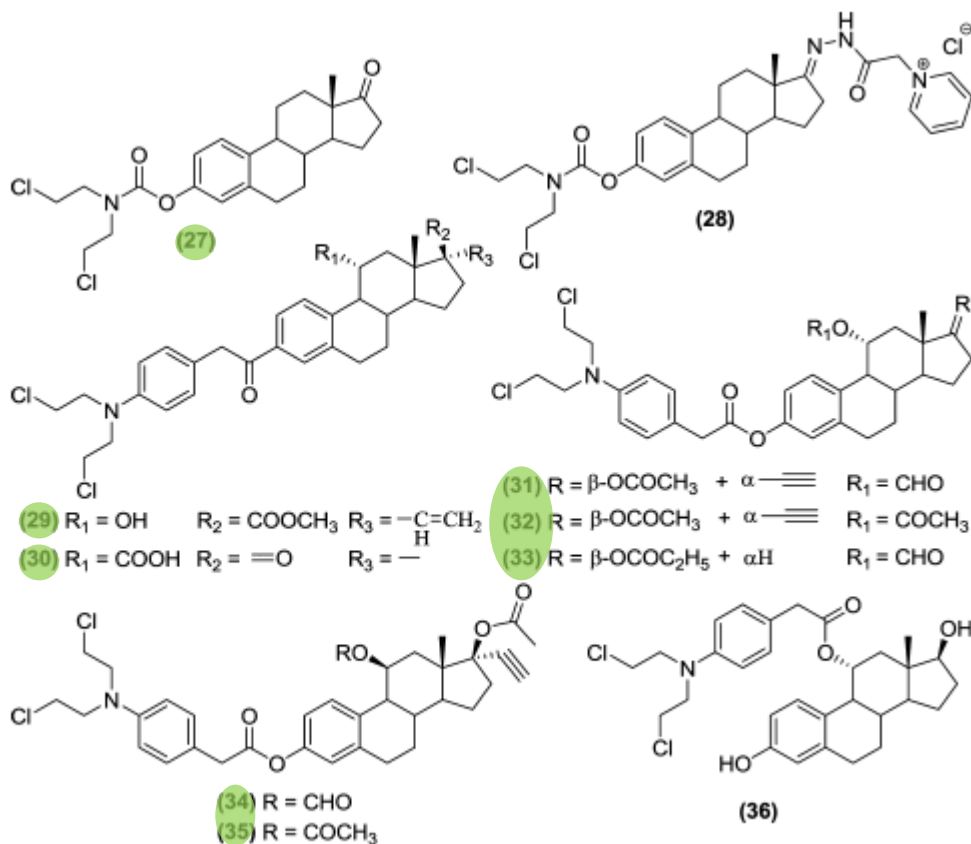
25 = anti-tumour activity, keto 23¹⁰⁹

26 = no anti-tumour activity, twice as good as others^{60,108}

Here the keto group has been shown to be important, increased activity between (23) and (24). Δ^5 -7-keto steroids are more toxic toward cancerous cells due to their ability to inhibit cell replication.¹⁰⁹ If the vinyl ketone is replaced on the steroid by just a ketone again we see reduced biological activity.



Estrane Derivatives



Steroidal alkylating agents with nitrogen mustards conjugated to C₃ of estrane nucleus.

27 = anti-tumour activity, L1210 and P388 leukemias, Lewis lung cancer, Ehrlich ascites tumours⁹⁷

