Electrostatic potentials of tumour promoters

Alastair F Cuthbertson and Colin Thomson

National Foundation for Cancer Research Regional Workshop, Chemistry Department, University of St Andrews, St Andrews, Fife KY16 9ST, UK

Molecular-modelling studies and quantum-chemical calculations have been undertaken on a variety of structurally unrelated tumour promoters. The modelling studies reveal some broad areas of agreement in the associated electrostatic potentials for the molecules TPA, teleocidin, aplysiatoxin and ingenol. Azulene-type derivatives were used as models for inactive and active tumour promoters in the quantum chemical studies. The results obtained from these calculations of the electrostatic potentials show very little difference between active and inactive compounds, thus suggesting that other factors are of importance in these systems.

Keywords: tumour promoters, electrostatic potentials, molecular modelling, quantum chemical calculations, quantum pharmacology

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Tumour promotion is only one step in the multistage process of chemical carcinogenesis, occurring after the initiation phase and before the progression phase. The initiation phase results in the production of a latent tumour cell, which is indistinguishable from a normal cell. Treatment of these initiated cells with a tumour promoter gives rise to predominantly benign tumour cells. However, further treatment by an initiator results in malignant tumours being formed.

The most widely studied system of chemical carcinogenesis is that of mouse skin where 12, 13 diesters of the tetracyclic diterpene alcohol phorbol are used as tumour promoters. However, there exist a number of structurally unrelated compounds such as indole alkaloids, aplysiatoxins and ingenols which also exhibit tumour-promoting activity. From the experimental work in the area, a wealth of quantitative structure–activity relationship data has been built up[1]. In addition, the discovery that protein kinase C (PKC) may be the receptor for tumour promoters as well as diacylglycerols (DAG)[2] and that both tumour promoters and DAGs stimulate the enzyme ornithine decarboxylase has opened up the field even further[3].

Our aim has been to try to rationalize some of the data by way of molecular modelling and quantum chemical calculation: a technique broadly referred to as quantum pharmacology.

Figure 1 shows the structural formulas for a number of tumour promoters and the numbering scheme used. One of the most potent is 12-O-tetradecanoylphorbol-13 acetate (TPA) which has a 4β hydroxyl group. However, the 4β methoxy compound and a conformational change at the 4 position to yield the 4α hydroxy compound result in greatly reduced activity. In addition, reduction of the 1–2 double bond, reduction or loss of the 3-carbonyl group or loss of the C20 hydroxyl function all result in a reduction or total loss of activity. This suggests that certain key residues play a crucial role.

Our investigations have been divided into two parts: one considering the molecules themselves, the other concentrating on model phorbol compounds. In the former case, some aspects of earlier work[4,5] have been repeated, but the molecular electrostatic potentials of the mole-

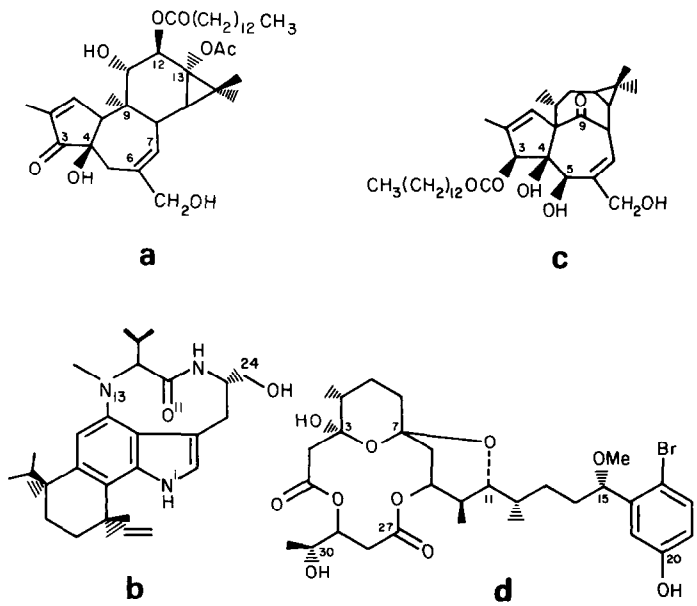

Figure 1. Structural formulas for: (a) 12-O-tetradecanoylphorbol-13 acetate (TPA), (b) teleocidin B, (c) 3-O-tetradecanoylingenol and (d) aplysiatoxin

cules have also been calculated. The latter case describes the use of and matching of electrostatic potentials for model active and inactive analogues.

