Articles

Effect of Steroids on DNA Synthesis in an in Vitro Replication System: Initial Quantitative Structure−Activity Relationship Studies and Construction of a Non-Estrogen Receptor Pharmacophore

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Received October 24, 1997

The molecular mechanism(s) by which steroids affect carcinogenesis is an active area of investigation. Recent studies with a series of related steroids in an in vitro DNA replication system produced a wide range of effects including enhancement and inhibition of DNA synthesis. The HeLa cell-free system used in these studies did not contain estrogen receptors. Since the majority of hormone effects on cellular replication have been attributed to interactions with estrogen receptors, an alternative description of the results was required. Quantitative structure−activity relationships (QSARs) were used to relate the observed bioactivity of these steroids with their structure. The results indicate that the percentage of DNA replication could be related to three parameters according to the following equation: %DNA = 23.9(±3.8)Xdipact + 57.8(±22.4)Hyd − 19.4(±10.4)Biophπ + 128.9, where Xdipact is the dipole moment on the *X*-axis, Hyd is the atomic hydrophobicity index, and Biophπ is the atomic π population on the heteroatom found in the pharmacophore. For each molecule, the orientation of the functional groups changed the dipole moment value, and this descriptor was used as a selector of active conformations. A 3D-QSAR model was then constructed combining pharmacophoric features and global properties, and the active space and inactive space were defined using a Boolean volumetric operation.

Introduction

Steroid hormones, in particular estrogens, have been related to the increased risk of some types of cancers, such as breast and ovarian cancers. Indeed several in vitro and in vivo studies indicate a correlation between estrogens and carcinogenesis;[1] in particular, estrogens have been associated with increased cellular proliferation.[2]

The effects of estrogens in carcinogenesis have been primarily attributed to their action on estrogen receptors. Several groups have explored the interaction of steroids with estrogen, progesterone, corticoid, and androgen receptors using quantitative structure−activity relationships (QSARs)[3,4] and X-ray crystallographic[5] approaches. However, an absolute relationship between receptor binding and the activity has not been established, and other mechanisms of carcinogenesis have been proposed. For example, it has been postulated that estrogens and other steroids directly interact with DNA, through intercalation into the DNA structure.[6−9]

Since cellular proliferation, and therefore DNA replication, is a common feature associated with the carcinogenic effects of steroids, the effect of a series of steroids on DNA synthesis[10] has been recently studied in an in vitro DNA replication system.[11] The effect of 17 structurally related steroids on the in vitro replication of a bacterial plasmid containing a specific mammalian origin of DNA replication from the hamster dihydrofolate reductase (DHFR) locus was analyzed. Of the 17 test compounds, 4 increased DNA replication and 6 decreased replication while 7 had no effect, in comparison to the in vitro replication of the same plasmid in the absence of any steroid treatment.

Unlike other systems, the DNA replication system used in the above study did not contain progesterone or estrogen receptors.[10,12] Thus, an alternative interpretation of the data was required, and a chemometric approach including a 3D-QSAR analysis was undertaken. QSARs are a useful tool in medicinal chemistry in the explanation of the forces governing the pharmacological activities of a particular class of compounds. In addition, when the crystallographic structure of the pharmacological target is unknown, it is possible to use molecular modeling techniques to construct models of receptor sites or a graphical pharmacophore[13] and use these models to improve description and prediction of activity. There are successful methods that have been used in constructing practical receptor models[14] which

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S0022-2623(97)00725-5 CCC: $15.00
Published on Web 04/03/1998

are mainly based on a surface or grid point surrounding the ligand.[15,16]

More recent studies[17,18] have focused on the use of molecular modeling to explain ligand structure−estrogen receptor binding affinity relationships. In describing the binding of 44 halogenated estradiol derivatives to the estrogen receptor, Gantchev et al.[19] used comparative molecular field analysis (CoMFA) to derive steric and electronic molecular field lattices. Another approach[6] was based on the computer molecular docking of various compounds into a partially unwound DNA fragment. This method showed a correlation between the estrogenic (uterotropic) activity of the compounds and the fit to the estrogen pharmacophore.