28 = anti-tumour activity, boosts activity¹⁰⁸

29 = anti-tumour activity, x3 inhibition¹¹²

30 = anti-tumour activity, x2.6 inhibition¹¹²

31-33 = very anti-tumour activity, significant reduction of toxicity⁹⁸

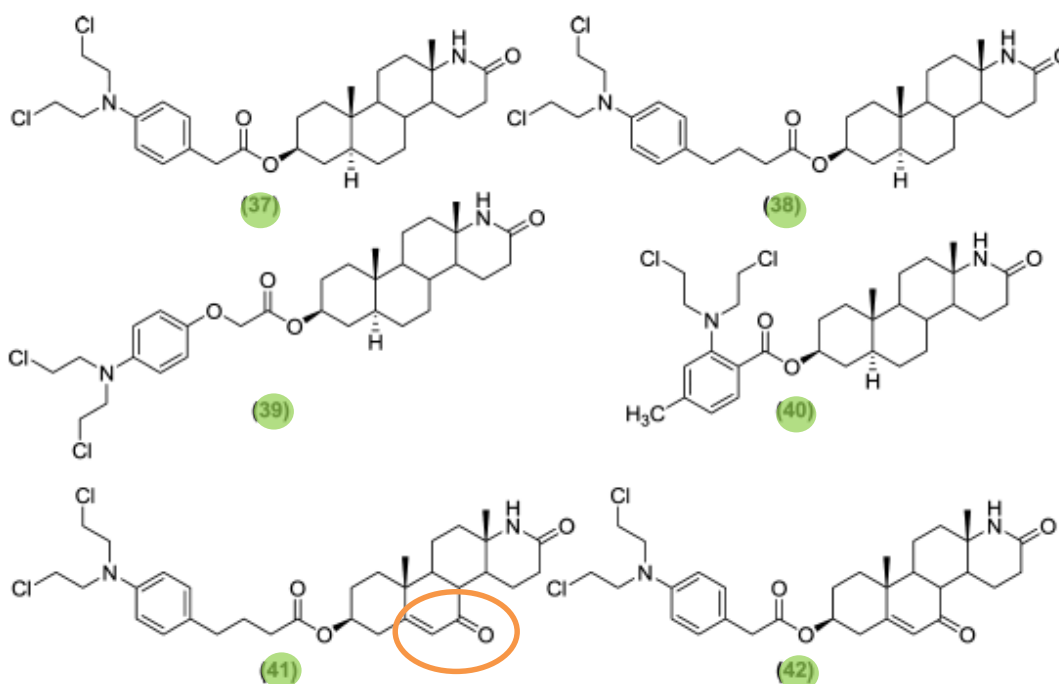
34-35 = anti-tumour activity¹¹⁴

36 = no anti-tumour activity, twice as good as others^{60,108}

Overall the compounds with the alkylating group attached to C₃ of steroid are more active than those attached to C₁₇. This has been rationalised to the less stable ester bond involving the C₃ phenolic -OH group, thus favouring an the cleavage of the linker between the steroid and the nitrogen mustard moiety at the tumour site.¹¹⁵



D-Ring Modified Steroids



D-Ring modified steroids with nitrogen mustards conjugated to C₃ position.

37 = anti-tumour activity, much better than giving the combo^{124,125}

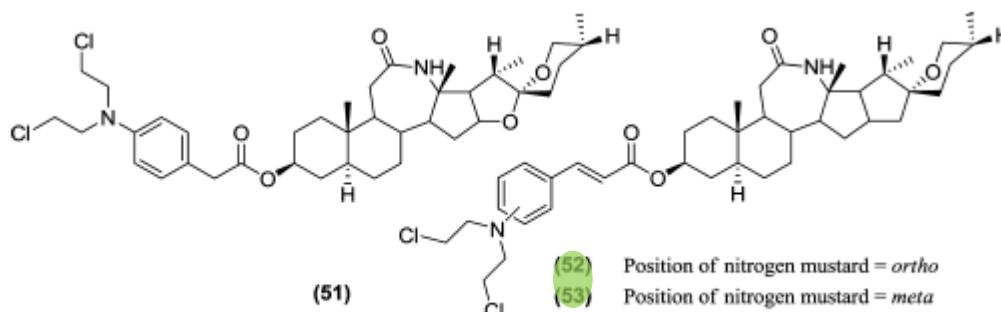
38 = anti-tumour activity, much better than giving the combo^{124,125}

