Ligands of the colchicine site of tubulin: a common pharmacophore and new structural classes

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Structure—activity relationships for ligands of the colchicine site of tubulin were analyzed based on their common pharmacophore. The role of the elucidation of the three-dimensional structure of the colchicine site of tubulin on the development of studies aimed at the search for the ligands of this site is analyzed.

Key words: structure—activity relationship, pharmacophore, colchicine, podophyllotoxin, combretastatin, tubulin polymerization inhibitors.

Introduction

The cell protein tubulin is one of the most important molecular targets of antitumor agents. Two subunits of this protein, *viz.*, α- and β-tubulins, form α,β-dimers, which can undergo polymerization to give microtubules. The control over cell division processes is among their important and various functions in cells. Inhibition of these processes by intervening into the tubulin system of tumor cells provides the basis of one of types of antitumor therapy.[1]

Antitumor agents can bind to different sites of tubulin and cause either its uncontrolled polymerization (for example, taxol and its analogs) or inhibit tubulin polymerization. The alkaloids vincristine and vinblastine belong to the second type of agents.[1] Tubulin polymerization is inhibited also by colchicine (**1**) and its analogs. The action of colchicine is based on binding in a particular site of tubulin (the colchicine site), resulting in deformation of the α,β-dimer structure, which hinders the tubulin assembly into microtubules.

Numerous natural and synthetic ligands of the colchicine site of tubulin are exhaustively listed in a review[2] (including structural classes that are beyond the scope of the present review). There is also a group of compounds for which binding to the colchicine site is only assumed (for example, the toxin 7,11-*epi*-thyrsiferol[3] or pyrimidinylpyrazole derivatives[4]).

It should be emphasized that the vast majority of the aforementioned compounds were found by screening or with the use of purely empirical structure—activity relationships. Quite a few attempts to quantitatively describe these relationships and to use them for the prediction of active compounds were made (see, for example, Refs 5 and 6). The reason for this is the absence, for a long time, of complete and reliable data on the three-dimensional structure of the colchicine site of tubulin. In 2004, the structure of a complex of a colchicine derivative with β-tubulin was established by X-ray diffraction.[7] In our opinion, this publication has marked a new stage in studies aimed at the search for active tubulin polymerization inhibitors. The present review gives a brief illustration of the possibilities provided by this publication both for explaining the known empirical structure—activity relationships for ligands of the colchicine binding site and analyzing recent trends (2004—2006) in the search for such compounds.

Pharmacophore groups of inhibitors of the colchicine site of tubulin

The structure elucidation of the α,β-tubulin complex with *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (DAMA-colchicine) and the α,β-tubulin complex with podophyllotoxin[7] confirmed the earlier hypothesis that colchicine (**1**) and podophyllotoxin (**2**) bind to β-tubulin at its interface with α-tubulin. An X-ray diffraction study demonstrated that the trimethoxyphenyl groups of both DAMA-colchicine and podophyllotoxin are located in the β-tubulin structure in the vicinity of the amino acid residue Cysβ239.* The width of the colchicine site is approximately 4—5 Å, and the volume of this site is confined in β-tubulin by helix 7 (H7) containing Cysβ239. The involvement of α-tubulin in the formation of the

* In the study,[7] this amino acid residue was denoted Cysβ241; however, this residue is numbered differently in some other publications.

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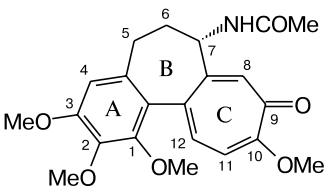

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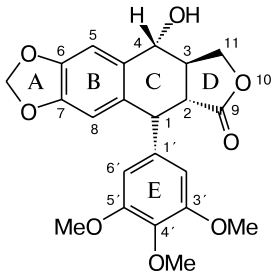

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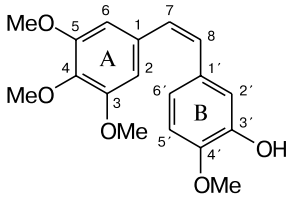