In this study, the Apex-3D expert system was used to find a pharmacophore for the test set of steroids. Based on the logicostructural approach,[20] Apex-3D identifies biophoric (i.e., pharmacophoric, toxicophoric) structural patterns responsible for manifesting certain types of biological activity. Descriptor centers can be either atoms or pseudoatoms that can participate in the ligand−receptor interactions based on a number of physical properties such as electrostatic interactions (charges, electron acceptor or donor), hydrogen bonds (presence), charge-transfer complexes (HOMO, LUMO), hydrophobic interactions, and van der Waals (London) dispersion forces (π-electron density on atoms).

The results of these studies indicate that the observed in vitro biological activity could be correlated to electrostatic interactions and hydrophobic interactions between the ligands and the target. The *X* component value of the dipole moment vector was identified as the most significant descriptor, and its absolute value is directly related to DNA replication. The magnitude of this variable for each ligand was dependent upon the compound's conformation and could be changed by rotating the substituents on the D- or A-ring. Since the crystallographic structure of the receptor was not available, the *X* dipole moments of the test compounds were compared by assuming that a common mode of binding existed for all of the ligands. This descriptor was then used as an active conformation selector. The results produced by this approach are presented below.

Materials and Methods

Compounds. The set of 17 test compounds belonged to different subfamilies of steroids, i.e., estrogen, androgen, glucocorticoid, and progestin. The compounds were purchased from Sigma (Mississauga, Ontario, Canada) and Steraloids Inc. (Wilton, NH). The compounds were dissolved in 100% ethanol and diluted with water. The amount of ethanol added to the biological system never exceeded 0.1%. Physiologically relevant steroid concentrations (10 nM) were used in assay.

Biological Data. The biological data is the relative effect of these steroids on DNA replication. An in vitro replication assay has been used to obtain this parameter. In vitro replication was carried out as described in Diaz-Perez et al.[12] Standard in vitro reactions are basically composed of equimolar amounts of a supercoiled plasmid, either plasmid pX24 or plasmid 30.4, HeLa nuclear and cytoplasmic extracts, an ATP-regenerating system, PEG, a mixture of nucleotides (ATP, CTP, GTP, UTP, dATP, dGTP, dTTP, dCTP), and 10 μCi of [α-^{32}P]dCTP and [α-^{32}P]dTTP. Plasmid pX24 contains a specific origin of replication from the hamster dihydrofolate reductase locus, and plasmid 30.4 contains a fragment of cDNA randomly selected from a human cDNA library.[10] The HeLa cell extracts have been tested for receptors and are estrogen and progesterone receptor-negative.[10,12] The compounds were preincubated with the template DNA and the precursor nucleotides, followed by addition of the remaining components of the in vitro reaction to initiate DNA replication. DNA was purified, and DNA synthesis was measured using the *Dpn*I resistance assay. DNA replication products were quantitated by densitometry of a phosphoimager screen using the Fuji BAS 2000 analyzer.

The data are expressed as the percentage of the non-drug-treated control (100%) and represent the average of at least two separate experiments (mean ± SD).

Molecular Modeling. The compounds used for our data set (testosterone, progesterone, estrone, estriol, 2-hydroxyestradiol, 4-hydroxyestradiol, 2-hydroxyestrone) were retrieved from the Cambridge Data Base[21] available via the Quest program. The compounds for which the crystallographic data did not exist were built using a fragments library on the InsightII 95.0 program (MSI, San Diego, CA) running on an IBM Risc6000 computer.