39 = anti-tumour activity, x3 inhibition¹¹²

41 better than 40 due to C7 keto group¹⁴³

42 better than 37 due to C7 keto group⁶⁰

C-Ring Modified Steroids

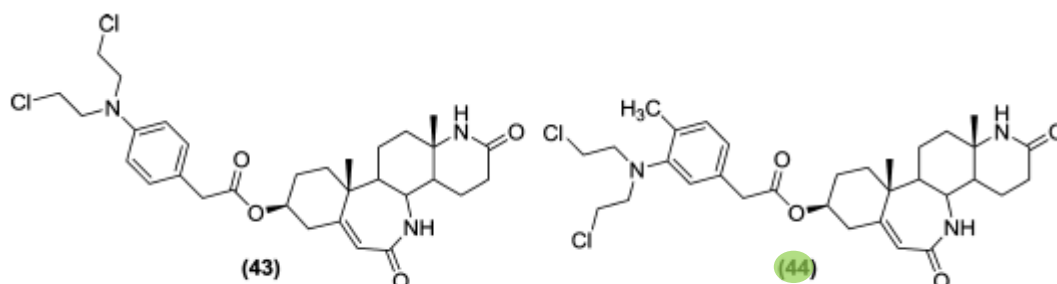


C-Ring modified steroids with nitrogen mustards conjugated to C₃ position.

The structure of the alkylating agent also plays a role, **(52)** and **(53)** more active than **(51)** as the aromatic nitrogen mustard is ortho/meta.¹⁴⁸ This is a common structural feature as we can see below.



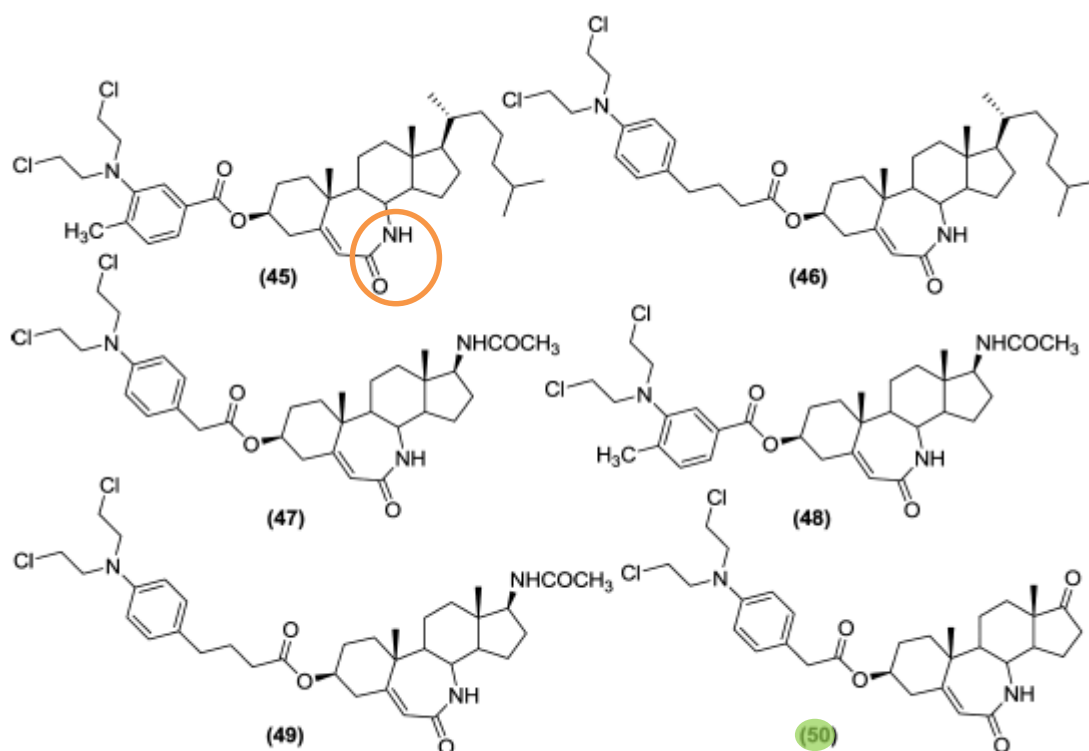
B-Ring and D-Ring Modified Steroids



B-Ring and D-ring modified steroids with nitrogen mustards conjugated to C₃ position.

Again compound (44) acts as a better anticancer agent than (43) due to the position of the alkylating agent being meta on the aromatic ring.¹⁴⁴

B-Ring Modified Steroids



B-Ring modified steroids with nitrogen mustards conjugated to C₃ position.