METHOD CASE 1

The crystal structures of phorbol[6], teleocidin[7], ingenol[8], aplysiatoxin[9] and sn 1-2 dilauroylglycerol[10] were obtained mainly from the Cambridge Crystallographic Database and displayed with the molecular modelling and display program Chem-X*. In order to obtain TPA from phorbol etc., the missing hydrogens and the alkyl chains were built up and added on using the Modify facility in Chem-X. Charges were calculated by the Skorczyk algorithm[11] used in Chem-X. Matching of the relevant atoms was achieved with the Fly option of the program. Connolly dot surfaces[12] were then produced and colour coded according to the simple electrostatic potential ($q*q/r$).

METHOD CASE 2

Bicyclic structures, which are essentially derivatives of azulene, were chosen as models. By changing the substituents attached to the rings we were able to produce both active and inactive model compounds. The reason for using a model system is that the actual molecules are all too large to allow quantum-chemical calculations to be undertaken in a reasonable time and also that the key residues are attached only to the bicyclic system.

The geometries of the model compounds were fully optimized using the MNDO[13] Hamiltonian and published parameters for H, C, N and O[13] incorporated in Mopac version 3.0[14]. The final optimized geometries were then used as input for a single point *ab initio* STO-3G calculation with the UCSF Gaussian 80 program[15] in order to compute the electrostatic potential on a van der Waals dot surface. The points on the surface were obtained with Connolly's MS program[12]. The results were displayed on a computer graphics system using an in-house program[16].

RESULTS CASE 1

The atoms used in the fitting process were: for phorbol and ingenol O3, O4, O9, O20; for teleocidin O11, N13, N1, O24; and for aplysiatoxin O27, O3, O11, O30, respectively[4]. The geometries of these four atom sets are given in Figure 2. In the fitting process, equal weights were used. The results can be summarized as follows.

The best fit is obtained, not surprisingly, with the TPA–ingenol system since these are the most closely related structurally, the mean deviation being 0.71 Å. The next best pair is aplysiatoxin–teleocidin, with a deviation of 1.21 Å, followed by TPA — teleocidin with 1.52 Å and finally TPA — aplysiatoxin with 2.12 Å.

The associated electrostatic potentials do show some broad areas of agreement, especially around the regions of the fitted atoms. This is because they are either nitrogens or oxygens, which give rise to a negative electrostatic potential to which a positive charge will be attracted. In TPA, the dimethylcyclopropane ring has a negative electrostatic potential as does the methyl group attached to the cyclohexane ring. The rest of the