Conformational analysis was done with the Search/Comp module. The rotatable bonds were defined in the range of 0−360° using 10°−80° as an increment angle. For each compound, a systematic search and energy optimization was performed. Several optimizations were used in sequence: steepest descents, followed by conjugate gradients, followed by a quasi-Newton−Raphson method with a maximum number of iteration set at 500. A cff force field with charge and cross-terms was used, and the thresholds for removing duplicate conformers were specified using the Dupl_E_threshold at 0.01 kcal/mol and Dupl_RMS_threshold at 0.01 Å. The Dupl_E_threshold parameter specifies the maximum energy difference in kcal/mol, and the Dupl_RMS_threshold parameter specifies the maximum root-mean-square difference (in Å) for which two conformers are considered to be the same.

Tsar V2.41 software (Oxford Molecular Ltd., Oxford, U.K.) was used to calculate molecular descriptors (electronic, steric, or lipohilic descriptors); Tsar was also used for statistical analysis of the data. All charges and dipoles moments were calculated using the Mopac V6[22] program. The origin of the dipole moment vector was set to the center of mass for each compound.

A routine written in C-shell was useful for extracting the coordinates of each conformation from the Search/Comp archive file. The routine splits the .arc file into .car files and converts the .car format to .dat (Mopac's internal coordinates format) and then prints and sorts the Mopac results (heat of formation and dipole moment components) to a result file.

The pharmacophores were built using Apex-3D 95.0 software (MSI, San Diego, CA) running on a Silicon Graphics Indy workstation. The pharmacophores selected included all of the compounds in the set with a match superimposition greater than 0.7. The overall match quality calculation[23] is based on all pairwise molecular similarities according to:

$$match = \frac{2}{n(n-1)} \sum_{i=1}^{n} \sum_{j=1+1} \frac{MolSim(i,j)}{MolSim(i,i) + MolSim(j,j) - MolSim(i,j)}$$

where n is the number of compounds and the $MolSim(i,j)$ function is calculated according to

$$MolSim(i,j) = \sum_{a_i=1}^{n} \sum_{a_j=1}^{m} \frac{AtSim(a_i,a_j)}{W_{3D}(a_i,a_j) \times W_{2D}(a_i,a_j)}$$

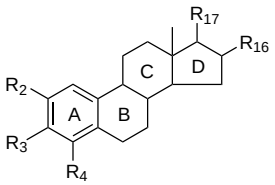
where n is the number of atoms in molecule i, m is the number of atoms in molecule j, $AtSim(a_i,a_j)$ is a function that calculates the similarity of atoms a_i and a_j, $W_{3D}(a_i,a_j)$ is a weighting function for matching atoms based on the Cartesian distances between them, and $W_{2D}(a_i,a_j)$ is a weighting function based on the topological distances of atoms from the biophore.

The 3D-QSAR equation was derived with the site radius set at 1.2, the occupancy at 5, the sensitivity at 2.5, and the randomization at 500. The total hydrophobicity and *X* dipole

Table 1. Compounds Studied

no.	compound	R_2	R_3	R_4	R_{16}	R_{17}
1	testosterone	H	O	H	H	OH (β)
2	progesterone	H	O	H	H	CH_3−CO (β)
3	estrone	H	OH	H	H	O
4	estriol	H	OH	H	OH (α)	OH (β)
5	16-epiestriol	H	OH	H	OH (α)	OH (β)
6	17-epiestriol	H	OH	H	OH (α)	OH (α)
7	16,17-epiestriol	H	OH	H	OH (β)	OH (α)
8	16-keto-β-estradiol	H	OH	H	O	OH (β)
9	2-OH-estradiol	OH	OH	H	H	OH (β)
10	4-OH-estradiol	H	OH	OH	H	OH (β)
11	16α-OH-estrone	H	OH	H	OH (α)	O
12	16β-OH-estrone diacetate	H	CH_3−CO	H	CH_3−CO (β)	O
13	β-estradiol-17-acetate	H	OH	H	H	CH_3−CO (β)
14	estrone acetate	H	CH_3−CO	H	H	O
15	2-OH-estrone	OH	OH	H	H	O
16	4-methoxyestrone	H	OH	CH_3−O	H	O
17	2,3-methoxyestrone	CH_3−O	CH_3−O	H	H	O