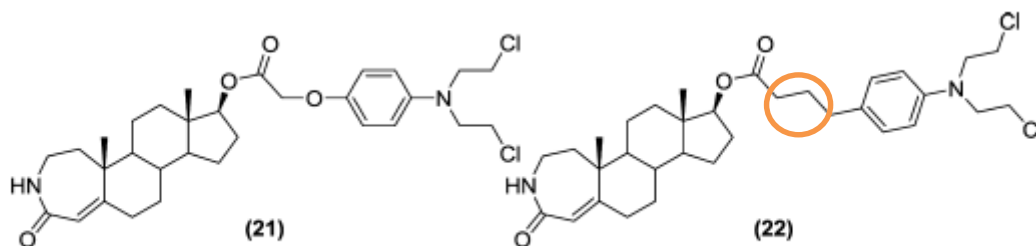
Profound antileukemic activity.^{59,60,109,144,146}

50 = best, reduced toxicity levels.¹⁴⁴

Amide within the steroid ring is the key, without it less activity than simple C7 ketone. The reason is the reduced peripheral hydrolysis by esterases leading to a greater concentration of the alkylating moiety at the binding site



A-Ring Modified Steroids



A-Ring modified steroids with nitrogen mustards conjugated to C₁₇ position.

Even a simple change on the linker of the alkylating agent (**22**) effects selectivity of the drugs, now (**22**) being inactive against L1210 leukemia compared to (**21**).¹⁰³

Information Overload

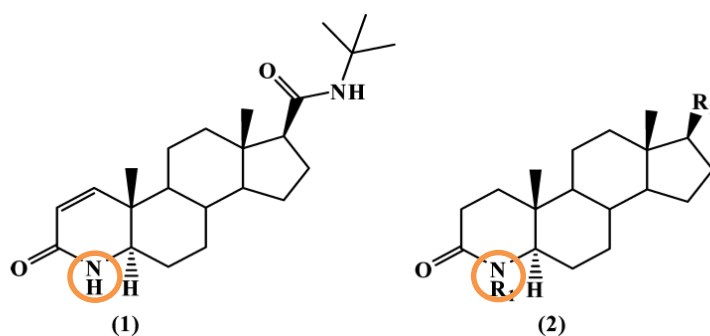
As you can see the slight adjustment of a steroid core can have huge effects on the activity of these drugs. Sometimes there is a clear distinction to what is causing the effects but other times it is hard to rationalise why a simple change has such a profound effect on activity/toxicity of these drugs.

Computational drug design

An alternative approach is to design the steroid to fit to the exact structure of a site (such as an enzyme) which is the cause of the cancer growth. Unfortunately not many crystal structures are available for these targets, which is needed for rational design of novel selective inhibitors^{151,152}

Presently design based on modifying structures of natural substrates and testing activity if the binding site not known. However a new technique that tries to predict the best structure by computer modelling with the available amino acid sequence data is available.¹⁵³ Steroidal and nonsteroidal compounds have been developed using this method but more attention given to steroidal compounds as small modification of the steroid core results in substantial changes in activity¹⁵⁴

5 α -reductase inhibitors Finasteride (**1**), a 4-azasteroid (**2**), nitrogen C₄ at position



Heterosteroid scaffolds (**1** and **2**) have 10 times higher selectivity to 5 α -reductase type II than type I, this must be due to the formation a more stable ligand complex with the enzyme.



Ligand-based drug design

Ligands similar to an active ligand are more likely to be active than random ligands.¹⁵⁵ Ligand similarity approaches require only one active molecule to start from, although a larger field is preferred. The approach consider two- or three-dimensional chemistry, steric, electrostatic, and interaction points (e.g., pharmacophore points) to assess similarity.¹⁵⁶

Quantitative structure–activity relationship (QSAR)

Ligand-based approach which correlates pharmacological activity with chemical properties of libraries of molecules.¹⁵⁷ Advantages of QSAR, time and cost of trial and error synthesis, guideline structural changes to increase activity with fewer side effects.¹⁵⁸

Self-organizing molecular field analysis (SOMFA)

3D approach which uses intrinsic molecular properties (electrostatic and steric potential) around a set of ligands and constructs 3D-QSAR models by correlating these 3D fields with the corresponding experimental activities of ligands interacting with a common target receptor.^{159,160} Analysis involves the alignment of molecules in a structurally and pharmacologically reasonable manner on the basis of the assumption that each compound acts via a common macromolecular target binding site.