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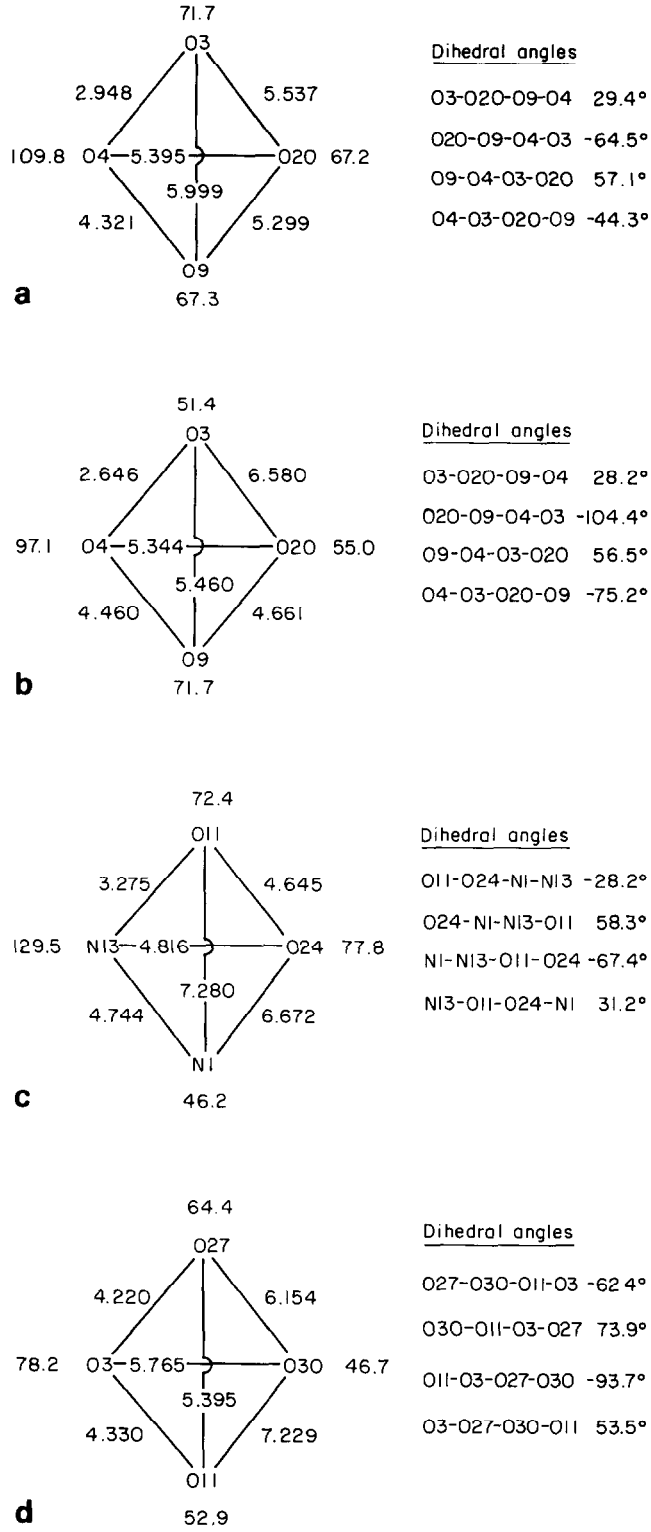


Figure 2. Geometries for the four atom sets important in the fitting process for TPA, ingenol, teleocidin and aplysiatoxin; (a) phorbol, (b) ingenol, (c) teleocidin, (d) aplysiatoxin

ring system has a slightly positive electrostatic potential, and the tetradecanoyl chain gives rise to negative carbons and positive hydrogens. Ingenol has a similar distribution of electrostatic potential to TPA except around the 6–7 double-bond region, which is more negative than that in TPA. On the other hand, teleocidin appears to be dominated by all the heavy atoms having a negative electrostatic potential and the hydrogens a positive potential. Similarly, aplysiatoxin has most of

the heavy atoms with a negative electrostatic potential except for the methylene carbons in the ring systems, which have a positive electrostatic potential.

The O11, O22 and O33 atoms of sn 1–2 dilauroylglycerol do show a good overlap with the O9, O12 carbonyl and O13 carbonyl of TPA[5] with a mean deviation of 0.7 Å. The long alkyl chains of the DAG can be superimposed on the O12 tetradecanoyl chain in TPA. The electrostatic potential of the sn 1–2 dilauroylglycerol shows positive methylene carbons at the 1 and 2 positions as well as the first methylene carbons in the alkyl chains. The other heavy atoms have a negative electrostatic potential and the hydrogens a positive potential.

A corresponding fit but using O4 or even O20 of TPA results in poorer mean deviations thus corroborating Brockerhoff's work with CPK models, which suggests that the O9 hydroxyl in TPA is the hydrogen-bond donor in the system[5].

RESULTS CASE 2

Table 1 lists the heats of formation of the eight systems studied, along with the respective total energies (STO-3G/MNDO). In Table 2, the geometries for the MNDO model compound and the relevant portion of the crystal structure pertaining to the model compound for the 4β hydroxy phorbol molecule are presented.

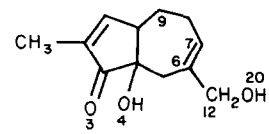
Comparing the two geometries reveals that the bond lengths are quite similar, the MNDO calculated values being in general slightly longer than the crystal-structure values. The bond angles compare well, differing by only a few degrees. The main discrepancy, however, occurs with the dihedral angles. The crystal structure has the seven-membered cycloheptene ring in a boat conformation, whereas the MNDO optimized structure is in the chair conformation. This is because we are looking at model compounds that contain only two of the four rings present in the phorbol skeleton. The addition of the cyclohexane and cyclopropane rings adds more rigidity to the skeleton and fixes it in the observed conformation. However, we feel that the differences between the observed and calculated conformations will not affect the electrostatic potential to any major degree. In particular, the conformation of the five-membered ring for the MNDO optimized structure is the same as that in the crystal. The main difference lies in the position of the 6,7 double bond and the associated CH_2OH group attached to C6, which in the crystal structure is bent underneath the seven-membered ring, whereas in the MNDO optimized structure it is more in line with the main ring plane; in fact, the two rings form a fairly planar skeleton.

