QSAR of Microtubule Stabilizing Dictyostatins

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Abstract

Microtubule stabilization is a validated mechanism for cancer chemotherapy. Dictyostatin, an analog of the failed drug discodermolide, binds to the β-tubulin subunit of microtubules, inhibiting cell growth by blockage at the G2/M phase of the cell cycle. Dictyostatin and analogs were synthesized and their antiproliferative activities against ovarian cancer cells were measured. These data, along with that from some discodermolides, were used to determine a quantitative structure-activity relationship (QSAR). Molecular models of the dictyostatins were built from NMR coordinates of discodermolide and their global minimum energy conformations determined. Models were superimposed to provide maximum structural overlap and a collection of electronic, thermodynamic and steric descriptors were calculated for each model. A special multiple linear regression analysis, the genetic function approximation, was then used to find the descriptors that best explained the differences in activity. A population of statistically-compelling QSAR equations was found and these may be useful in future analog design.

Background

The discovery and advancement of new chemotherapeutic agents for the treatment of cancer is currently of high significance. Some of the most useful chemotherapeutic agents are natural products or natural product analogs. For example, paclitaxel is a natural product that is currently being used to treat patients with breast, lung and ovarian cancers. Paclitaxel belongs to a group of chemicals known as taxanes, which functions through binding to the β-tubulin subunits of microtubules. A number of analogs of paclitaxel, including docetaxel, are also clinically useful anticancer agents.

The mechanism by which paclitaxel hinders cancer cell growth is the stabilization of microtubules. Microtubules are polymers made up of α- and β-tubulin heterodimers. Tubulin polymerizes at each end with the α-subunit of one tubulin dimer connecting to the β-subunit of the next. Therefore, one end of the microtubule will have the α-subunit (minus or – end, where microtubule shrinking can occur) exposed, while the other end will have the β-subunit (plus, +, or growing end) exposed.

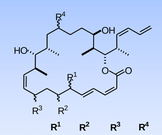
The α- and β-tubulin subunits each bind one mole of guanosine triphosphate (GTP). The GTP bound to α-tubulin is stable, but the GTP bound to β-tubulin can be hydrolyzed to guanosine diphosphate (GDP) shortly after a heterodimer adds to the growing polymer. The GTP-bound β-tubulin therefore forms a cap at the (+) end of the microtubule, keeping it from disassembling. When hydrolysis catches up to the tip of the microtubule, it begins to quickly depolymerize and shrink. GTP-bound tubulin can begin adding to the tip of the microtubule again, providing a new cap and protecting the microtubule from shrinking. However, when a drug such as the taxanes is attached, it hyperstabilizes microtubules by binding to the β-tubulin of the microtubule and preventing the disassembly from the (–) end.

Like paclitaxel, discodermolide, a polyketide natural product, was discovered to be a very potent inhibitor of cancer cell growth. It has been proven to inhibit the growth of human cells by blocking them at G2/ M phase of the cell cycle. It was a clinical candidate for cancer chemotherapy due to its high potency in microtubule stabilization and its strong activity against multiple drug resistant cancers. Unfortunately, discodermolide only made it to Phase II clinical trials when tested in humans, where it failed due to unexpected toxicity.

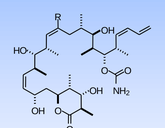
Because discodermolide showed promising effects, it was important in the fields of chemotherapy and drug discovery to uncover an agent quite similar in structure and activity. It was determined that another marine sponge-derived natural product discovered in 1994, dictyostatin, shares much structural similarity to discodermolide, including identical configurations at all common stereocenters. Dictyostatin also has very similar biological activity to discodermolide. It is active against paclitaxel-resistant cell lines and is one of the most potent microtubule stabilizers known, potently competing with paclitaxel and discodermolide for the taxoid binding site on microtubules. With the recent withdrawal of discodermolide from clinical development, the importance of uncovering a dictyostatin with the potential for clinical development has increased. Several analogs of dictyostatin have been synthesized and some of their biological activities have been measured. Using the structures of these analogs and their biological activities, along with those of discodermolide and a potent, structurally-related analog, the purpose of this work will be to develop a quantitative structure-activity relationship (QSAR) useful in further analog design.