Chart 1. Structure of the Steroids Studied



Testosterone: R_{16} = H; R_{17} = OH (β)
Progesterone: R_{16} = H; R_{17} = HCH_3-CO (β)

moment values were selected as global properties. The biophoric centers and secondary sites combined to global properties (total hydrophobicity, molecular refractivity, and components of the dipole moment) were used to obtain an equation to predict the percentage of DNA replication. The biophoric sites were set to charges, π-population, HOMO, LUMO, hydrogen acceptor, hydrogen donor, and hydrophobic site. The secondary sites were set to hydrogen acceptor, presence; hydrogen donor, presence; heteroatom, charge; hydrophobic, hydrophobic; steric, presence; ring, π-sum.

Volumes were generated using the Volume/Create option of InsightII; the van der Waals scale was set to 1 Å and the van der Waals increment to 0.

Results

Conformational Analysis. The molecular structures of the 17 steroids used in this study are shown in Chart 1, and the nature of the functional groups for each compound is presented in Table 1. For 9 of the 17 steroids used, the substitution on the D-ring included a hydroxyl group at position 16 and/or at position 17, and 12/15 had a hydroxyl function at position 3 on the A-ring. Three compounds contained an acetoxy moiety at position 16 (two compounds) or position 17 (one compound).

All the compounds were subjected to a conformational search using the Search/Comp module (MSI), containing an algorithm to eliminate high energy due to the steric effects. This effort produced a number of acceptable conformations which were dependent upon the shape of the rotatable group. The estimated and accepted conformations after optimization are reported in Table 2. Only nonduplicate conformations with values of less than 10 kcal/mol were allowed. The number of acceptable conformations after the energy optimization varied between 2 and 25. These conformations were used to build the QSAR models.

Table 2. Conformational Analysis Results[a]

compd	IncD	IncA	estimated	accepted
1	10		22	3
2	10		14	3
3		10	36	2
4	20	180	144	18
5	20	180	144	16
6	20	180	144	16
7	20	180	144	12
8	10	180	52	6
9	10	90	352	25
10	10	90	264	18
11	10	180	62	6
12	20	20	216	18
13	10	90	48	4
14	10		36	2
15		30	144	4
16	20	20	187	6
17	20	20	289	7

[a] IncD: The increment angle in degrees for the rotatable bonds on the D-ring. IncA: The increment angle in degrees for the rotatable bonds on the A-ring. Estimated: The number of conformations estimated after steric evaluation. Accepted: The number of conformations estimated after optimization and removing duplicate conformations.

1D-QSAR Results. The results from the DNA replication studies are presented in Table 3 as the percentage of DNA replication with the standard deviation and the number of assays. The experimental control was set at 100%, and the average of the standard deviation for all the compounds is approximately 20%. Thus, compounds producing a DNA replication > 120% (compounds **4**, **7**, **11**, and **12**; Table 2) were considered promoters, compounds producing DNA replication of <80% (compounds **1**, **9**, **10**, **13**, **16**, and **17**) were considered inhibitors, and compounds producing DNA replication of 80−120% (compounds **2**, **3**, **5**, **6**, **8**, **14**, and **15**) were deemed inactive.

The position and the number of the hydroxyl groups on the D-ring seemed to affect the observed activity, since all the inhibitors were unsubstituted at position 16 (Chart 1) on the D-ring as were four inactive molecules (compounds **2**, **3**, **14**, and **15**). The inhibitors testosterone, 2-OH-estradiol, and 4-OH-estradiol, contained one β-hydroxyl group in position 17 of the D-ring.