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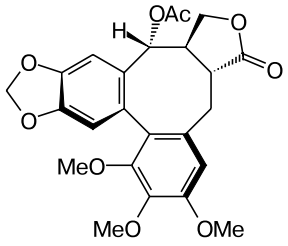

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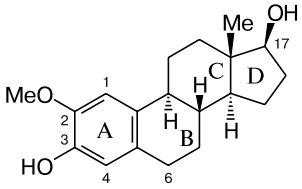

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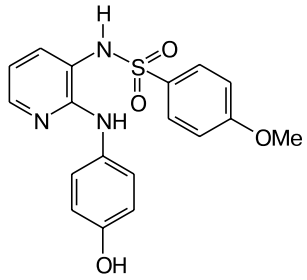

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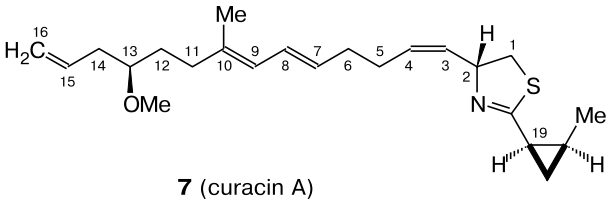

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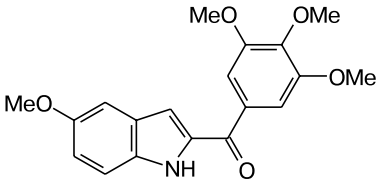

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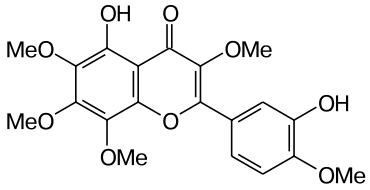

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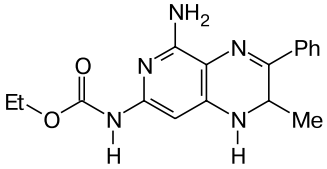

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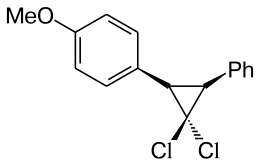

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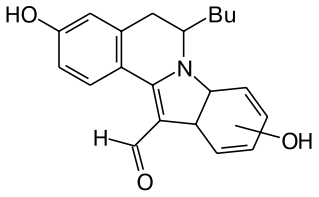

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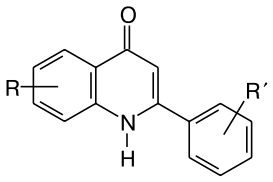

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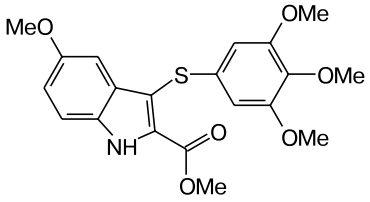

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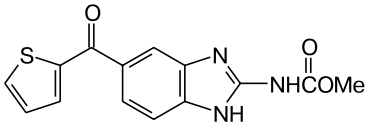

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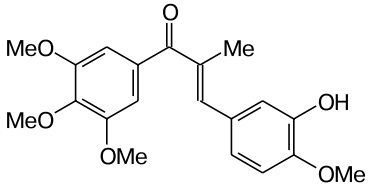

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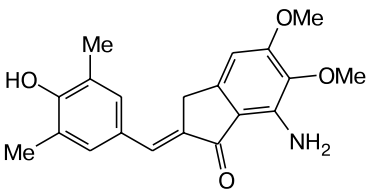

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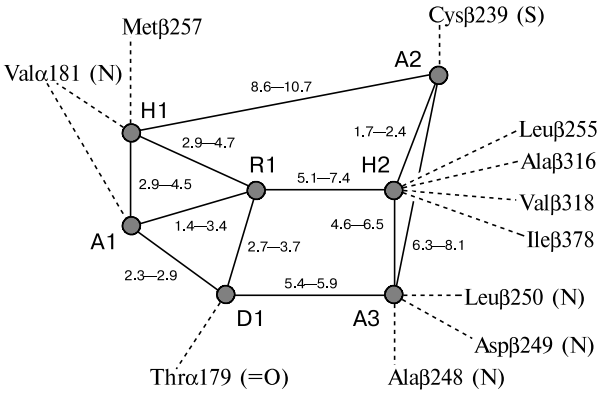