A previous MNDO/3 study by Pack[17] on the parent alcohol, phorbol, showed that the calculated geometry was similar to that of the crystal structure; in particular the ring puckers were the same.

As was noted above, certain functionalities play a key role: namely, O4, O20, O9 and O3. In our calculations with O4 and O20 groups, we find that they adopt a trans conformation with respect to each other. In other words, if the O4 group is above the ring plane, then the O20 substituent will be pointing downwards.

Concentrating on the four key functional groups, O3, O4, O9 and O20, Table 3 lists their charges calculated both with MNDO and with an STO-3G basis set for

Table 1. Heats of formation and total energies for eight model systems



	MNDO Δ $Hf^{\ominus}$ kcal/mol	STO-3G/MNDO, au
4OHβ	−103.7	−679.6518
4OHβ	−108.1	−679.6599
4Hβ	−68.1	−605.8308
4Hα	−69.9	−605.8338
4OHβ, 9OH	−142.7	−753.4766
4OHβ, 6Me	−61.6	−605.8327
4OHβ, 9OH, 6-7 epoxide	−162.2	−827.3287
4OMeβ, 9OH	−132.2	−792.0512

the model compounds 4OHβ and 4OMeβ. These represent an active and inactive species, respectively, and are the best to compare for differences in electronic properties as conformational changes are negligible.

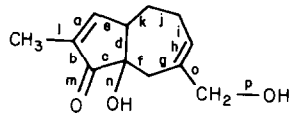
The charge on the oxygen of the 3 carbonyl group is the least negative for both species and methods of calculation. The differences in the charges between the species for a specific oxygen atom are small except for the O4 atom which is of course where the change of hydrogen to methyl occurs. Unfortunately, the calculations differ as to the ordering of the charges for the O4 atom, with MNDO having a greater negative charge for the methyl species while the STO-3G/MNDO result has the hydrogen species carrying the greater negative charge. The charges on the O9 and O20 groups are very similar, irrespective of species or method of calculation.

An analysis of the electrostatic potential calculations on the basis of percentage of positive and of negative points is presented in Table 4. The calculations can be conveniently split into two groups: those with the 9 OH group and those without. In either case, the active compounds all have a higher percentage of negative electrostatic potential points than positive ones.

Colour Plate 1 shows the electrostatic potential on a van der Waals surface for the 4OHβ and 4OMeβ model compounds. These pictures, and similar ones for the other molecules studied, reveal that there are many similarities between them, especially when the difference between adjacent colours on the scale is approximately 10 kcal/mol. The pictures corroborate in a more obvious form the results that were presented in Table 4. Most of the surface has a slightly positive electrostatic potential, the all-important negative regions are located at the oxygen atoms and, to a lesser extent, at the double bonds where present. These mimic the charges obtained with STO-3G/MNDO. The O3 carbonyl oxygen has the least negative electrostatic potential, whereas the major difference is around the O4 atom. This can easily be seen by choosing finer contour levels for the negative values: a difference of 3 kcal/mol between adjacent colours is reasonably effective.

A calculation of the electron density similarity index[18] for the 4OHβ and 4OMeβ model compounds using the

Table 2. Geometries for the 4β hydroxy phorbol molecule as calculated by MNDO and the relevant portion pertaining to the crystal structure