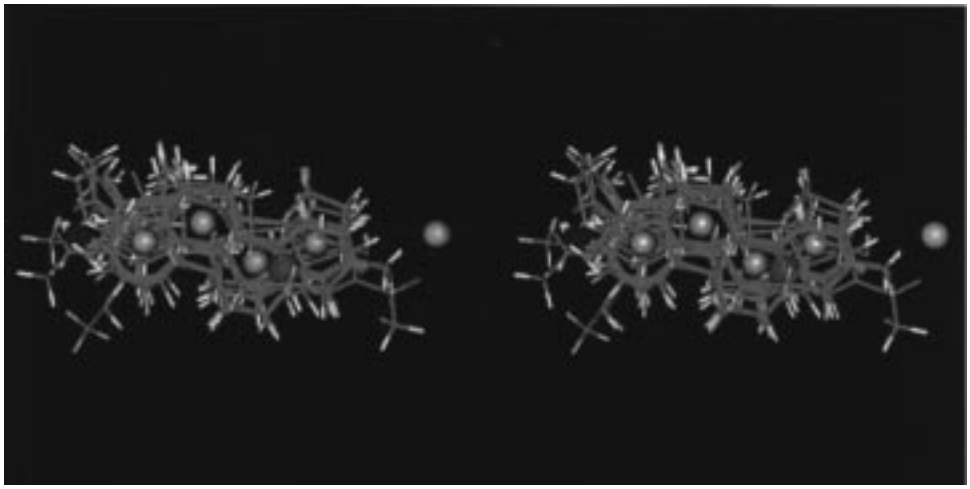


Figure 1. Stereoview for the best pharmacophore selected. Features color code: white, center of ring; yellow, hydrogen-bonding site; purple, hydrophobic site; blue, heteroatom.

Table 3. Percentage of DNA Replication[a]

compounds	%DNA	SD	*n*
1	72	17	4
2	115	9	3
3	108	17	2
4	144	19	2
5	118	26	2
6	103	19	3
7	200	6	2
8	99	25	2
9	52	16	2
10	53	6	2
11	150	26	3
12	157	41	2
13	67	17	2
14	111	3	2
15	92	19	4
16	53	1	2
17	63	1	2

[a] %DNA: The percentage of DNA replication. SD: Standard deviation. *n*: Number of assays.

All the promoters had two oxygens on the D-ring (as well as one oxygen moiety on the A-ring). When two hydroxyl groups were present on the D-ring, the molecules containing 16α,17β or 17α,16β configurations promoted the DNA replication (compounds **4** and **7**), while compounds with 16α,17α or 16β,17β configurations were inactive (compounds **5** and **6**).

When the observed activity in the DNA replication system was correlated to the calculated descriptors for all 17 test steroids, no significant multiparameter relationship was found. However, the dipole moment on the *X*-axis of the minimum energy conformation (Xdip) was significantly correlated to activity ($R = 0.77$) for 12 compounds (compounds **1**−**3**, **5**, **6**, **10**, **11**, and **13**−**17**: set 1), eq 1:

$$\%\text{DNA} = 28.9\text{Xdipmin} + 128.8 \quad (1)$$
$$n = 12,\ R = 0.77$$

While the steroid backbone is rigid, the substituents on the D-ring have a great deal of conformational mobility. Since the magnitude and orientation of the *X*-axis dipole moment can be changed by small alterations in the orientation of these substituents, the assignment of an "active" conformation is not a trivial operation. In this study, it was assumed that for set 1, the minimum energy conformation produced the optimum ligand conformation for biological activity. While for the other five compounds (compounds **4**, **7**−**9**, and **12**: set 2), the minimum energy conformation did not approximate the "active" conformation. Optimum binding conformations for the five compounds in set 2 were chosen using Apex-3D, and these "active" conformations were included in the total data set used to produce the best superimposition for all of the compounds. Table 4 contains the values of the *X* dipole moment for the minimum energy conformations and for the low-energy "active" conformations. The energetic cost required to adopt the active conformations for set 2 compounds ranged from 0 to 3.6 kcal/mol, which is generally acceptable in QSAR studies.