Fig. 1. Common pharmacophore of ligands of the colchicine site of tubulin based on the published data.[8] The distances between the pharmacophoric points are given in Angstroms.

colchicine site is primarily confined by the loop between sheet 5 and helix 5 containing the amino acid residues Thrα179 and Valα181. Soon after the publication of data on the structure of the colchicine binding site, a fundamental study appeared describing a common pharmacophore of ligands of this site.[8] The computer simulation (docking) of a set of structures including, in particular, compounds **1—9** and **14—17**, newer data on the steric and electronic characteristics of the binding region of colchicine to the protein being taken into account, revealed pharmacophore groups that are important for this binding.

According to these data, the common pharmacophore of ligands of the colchicine site contains the following seven pharmacophoric points: three hydrogen bond acceptors (A1, A2, and A3), one hydrogen bond donor (D1), two hydrophobic centers (H1 and H2), and one planar group (R1) (Fig. 1). These points can be distributed between two planes located at an angle of about 45° (the points A1, D1, and H1 and R1 are in the plane A, and the points A2, A3, and H2 are in the plane B, see Fig. 1). Interactions between the pharmacophore groups and the tubulin structure are presented in Fig. 1. The hydrophobic center H1 is wedged between the side chains of Valα181* and Metβ257. The group H1 generally consists of the carbon atom of the methoxy group. The group H2 (generally, the aromatic ring) forms a hydrophobic contact with the side chains of Leuβ255, Alaβ316, Valβ318, and Ileβ378. In addition, there are six potential hydrogen bonds: (1) between A1 and the amide hydrogen atom of Valα181 (A1—N, 3.3—4.6 Å), (2) between A2 and the thiol hydrogen atom of Cysβ239 (A2—S, 3.2—4.2 Å), (3—5) between A3 and the amide hydrogen atoms of Alaβ248, Aspβ249, and Leuβ250 (A3—N, 3.9—6.4 Å), and (6) between D1 and the carbonyl oxygen atom of Thrα179 (D1—O, 3.0—4.9 Å). It should be noted that the distance for the contact between A3 and the amide groups of Alaβ248, Aspβ249, and Leuβ250 is longer than that commonly accepted for hydrogen bonds. Their formation was attributed[8] to either conformational changes in the loop containing the above-mentioned amino acid residues and a decrease in the distance between the hydrogen bond donors and A3 or the insertion of water molecules between this region of hydrogen bond donors and A3.

Ligands **1—9** and **14—17** are characterized by the number of pharmacophoric points from 5 to 6. All these ligands contain the fragments A2, H2, and R1. In most of the ligands, H1 is present. Therefore, the hydrophobic fragment H2 and the planar fragment R1 apparently serve as a rigid molecular basis providing the required spatial arrangement for the groups A1, A2, A3, D1, and H1 forming specific interactions with the protein. An A2—Cysβ239 hydrogen bond is present in all structures considered in the study;[8] in most structures, there is also an A2—Valα181 hydrogen bond.

An analysis of the literature data shows that the above results of computer simulation provide an explanation for many known and new data on the structure—activity relationships for ligands of the colchicine site. In addition, the fact that none of the known structures contains all seven pharmacophore groups suggests definite modifications of known ligands that could, in principle, result in an increase in their activity.

Structure—activity relationship for the prototypical group of colchicine ligands

According to the nomenclature used in the study cited (Ref. 8), the prototypical group of ligands of the colchicine site includes structures, which are essentially similar to that of colchicine and have the following three characteristic features: 1) the biaryl structure; 2) the presence of the trimethoxyphenyl group; 3) rather limited conformational mobility (for example, **1—4**, **8**, **9**, **14**, and **16**).