Method

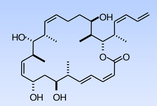
The genetic function approximation, which is a special multiple linear regression analysis, was used to find a minimum number (the number four was chosen) of descriptors that best explained the difference in activity. The classes of descriptors include structural, thermodynamic, spatial, electrical, and quantum mechanical, for all properties studied, the best models were selected from the group of calculations. After the population of equations was developed, the best five equations (i.e., those with the best statistical scores, particularly Friedman's lack of fit and R2) were then studied. The five equations produced a set of predicted activities. The predicted activity was plotted as a function of the actual activity on a scatter graph to determine the differences between the actual and predicted values.



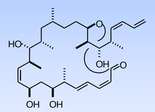
	R^1	R^2	R^3	R^4
JMC1	*R*-Me	*S*-OH	*S*-OH	*S*-Me
JMC4	*R*-Me	*S*-OH	*S*-OH	H
JMC5	*R*-Me	*S*-OH	*S*-OH	*R*-Me
JMC54	*R*-Me	*S*-OH	*R*-OH	H
OL22	*S*-Me	*R*-OH	*S*-OH	*S*-Me
OL23	*S*-Me	*S*-OH	*S*-OH	*S*-Me
OL24	*R*-Me	*R*-OH	*S*-OH	*S*-Me



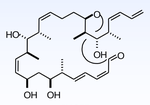
JMC2 Me
JMC3 H



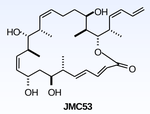
JMC6



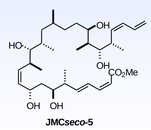
JMC27



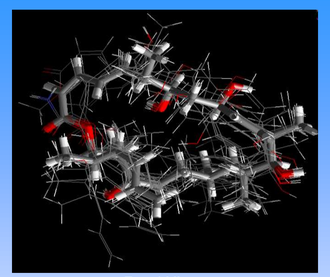
JMC55



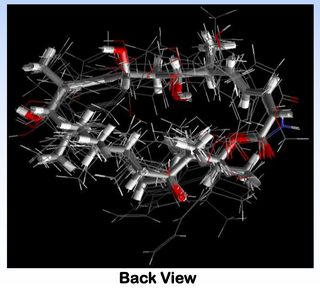
JMC53



JMCseco-5



Front View



Back View

QSAR Equations

A *descriptor* is any of a number of built-in molecular properties that can be calculated and used to determine QSAR relationships.

Index	Equation (Activity)
1 (100)	= -0.017328 - 0.41287 * "*RotIbonds*" + 0.091723* "*HF_MOPAC*" – 0.223325 * "*HF*" + 0.0611096 * "*Dipole_Mag*"
2 (99)	= -26.5019 +0.03692 * "*Area*" – 0.328785 * "*RotIbonds*" – 0.04374 * " *HF*" + 0.924229 * "*Dipole_Mopac*"
3 (98)	= -26.3842 + 0.1088 * "*Area*" + 1.31599 * "*Dipole_Mopac*" - 0.353032 * "*MR*" + 0.654779 * *LogP*
4 (97)	= 1.694349 – 0.305704 * "*RotIbonds*" – 0.135425 * "*HF*" + 0.605182 * "*Dipole_Mopac*" + 0.053736 * "*HF_Mopac*"
5 (96)	= -48.6935 – 0.200571 * "*MR*" – 0.270041 * "*RotIbonds*" + 1.27617 * "*Dipole_Mopac*" + 0.117247 * "*Area*"

Rotatable bonds (RotIbonds) are *structural* descriptors. The number of rotatable bond descriptor counts the number of bonds in the current molecule having rotations that are considered to be significant in molecular mechanics.

The molecular surface area (Area) descriptor is a 3D *spatial* descriptor that describes the van der Waals area of a molecule.

The heat of formation (Hf) is a *thermodynamic* descriptor. It is the enthalpy for forming a molecule from its constituent atoms.

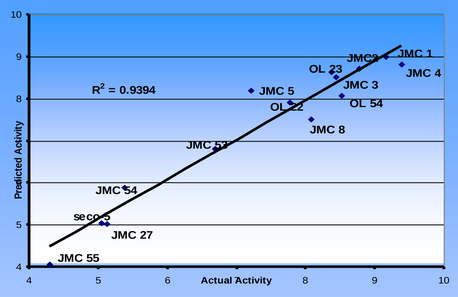
HF_Mopac and Dipole_Mopac are *quantum mechanical* descriptors.