	MNDO, Å	Crystal structure, Å
a	1.359	1.341
b	1.509	1.460
c	1.583	1.533
d	1.587	1.559
e	1.518	1.515
f	1.564	1.520
g	1.520	1.508
h	1.354	1.327
i	1.506	1.518
j	1.541	1.546
k	1.538	1.548
l	1.494	1.504
m	1.219	1.220
n	1.404	1.427
o	1.533	1.518
p	1.397	1.431
ab	109.0	108.5
bc	106.9	109.9
cd	100.7	103.1
de	103.6	103.6
ae	112.8	114.0
df	110.6	111.4
fg	114.7	116.9
gh	126.0	127.9
hi	131.8	127.5
ij	119.4	115.8
jk	113.5	108.1
dk	116.3	117.9
ek	118.5	121.4
al	127.8	127.5
bl	123.2	123.9
bm	127.6	126.7
cm	125.5	123.3
cn	106.6	105.5
dn	109.8	110.6
fn	112.0	112.8
go	115.4	112.4
ho	118.6	119.6
op	109.3	109.4
abc	12.5	4.9
bcd	−23.3	−9.0
cde	25.1	9.3
dea	−20.1	−7.3
eab	5.0	1.7
dfg	73.2	40.5
fgh	−47.6	11.1
ghi	−2.1	9.1
hij	44.6	−68.2
ijk	−58.7	43.2
jkd	69.9	40.1
kdf	−79.3	−92.2
eal	−176.3	−177.2
cbl	−166.2	−176.1
abm	−168.9	−177.3
dcm	158.1	173.1
cdk	157.0	146.5
edf	148.8	130.6
edn	−87.1	−103.1
kdn	44.8	34.1
lbm	12.3	1.7
mcn	−87.3	−70.8
gop	83.8	73.6
hop	−95.2	−102.1
bcn	91.3	107.1
gfn	−49.6	−84.7

Table 3. Charges the four oxygen atoms for the 4OHβ and 4OMeβ model compounds as calculated by MNDO and MNDO//STO-3G

Atom label model	MNDO	MNDO/STO-3G
O3, 4OMeβ	−0.2719	−0.2316
O3, 4OHβ	−0.2775	−0.2371
O4, 4OMeβ	−0.3507	−0.2872
O4, 4OHβ	−0.3123	−0.3317
O9, 4OMeβ	−0.3295	−0.3323
O9, 4OHβ	−0.3283	−0.3320
O20, 4OMeβ	−0.3224	−0.3239
O20, 4OHβ	−0.3216	−0.3236

Table 4. Percentage of positive and negative points of electrostatic potential on the van der Waals surface for the model systems studied

	Positive percentage	Negative percentage
4OMeβ/9OH	69	31
4OHβ/9OH	67	33
4OHβ/6,7 epoxide/9OH	69	31
4OHα	75	25
4OHβ	72	28
4OHβ/20Me	76	24
4Hα	77	23
4Hβ	78	22
4OMeβ/9OH/2vdw	50	50
4OHβ/9OH/2vdw	44	56

CNDO style approximation (charge × overlap integral[18,19]) reveals a high value for the similarity, whether the charges are taken from MNDO or from STO-3G, the values of the index being 0.95 and 0.96, respectively (the index lies between 0, no similarity and 1, perfect similarity). An identical style calculation but using the 4OHα and 4OHβ model compounds results in a much lower value of around 0.3. However, much of this difference can be attributed to the conformational change in going from the α to the β conformation.

In order to highlight the differences more clearly, calculations were made using the 4OHβ wavefunction but computing the electrostatic potential on the 4OMeβ surface. This involves a direct superposition or fit of the corresponding atoms in the two molecules, which in this case are almost geometrically identical. A direct comparison can then be made, and a difference electrostatic potential can be calculated. By counting the number of points that lie within a specific range in the difference computation and expressing this as a percentage or fraction of the total number of surface points, a crude form of electrostatic potential similarity index can be obtained.

This shows that, at the van der Waals surface level, over 90% of the molecules' electrostatic potential is the same within a range of ±6 kcal/mol. This form of analysis can be performed not only on the difference electrostatic potential but also on the actual electrostatic potential calculated. Further, it can be extended, the values being subdivided into atom contributions, and so on.

CONCLUSIONS

The results presented in Case 1 show that there are areas of similarity both in geometry and in electrostatic potential for a range of tumour promoters. The active and inactive models in Case 2 are somewhat more disappointing in that the electrostatic potentials are very similar and hence cannot be used to discriminate between active and inactive compounds. Therefore, the electrostatic potential is not a good property to use in this case. We are, of course, looking at model compounds, and factors such as solvation and Ca^{+2} have not been considered. In addition, steric requirements may be the important factor in receptor binding.

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