Colchicine and its derivatives. Colchicine (**1**) contains five pharmacophore fragments: A2 (the oxygen atom of the methoxy group at the C(2) atom), H2 (the ring A), R1 (the ring B), A1 (the carbonyl oxygen atom at the C(9) atom), and H1 (the methyl group of the methoxy substituent at the C(10) atom). The correlation of this model with the known structure—activity relationships* for numerous colchicine analogs[2,5,9] provides an explana-

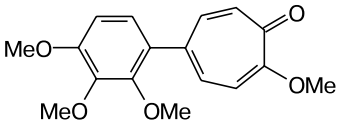
* The amino acid residues, which were numbered Thrα177 and Valα179 in the study,[8] should be numbered Thrα179 and Valα181 (see Additions and Corrections, *J. Med. Chem.*, 2005, **48**, 7917).

* When considering such relationships, we compared the degrees of inhibition of tubulin polymerization rather than the cytotoxicities as a measure of activity, because the former values are associated only with binding to this protein. (It is well known that the degree of cytotoxicity not necessarily correlates with affinity.)

tion for a decrease in activity upon the replacement of methoxy groups (especially, at the C(2) atom) by other substituents (for example, by alkyl groups) and the fact that the nature of the substituent at the C(10) atom of colchicine has only a slight effect on the ability of *in vitro* inhibition of tubulin polymerization, except for a decrease in activity in the presence of a very bulky or carbonyl group at the C(10) atom. A computer simulation showed that this substituent is not involved in hydrogen bonding with an amino acid residue of the colchicine binding site or in any polar interaction.

The fact that the ring C or B of colchicine can be contracted to the corresponding six-membered fragments with retention of high activity, whereas similar phenanthrene and dihydrophenanthrene analogs of colchicine are inactive,[5,10] might also be explained in the framework of the concept on the "two-plane" structure of the binding site and thus the advantage of a nonplanar structure of its ligand (see Fig. 1).

A complication of the ring B as a result of addition of a heterocycle at the C(6)—C(7) bond or, on the contrary, the complete removal of this ring (structure **18**) result in retention of high activity of the parent molecule.[11,12]



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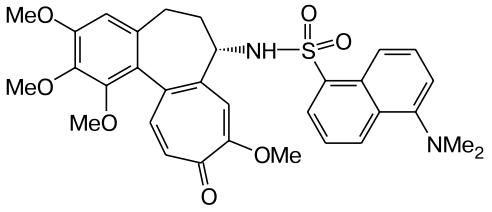

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Assumptions that only the rings A and C are involved in binding of colchicine to tubulin, which were made based on the above fact, were confirmed also by newer results of simulation. The ring B ensures conformational rigidity of the system consisting of the rings A and C.

The important role of the carbonyl group at the C(9) atom as a hydrogen bond acceptor (A1), which was revealed for the colchicine molecule by X-ray diffraction, explains to a considerable degree the earlier observed inactivity of isocolchicine characterized by the inverse order of substituents at the C(9) and C(10) atoms (and, in general, sensitivity of the activity of the ligand to the nature of the substituent at C(9)).[13] Nevertheless, the first isocolchicine analog (**19**) exhibiting certain activity has been recently found.[13,14] However, the presence of a bulky aromatic substituent most likely substantially changes the mode of binding of compound **19** to the protein compared to colchicine (probably, compound **19** interacts with the colchicine site analogously to sulfonamides structures, see below).

Podophyllotoxin and its derivatives. Podophyllotoxin (**2**) contains the following six pharmacophore fragments: A2 (the oxygen atom of the methoxy group at the C(4′) atom), H2 (the ring E), R1 (the ring B), A3 (the carbonyl oxygen atom at the C(9) atom), H1 (the methylene group in the ring A), and D1 (the hydroxy group at the C(4) atom). The correlation of this model with the structure—activity relationships for podophyllotoxin derivatives presents difficulties because of contradictory data and the lack of data on activity of many compounds (in many cases, only cytotoxicity was measured).[15] Nevertheless, the following conclusions can be drawn.