Statistical parameters

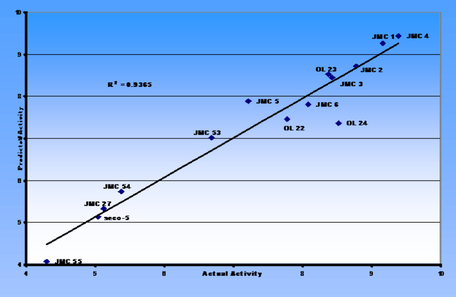
	LOF	r^2	r^2-adj	F-test	N Obs	N Vars	LSE	r	C(P)
1 100)	0.832	0.943	0.918	37.425	14	5	0.153	0.971	- 3.940
2 (99)	0.889	0.939	0.912	34.867	14	5	0.163	0.969	- 3.935
3 (98)	0.930	0.937	0.908	32.210	14	5	0.171	0.968	- 3.932
4 (97)	0.932	0.936	0.908	33.161	14	5	0.171	0.968	- 3.932
5 (96)	0.964	0.934	0.905	31.971	14	5	0.177	0.967	-3. 930

Results

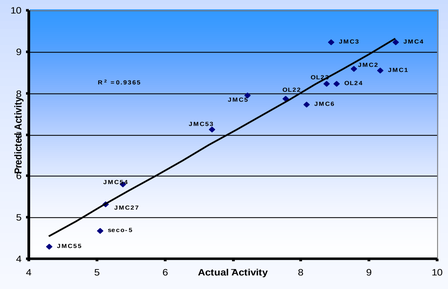
Using plots of predicted versus actual activities and the regression line for each equation, the outliers were further examined to determine the role each descriptor played in causing a poor prediction of activity. A "leave one (descriptor) out" exercise was performed to identify descriptors with the highest influence on predicted activity.



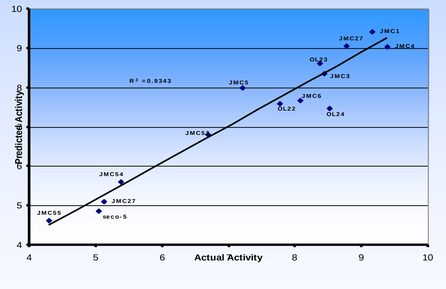
The descriptor "Area" played a major role in equation 2. Predictions for outliers such as JMC 5 were most wrong (e.g., a Δ of 25) without taking into account the contribution from the "Area" descriptor. The "Area" descriptor was the most important for correct best predictions of activity for all structures under study in equation 2, such as JMC 54 (Δ 25), JMC 27 (Δ 27), and seco-JMC5 (Δ 28).



Similar to what occurred in equation 2, "Area" along with molar refractivity "MR" were the most significant contributors to activity predictions in equation 3. For example, JMC 5 had a Δ of 77 when "Area" was left out, and a Δ of 55 when "MR" was excluded. JMC 54 had a Δ of 75 without "Area" and a Δ of 54 without "MR"; seco-5 had a Δ of 84 without "Area" and Δ of 60 without "MR"; and OL 24 had a Δ of 78 and a Δ of 55 without "MR".



The heat of formation "HF" and "HF_Mopac" proved to be important descriptors in equation 4, causing the largest change in values when left out. Examples are JMC 3 with a Δ of 28 without the presence of "HF" and Δ of 19 without "HF_MOPAC", JMC 5 had a Δ of 18 without the presence of "HF" and a Δ of 14 without "HF_MOPAC", JMC 27 had a Δ of 19 without the "HF" and a Δ of 14 without "HF_MOPAC", JMC 54 had a Δ of 16 "HF" and a Δ of 13 without "HF_MOPAC", and seco-5 had a Δ of 26 without the "HF" and Δ of 17 without "HF_MOPAC".



Like equation 3, the descriptors that were noteworthy in Equation 5 were "Area" and "MR". For example, JMC 5 had a Δ of 82 without "Area" and Δ of 31 without "MR"; JMC 27 had a Δ of 85 without "Area" and Δ of 31 without "MR"; JMC 54 had a Δ of 81 without "Area" and Δ of 30 without "MR"; and seco-5 had a Δ of 90 without "Area" and a Δ of 34 without "MR".

Conclusion

The understanding of descriptors used in QSAR equations can provide excellent opportunity for identifying their features and becoming aware of how they affect each compound.

Furthermore, the simpler the equation, the easier it is to use that equation to make chemical modifications; and, in general, the more likely it will be useful in drug design.

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2) **Billy Day**
3) **University of Pittsburgh**
4) **Grambling State University**

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