Table 4. Molecular Descriptors Found in Eq 2 for Minimum Energy Conformation and Active Conformation

compd	E_{min}	Xdipmin	E_{act}	Xdipact	ΔE	Biophπ	Hyd
1	−88.64	−2.25	−88.64	−2.25	0	0.056	−0.14
2	−72.45	0.11	−72.45	0.11	0	1.036	−0.22
3	−64.12	−1.32	−64.12	−1.32	0	0.196	−0.14
4	−128.86	−1.41	−126.99	1.05	1.87	0.056	0.16
5	−129.65	−1.25	−129.65	−1.25	0	0.056	0.16
6	−129.00	−1.00	−129.00	−1.00	0	0.056	0.16
7	−128.66	−1.23	−127.35	2.34	1.31	0.056	0.16
8	−106.21	1.80	−103.65	0.36	2.56	1.064	0.16
9	−128.02	0.55	−124.44	−1.08	3.58	0.196	−0.48
10	−126.88	−1.76	−126.88	−1.76	0	0.196	−0.48
11	−105.89	−0.49	−105.89	−0.49	0	0.056	0.16
12	−86.04	−0.22	−84.23	1.39	1.81	1.036	0.16
13	−68.54	−1.83	−68.54	−1.83	0	1.036	0.16
14	−54.91	−0.34	−54.91	−0.34	0	1.036	−0.14
15	−107.42	−0.64	−107.42	−0.64	0	0.196	−0.14
16	−97.20	−1.94	−97.20	−1.94	0	0.196	−0.14
17	−87.46	−2.49	−87.46	−2.49	0	0.168	−0.14

[a] E_{min}: The energy of the minimum conformation in kcal/mol. Xdipmin: The dipole moment on the *X*-axis for the minimum energy conformation. E_{act}: The energy of the active conformation in kcal/mol. Xdipact: The dipole moment on the *X*-axis for the low-energy active conformation. ΔE: $E_{act} - E_{min}$. Biophπ: The π-population on the heteroatom. Hyd: The hydrophobicity index.

3D-QSAR Results. Using Apex-3D software, over 50 pharmacophores were found with different sizes and arrangements (center of aromatic ring, center of non-aromatic ring, hydrogen bond donor, hydrogen bond acceptor, hydrogen bond site, methyl group). We selected the ones with the best superimposition (match > 0.7) and with the best active conformation which fit the

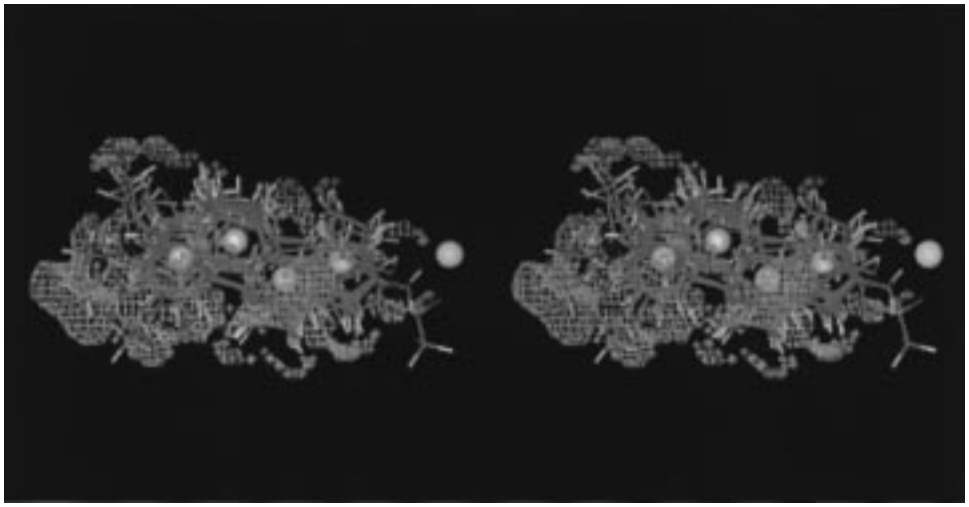


Figure 2. Stereoview of the inactive volume.