An important role of the oxygen atom of the methoxy group at position 4′ accounts for the fact that analogs containing very bulky substituents at this position lose activity. A great role of the lactone carbonyl (A3) in binding explains the fact of a twofold loss of the inhibitory ability of the analog containing the methylene group instead of the carbonyl and also a loss of activity of analogs containing C=S and SO_2 groups instead of C=O.[15]

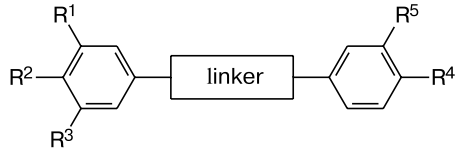
The earlier hypothesis on steric hindrance in the vicinity of a substituent at position 4 of the ring C can be refined based on X-ray diffraction data and the results of simulation. The role of the hydroxy group at this position as a hydrogen bond donor in binding to the protein explains a decrease in activity not only upon the introduction of bulky substituents at this position, but also upon the removal of the hydroxy group.* Based on the common pharmacophore, the earlier observed decrease in cytotoxicity upon opening of the ring A (see Ref. 15) giving rise to one or two hydroxy groups is attributed to the fact that this replacement is accompanied by violation of favorable hydrophobic interactions of H1.

Combretastatins and their derivatives. This group of ligands of the colchicine site is the most representative because of the rather simple structure of compound **3** and its very high activity due to which combretastatin A-4 (**3**) is the leader in the design of many structural classes of ligands of the colchicine site (see the reviews[16,17]).

Studies of the structure—activity relationships for analogs **3** (general scheme **20**) showed that the highest activity is observed for $R^1 = R^2 = R^3 = R^4 = OMe$ and $R^5 = H$, OH, or NH_2, whereas the introduction of small substituents at other positions of the rings A and B does not generally lead to enhancement of activity.[16—19] The ring B can be replaced by nitrogen-containing heterocycles

* It should be noted that some podophyllotoxin derivatives containing very bulky substituents at the C(4) atom are highly cytostatic agents due to interactions with another biological target.

with retention of activity. A recent study of the structure—activity relationships for a series of naphthalene analogs of combretastatin demonstrated that the 2-naphthyl group (structure **21**)[18] is a good bioisostere of the 3-hydroxy-4-methoxyphenyl (but not of the 3,4,5-trimethoxyphenyl) group of combretastatin. In terms of the common pharmacophore, these data are explained by the presence of the pharmacophoric points A1, H1, A2, and H2 in the combretastatin molecule analogous to those in colchicine.



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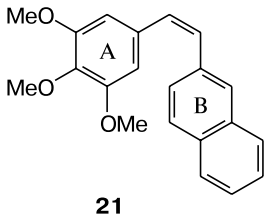

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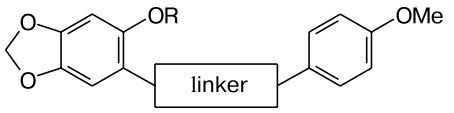
The spatial arrangement of the two aromatic rings is an important structural feature of combretastatin **3** and its derivatives **20**. This arrangement determines to a large extent their correspondence to the common pharmacophore. The *cis* configuration of the linker is favorable. Most of analogs with the *trans* arrangement of the rings are inactive or serve as weak tubulin polymerization inhibitors. An analysis of the main tendencies upon the replacement of the *cis*-olefin linker by the ether (—O, —CH_2O, —OCH_2), carbonyl, sulfonamide (—SO_2—NH—), sulfonate (—SO_2O—), or cyclopentane groups or by heterocycles (pyrazole, thiazole, triazole, tetrazole, oxazole, imidazole, thiophene, furan, furanone, dioxolane, indole, *etc.*) shows that activity is observed only for derivatives in which there is a fine balance of the distances between the rings (the highest activity was found for a linker containing two carbon atoms) and their spatial mutual orientation.[18] The results of simulation indicative of a small volume of the binding site and the two-plane common pharmacophore (see Fig. 1) confirm these features and explain, for example, a decrease in activity of combretastatin analogs containing the acetylene linker or with the directly linked rings A and B[10,18] and the fact that the dependence of the activity on the linker length for benzodioxole analogs (**22**) is different from that observed for combretastatin derivatives.[2] Higher activity of compounds **22** containing a longer linker than that in **21** (variations in substituents in these compounds being similar[20]) is apparently associated with the fact that the former compounds are bound in the colchicine site analogously to podophyllotoxin (*i.e.*, somewhat differently from combretastatin). This partially explains the fact that all open steganacin analogs **4** (structure **23**) are inactive regardless of the nature of the substituent R.[2]