ligand-based method such as SOMFA is widely used not only because it is not very computationally intensive but also because it can lead to the rapid generation of QSARs from which the biological activity of newly designed compounds can be predicted. Critical interpretation of SOMFA maps an give key structural features that could be modified to increase potency.¹⁶¹

3D-QSAR Models

Steric maps - indicated the presence of a sterically bulky/less bulky substituent

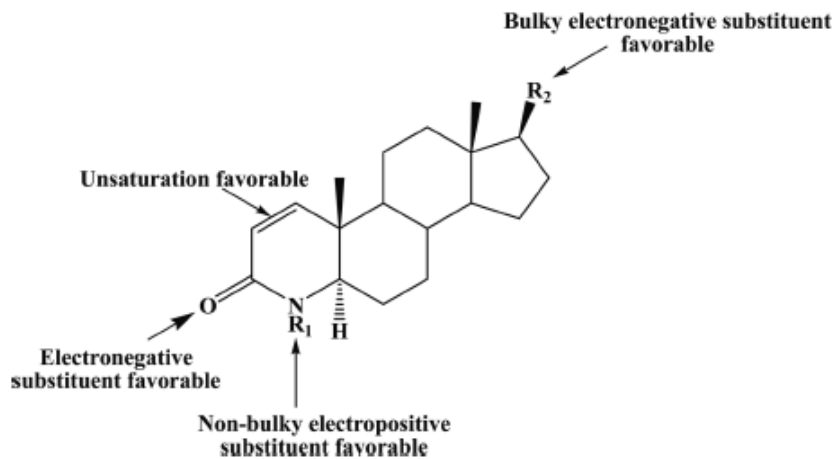
Electrostatic maps - indicated the presence of an electropositive/negative substituent

These computational maps provide a fast way to look at modifying the core of the steroid to enhance activities



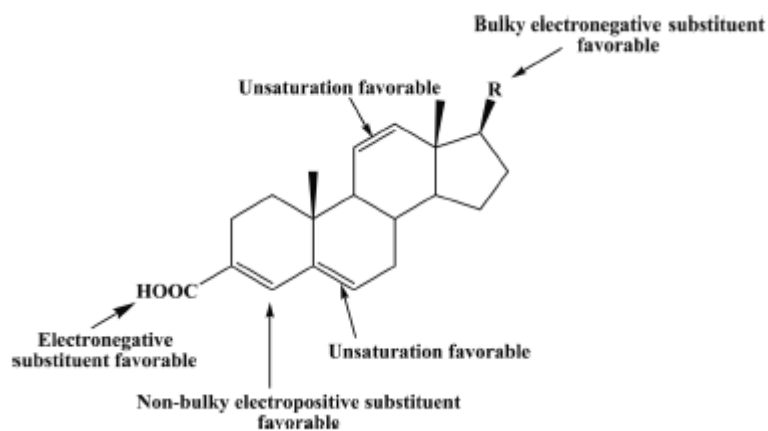
4-Azasteroids inhibitors of steroidal 5 α -Reductase-II

3D-QSAR study was performed finasteride analogues (4-azasteroids)^{162,163}



3-Carboxysteroids as Inhibitors of Steroidal 5 α -Reductase-II

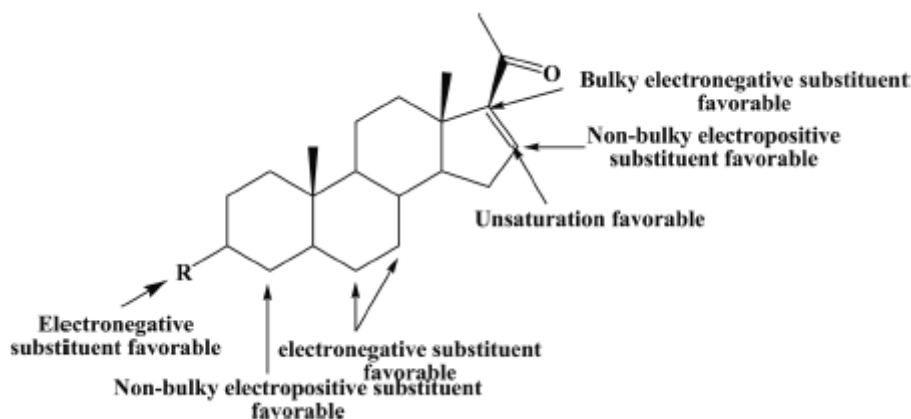
3D-QSAR study performed using epristeride analogues¹⁶⁴