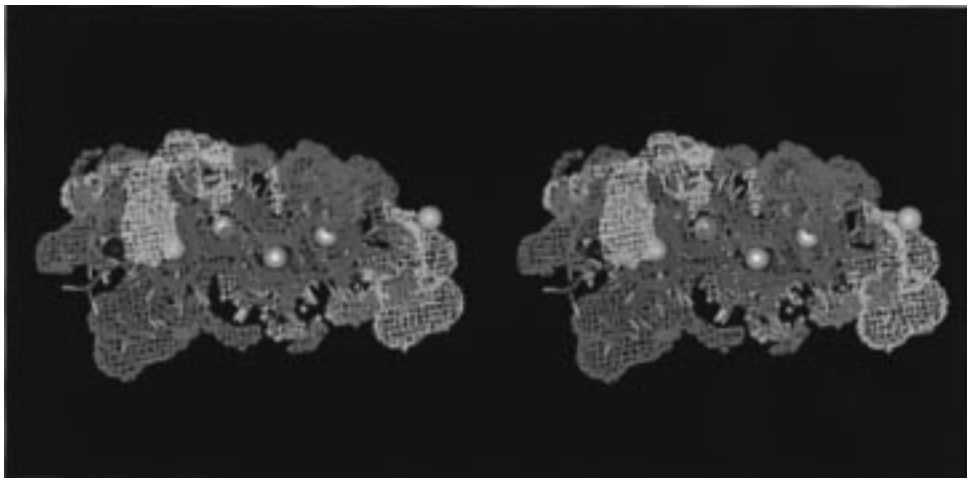


Figure 3. Stereoview for the inhibitor volume (blue) and promotion volume (yellow).

1D-QSAR model for the set 2 compounds (see Table 4). Then we performed a 3D quantitative analysis including the *X* dipole moment for the active conformations.

The best 3D pharmacophore selected (Figure 1) had six key structural features: four ring centers, one hydrogen bond site (HBS), and one heteroatom site. The quality of match for molecules having this common pharmacophore was 0.80. The distance between the heteroatom and the hydrogen bond site when all the compounds were superimposed was 13.59 Å. This distance was 13.61 Å when only inhibitors were superimposed. When only promoters were superimposed, the distance decreased to 13.31 Å, and when only inactive analogues were superimposed, the distance increased to 13.75 Å.

An interaction mechanism involving A-ring binding/D-ring activation is generally accepted as the source of the high affinity of steroids for estrogen, progestin, and corticoid receptors.[24] Androgen receptor binding data and molecular modeling studies suggest an opposite mechanism of D-ring binding/A-ring activation.[25] Both mechanisms are possible with the pharmacophore identified in this study where (1) D-rings of inhibitors are situated in the HBS region of the receptor except for the β-estradiol-17-acetate and testosterone which had this ring situated in the heteroatom region; (2) A-rings of promoters are near the HBS; and (3) for the inactive analogues, three had the D-ring (**3**, **14**, and **15**) and four the A-ring (**2**, **5**, **6**, and **8**) adjacent to the HBS.

In the HBS region of the pharmacophore, the orientation of the hydroxyl group is different for each class of compound. For promoters (**4**, **7**, and **11**) the hydrogen of the hydroxyl is oriented in the direction of HBS, while for inactive analogues (**5**, **6**, and **8**) and inhibitors (**9**, **10**, and **13**) the lone pairs of the oxygen are oriented in the direction of HBS. The value of the angle C−O−HBS is around 120° for inactive compounds and promoters. This value is 127° for β-estradiol-17-acetate and 107° for 2-OH-estradiol and 4-OH-estradiol. These results suggest that the HBS on the pharmacophore contains both a heteroatom and an acidic hydrogen.