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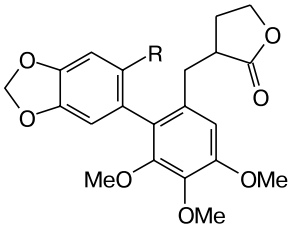

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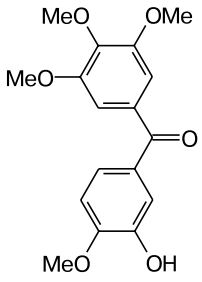

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The binding model of compound **3** to tubulin provides an explanation for the possible existence of additional interactions with the protein in combretastatin analogs. For example, phenstatin **24**, which is structurally similar to compound **3**, is approximately twice as active as the parent compound.[21] A simulation demonstrated that compound **24** has an additional hydrogen bond with the hydrogen atoms of the amide groups of Alaβ248, Aspβ249, and Leuβ250 through the hydrogen bond acceptor A3, *viz.*, the carbonyl oxygen atom.[8]

Arylthioindoles. Among known arylthioindoles,[22] compound **14** proved to be the most active in inhibition of tubulin polymerization (comparable in activity to combretastatin). It should be noted that the authors of this publication[22] were among the first to propose a binding model of compound **14** based on the structure of the DAMA—colchicine—tubulin complex.[7] More recently, when constructing the common pharmacophore for ligands of the colchicine site,[8] a similar model (containing the methoxy group of the aryl ring in the vicinity to Cysβ239) was proposed for arylthioindole. This model differs in the arrangement of the indole fragment relative to the C—S bond (the benzene ring of the indole serves as R1; the methyl group of the methoxy substituent, as H1; and the NH group of the indole, as A3). The latter model was used in the study[23] concerned with the refinement of the arrangement of ligand **14** in the colchicine binding site.

Structure—activity relationship for the atypical group of ligands of colchicine

In the group of ligands called atypical (for example, including compounds **5—7**, **15**, and **17**), at least one of

the above-mentioned three features of the prototypical series is absent. As a result, structurally more diversified compounds belong to this group.

Curacins. It is much more difficult to reveal pharmacophore groups in the curacin A molecule (**7**) than in "more conformationally restricted" ligands of the colchicine site. According the results of the study,[8] there are the following pharmacophore groups: A2 (the oxygen atom of the methoxy group at C(13)), H2 (the carbon chain C(7)—C(10)), R1 (the fragment of the thiophene ring C(2)—N=C(19)), A1 (the sulfur atom of the thiophene ring), and H1 (the methyl substituent in the cyclopropane fragment).

This curacin binding model provides an explanation for the presently known structure—activity relationships[24,25] in series of their analogs, such as the possibility of the replacement of the cyclopropane ring by other lipophilic groups (for example, by the *tert*-butyl group) and a sharp decrease in activity of analogs containing the corresponding methoxy substituent at the C(13) atom and analogs containing the open thiazoline ring.

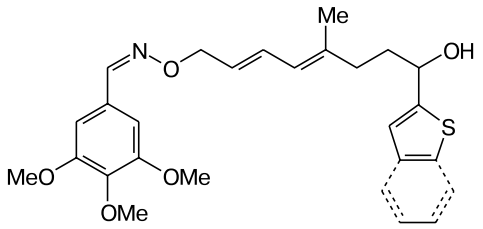
The fact that hydrogenation of the C(15)—C(16) bond is not accompanied by changes in activity, whereas hydrogenation of the C(3)—C(4) bond leads to a substantial decrease in activity and the sensitivity of the diene fragment C(7)—C(10) to structural modifications and isomerization is apparently attributed to an important role of the latter two fragments in the orienting arrangement of the ligand.

The use of the binding model of curacin A (**7**) to tubulin provides also an explanation for some facts that are far from evident, for example, a several-fold decrease in activity of a curacin analog containing no methyl groups at the C(10) atom. In curacin A, this group is located in the hydrophobic pocket formed by the side chains of Leuβ240, Leuβ250, and Leuβ253. The binding energy of the methyl group in the ligand—receptor interaction is estimated as 0.8 kcal mol^{-1}.[8] However, if the steric and electronic characteristics of the ligand are completely consistent with the structure of the receptor, the average binding energy can be substantially higher. Correspondingly, the above-mentioned methyl group in curacin A can provide additional stability upon binding of the ligand to the protein.