Pregnane Derivatives As Inhibitors of Human Steroidal 5 α -Reductase-II

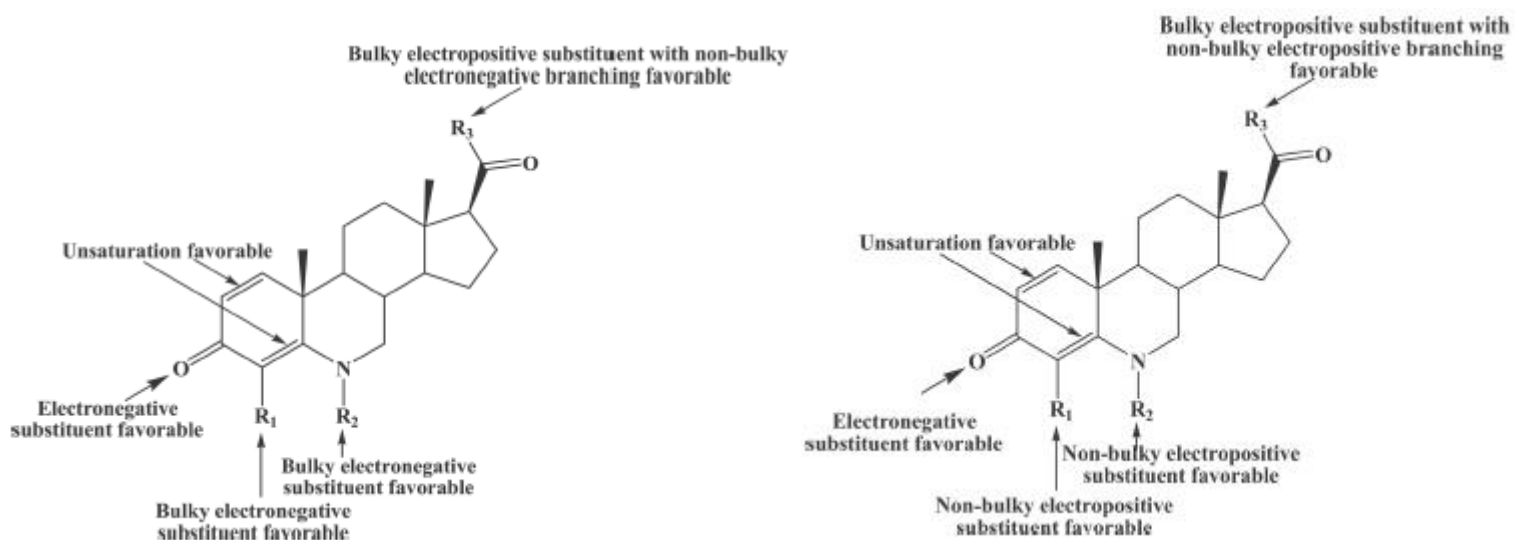
3D-QSAR study performed using pregnane derivatives¹⁶⁵



6-Azasteroids as Inhibitors of Both Isoforms of Steroidal 5 α -Reductase Type I and II

A 3D-QSAR study was performed on the selected azasteroidal data set to generate a comparative pharmacophoric model for both isoforms of steroidal 5 α -reductase using 6-azasteroids.¹⁵³

Replacement of -H at "R1" and "R2" with -CH₃ and -Cl gives 5 α -reductase-I inhibitory activity while having a slight decrease in steroidal 5 α -reductase-II inhibitory activity.



3D-NET (EU FP7 2013 - 612218/3D-NET)

3DNET SOPs

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Conclusions

The use of QSAR methods to help predict structures of active drug targets is a very powerful method. As shown in the first half, very simple modifications of steroid analogues can have massive effects on the performance of a drug. A few 3D-QSAR maps as shown above can combine huge amounts of data from the literature to give an easy to visualise way maps which help in the designing steroid analogues.

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