To derive a 3D multiparameter equation, the pharmacophore was used as a superimposition model. We found that the percentage of DNA replication was related to three parameters: the *X* dipole moment value for the active conformation, the atomic hydrophobicity index at the hydrophobic site (situated at 5.93 Å from the HBS and 7.93 Å from the heteroatom), and the atomic π-population on the heteroatom.

$$\begin{aligned}\%\mathrm{DNA} = {} & 23.9(\pm 3.8)\mathrm{Xdipact} + 57.8(\pm 22.4)\mathrm{Hyd} - \\ & 19.4(\pm 10.4)\mathrm{Bioph}\pi + 128.9 \\ & n = 17,\ R = 0.93,\ F = 26\end{aligned} \quad (2)$$

According to eq 2, electronic and hydrophobic interactions are primarily responsible for binding and activity. The electronic effects are represented by the *X* dipole moment (Xdipact) and the π-population on the heteroatom (Biophπ), while the hydrophobic effect is represented by the hydrophobicity index (Hyd).

The *X* component value of the dipole moment vector is the most significant descriptor in eq 2, and its absolute value is directly related to DNA replication. This parameter is a quantitative measurement of separation of charges along the *X*-axis as defined by the average plane of the four steroid rings. Since the magnitude of this variable for each ligand was dependent upon the compound's conformation and could be changed by rotating the substituents on the D- or A-ring, it was assumed that a common mode of binding existed for all of the ligands. The derived descriptors are presented in Table 4.

Unlike the *X* dipole moment an increase in the π-population on the heteroatom decreased DNA replication. This parameter characterizes the total electron population of the atomic orbitals with π-symmetry on the atom, and it reflects a local interaction with the receptor involving π-electrons such as dispersion interactions. The π-population of the oxygen decreases in this order: O of a ketone group > O in a hydroxyl group attached to the A-ring > O attached to the D-ring (Table 4). The hydrophobic index (Hyd) found in eq 2 is the carbon atomic increment calculated according to Ghose and Crippen.[26] According to the pharmacophore, most of the inactive compounds and inhibitors had a methyl group adjacent to the hydrophobic region of the target, while promoters had an aromatic carbon in this region. These results suggest the presence of a hydrophobic pocket on the target which is involved in the binding of the steroids and the resultant activity.

Receptor Mapping. The model discussed above defined only electrostatic, hydrophobic, and dispersive interactions; no steric parameter appeared in the equation. Since differences in activity may be related to access to the binding site of the target, "inactive", "promotion", and "inhibition" spaces were determined. An "inactive" space, or exclusion volume, is defined as the difference in van der Waals volume between inactive ligands (449 Å^3) and active ligands (540 Å^3) according to the pharmacophore model. The derived "inactive" space is presented in Figure 2 and contained a steric region with the total resultant volume around 38 Å^3. This region is adjacent to the heteroatom site and covers the four ring centers on the opposite side of the methyl groups at position 13 or position 10 (progesterone and testosterone). However steric constraints were absent around the hydrogen bond site.

The "promotion" space is defined as the difference in volume between promoters (358 Å^3) and the union volume of inhibitors and inactive analogues (530 Å^3), while the "inhibition" space is the difference in volume between inhibitors (464 Å^3) and the union volume of promoters and inactive analogues (511 Å^3). The values of the inhibition space and promotion space are respectively 67 and 49 Å^3.

As shown in Figure 3 the inhibitors occupy a larger space around the heteroatom site than the promoters, while the promoters have a large preferred region adjacent to the hydrogen bond site.

Conclusions

The 3D-QSAR methodology has been applied with a set of steroids which affected DNA replication in an in vitro test system. The active conformations of these compounds were identified using the dipole moment on the *X*-axis as a molecular descriptor, and an equation relating the percentage of DNA replication to electrostatic and hydrophobic parameters was developed. Since no crystallographic data of the target exist, the receptor mapping approach was used to define the binding and active regions for this set of compounds. These results may aid in the design of new compounds for use in the treatment of cancer (inhibitors of DNA synthesis) or wound healing (promoters of DNA synthesis). Shortly after submission of this manuscript, the experimental DNA replication of the 17β-estradiol was determined at 178%. Our model predicts the lowest-energy conformation (match = 0.81) of this compound as a promoter with a theoretical DNA replication of 153%.

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JM970725M