The simulation also clearly shows that the same group in close analogs of curacin can interact with the protein in different modes. For example, the hydroxy group in thiophene and benzothiophene analogs **25** (see Ref. 24) serves as a hydrogen bond acceptor (A1) and a hydrogen bond donor (D1), respectively.[8]

Since the simulation demonstrated that the pharmacophoric points A3 and D1 are absent in the curacin A molecule, the introduction of a hydrogen bond donor or acceptor would lead to an increase in the ligand affinity.

2-Methoxyestradiol and its derivatives. 2-Methoxyestradiol (**5**) is the main metabolite of the hormone



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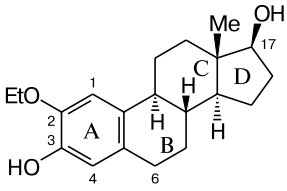

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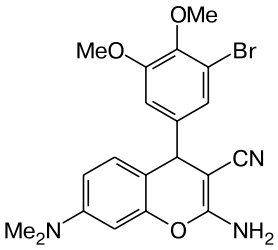
β-estradiol and is a weak competitive inhibitor of colchicine binding to tubulin. The most active derivatives **5** contain the ethoxy group at position 2 (**26**), whereas an increase or decrease in the chain length or chain branching results in a decrease in activity.[26,27] The earlier hypothesis that the ring A of the steroidal structure **5** occupies the same position in the protein as that of the ring C in colchicine and the ring B in combretastatin was confirmed by the results of simulation,[8] according to which the C—D rings and the hydroxy group at the C(17) atom (corresponding to the pharmacophore centers H1 and A2) in molecule **5** function as the colchicine trimethoxyphenyl group. The binding model of **26** to tubulin[8] shows that the ethyl group can be located in the colchicine site without considerable steric hindrance. Hence, a higher lipophilicity of the ethyl group (compared to the methyl group in compound **5**) in the pharmacophoric point H1 accounts for higher activity of **26** compared to **5**.

Sulfonamides. Compound **6** is a classical representative of sulfonamides. Numerous investigations[28,29] provided evidence that this compound binds to the colchicine site and, according to the results of simulation, contains six pharmacophore centers out of the possible seven centers. The unusual feature of this ligand is that the hydrophobic center H1 is absent.[8] Hence, the simulation showed that the affinity of this ligand for tubulin can be increased by introducing a lipophilic group into the pyridine ring. Therefore, methylated analog **6** was predicted to have higher activity in inhibition of tubulin polymerization compared to that of the parent molecule.

New structural classes

In recent years, several new structural classes of ligands of the colchicine bonding site of tubulin have been found. For example, this activity would be expected for compound **27** belonging to the 4-aryl-4*H*-chromene series[30]

and for a group of *N*-[(benz)imidazolylthiazolyl]phenylamines (**28**).[31]



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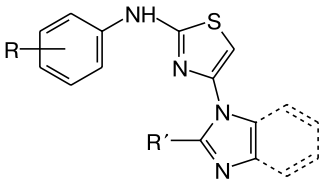

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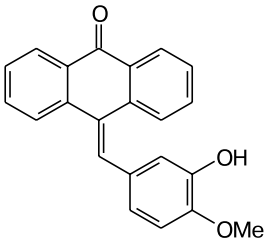
In experiments on binding to tubulin for the benzylidene-9(10*H*)-anthracenone series, compound **29** proved to be equal in activity to colchicine.[32] The fact that the —OH and —OMe groups are substituents of choice in the benzyl fragment (as in structure **29**) suggests that this ring binds to the same region of the protein as the ring C in colchicine (**1**).



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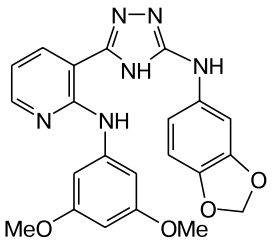

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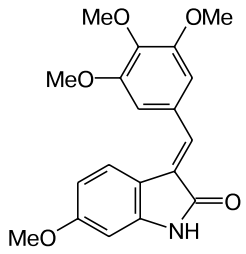
A new class of ligands of the colchicine site was found among substituted triazoles.[33] In this class, compounds containing cyclic alkoxy groups (for example, **30**) have the highest activity. More recently, the same research group has synthesized structurally similar compounds containing the oxadiazole fragment.[34] It was hypothesized[33] that binding of compound **30** to tubulin is analogous to that of podophyllotoxin (**2**). The computer superposition of the structures demonstrated that the benzodioxole fragment of **30** is superimposed onto the analogous fragment in podophyllotoxin. A comparison of the structures with the data on the common pharmacophore of podophyllotoxin[8] shows that the NH group adjacent to the triazole ring in compound **30** should function as D1 (the OH group at the C(4) atom in **2**), and the nitrogen atom of the pyridine ring in **30** acts as A3 (the lactone carbonyl at the C(9) atom in **2**).

New highly efficient conformationally restricted combretastatin analogs (**3**) of the 3-benzylidene-2-indolinone series were documented.[35,36] Compound **31**, which evidently belongs to the prototypical group of ligands of the colchicine site, exhibited the highest activity.

As can be seen from the above-considered newer studies, some research groups continue searching for active



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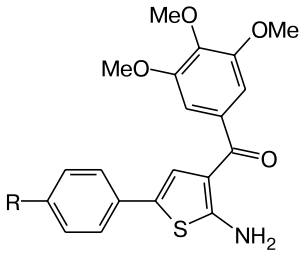

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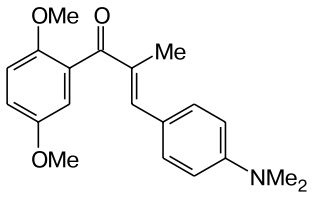
ligands of the colchicine site by empirical screening. Nevertheless, X-ray diffraction data on the structure of the colchicine site[7] have been already invoked by some researchers not only for the design of common pharmacophores[8] but also for an explanation of the structure—activity relationships in series of new compounds[22,23,37] and, which is the most important, for the prediction of new structures.[38]

A new structural class of phenstatin analogs (**24**) containing the thiophene fragment with the amino group and the aryl substituent (general formula **32**) was discovered.[37] High activity of derivatives **32** with R = F, Me, CF_3, or MeO was explained using docking of ligands into the binding model of colchicine. For example, higher inhibitory ability of compound **32** (R = F) compared to that of colchicine is attributed to the fact that compound **32** can form additional hydrogen bonds with the amino acid residues Glnβ11, Leuβ252, and Asnβ258 of tubulin (the atomic numbering scheme corresponds to that used in the study[37]).

A binding model of the colchicine site to chalcone **33** (MDL-27048) was proposed[38] based on the X-ray diffraction data for this site.[7] In terms of this model, the activity was for the first time predicted[38] for new chalcone derivatives **34**. For these compounds, the existence of several interactions with additional amino acid residues, which are not involved in binding to colchicine, was expected. Compound **34** synthesized based on these predictions actually showed noticeable activity as a tubulin polymerization inhibitor.[38]



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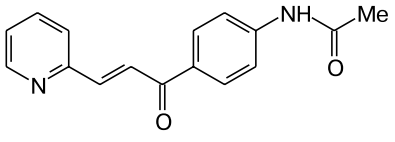

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Conclusions

In conclusion, the analysis of the published data shows that the knowledge of the structure of the binding site of the protein tubulin to colchicine and podophyllotoxin derivatives had a considerable influence on the development of studies aimed at the design of the corresponding ligands. The common pharmacophore of ligands of the colchicine site, which was constructed based on these data, provided an explanation for many of the previously known structure—activity relationships and allowed the prediction of chemical modifications of known compounds resulting in an increase in their affinity for tubulin. Although a number of questions concerning the conformations of some ligands in the protein require further refinement, the experimentally supported model of the colchicine site can, in principle, be actively used for the design of new (including unusual[39—41]) structural types. This allows the rationalization of a search for active compounds. Investigations on the molecular design of ligands of the colchicine site will be apparently intensified in future.

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