A New Generation of *N*-Aryl-*N′*-(1-alkyl-2-chloroethyl)ureas as Microtubule Disrupters: Synthesis, Antiproliferative Activity, and *β*-Tubulin Alkylation Kinetics

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New *N*-aryl-*N′*-2-chloroethylureas (CEUs) with enhanced cytotoxicity were developed as antimitotic agents potentially useful in cancer chemotherapy. Highly potent CEUs were obtained by introduction of a branched alkylating chain, the *N′*-(1-methyl-2-chloro)ethyl group. Their cytotoxic activity was enantio-dependent and induced through specific alkylation of *β*-tubulin, leading to microtubule disruption and mitotic arrest. Overall, the structural modifications of the CEUs described here significantly improved their kinetics of *β*-tubulin alkylation. In this new series, CEUs **16a** and **18a** displayed particularly enhanced antiproliferative activity related to a faster reaction with *β*-tubulin and merit further investigation as potential antitumor agents.

Introduction

Antimitotic agents have generated great interest as useful therapeutic drugs in clinical oncology, particularly since the successful development of taxanes in breast cancer treatment.[1] Their main target is microtubules, which are key components of the cell involved in structure/shape determination and in cell division.[2,3] Microtubules are linear polymers of α-/*β*-tubulin heterodimers constructed by a dynamic assembly/disassembly process. Microtubule dynamics play a pivotal role in mitotic spindle formation essential for the correct distribution of chromosomes.[3,4] Antimitotic agents are generally derived from natural products and fall into two classes: (i) microtubule disrupters, such as Colchicinium and Vinca alkaloids (e.g., colchicine[5] and vinblastine[6]), podophyllotoxin,[7] and combretastatin A-4,[8] which depolymerize tubulin; (ii) microtubule stabilizers including taxoids (e.g., paclitaxel and docetaxel[9]) along with the more recently discovered epothilones,[10] discodermolide,[11] and eleutherobin,[12] which block microtuble disassembly. These antitumoral substances interact with tubulin at distinct sites and thereby prevent the formation of functional microtubules, resulting in mitotic arrest and cell death.[13−15] Several of these antimitotic compounds have proved to be antiproliferative agents effective in the treatment of human malignancies.[1,2] In addition, other antimitotic drugs have been described that affect microtubule dynamics by another specific mechanism: alkylation of *β*-tubulin sulfhydryl groups, inducing inhibition of microtubule polymerization.[16] Such alkylating agents include iodoacetamide,[17] 1-fluoro-2,4-dinitrobenzene,[18] and 2,4-dichlorobenzylthiocyanate,[19] mainly used for demonstrating the regulatory role played by tubulin sulfhydryl groups in the formation of the mitotic spindle.[20−22] These compounds interact with several cysteine residues of tubulin, although 2,4-dichlorobenzylthiocyanate and a recently discovered sulfonamide derivative were found to be more selective.[19, 23]

We have developed new antiproliferative agents, *N*-(aryl)-*N′*-(2-chloro)ethylureas (CEUs), that prove to be microtubule disrupters, although they are derived primarily from DNA alkylators.[24,25] CEUs are highly selective: (i) they do not interact with DNA or glutathione but mainly with *β*-tubulin; (ii) they react specifically with the cysteine 239 of *β*-tubulin,[25] a critical sulfhydryl residue, the integrity of which is essential for microtubule assembly.[16,19,20,22] This property makes CEUs particularly attractive among the sulfhydryl-directed agents, which generally lack selectivity. Moreover, CEUs are nonmutagenic and maintain their cytotoxicity against various drug-resistant cells.[26] Additionally, compared with common antimitotic drugs, their relatively simple structures and low molecular weights may offer further advantages as easier preparation and potential oral administration. Finally, the profiles of CEUs appear to offer potential for their development as antitumor agents. In preliminary experiments, we identified the essential structural and functional requirements for cytotoxic activity, briefly summarized in Figure 1.[24] This study clearly optimized an important region of the CEUs that was assumed to fit into a hydrophobic cavity of the *β*-tubulin, i.e., the aryl substituent appended to the urea function, and also pointed out that the 2-chloroethyl moiety is a prerequisite for the alkylating activity and cytotoxicity. However, these CEUs still require a relatively long incubation with *β*-tubulin to disrupt microtubules completely.[25]

These findings prompted us to propose further improvements to the alkylating moiety, a crucial structural element of a CEU. We hypothesized that the introduction of an anchoring point in the structure of the alkylating moiety, appropriate to the spatial environment of the active site, would allow a closer and more prolonged contact between the drug and the sulfhydryl group of the cysteine residue of *β*-tubulin. The tubulin alkylation rate should therefore be increased and the

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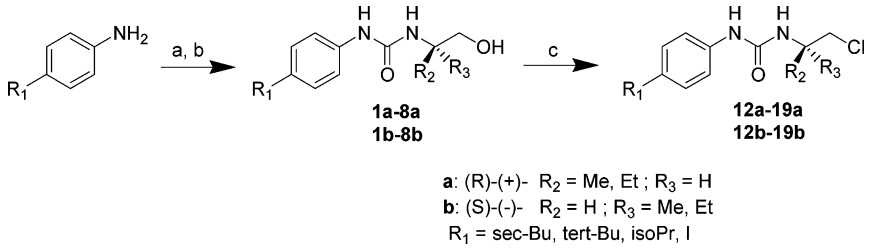
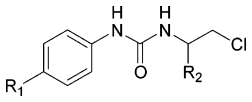
Scheme 1[a]



[a] Reagents: (a) Boc_2O, DMAP, CH_2Cl_2; (b) (*R*)- or (*S*)-$NH_2CR_2R_3CH_2OH$, CH_2Cl_2; (c) PPh_3, CCl_4/CH_2Cl_2.

Table 1. Cytotoxic Activity of *N*-(4-Alkyl)phenyl-*N*′-(1-alkyl-2-chloro)ethylureas



			IC$_{50}$ [a] (μM)						
compd	R_1	R_2	B16	HT29	K−562	L1210	MCF−7	T24	alk act.[b] (A_{540}, min^{-1})
23	*tert*-butyl	H	8.0	4.0	3.6	3.3	11	6.3	1.6
(*R*)-**12a**	*tert*-butyl	methyl	8.5	3.9	3.8	3.4	10	7.0	0.82
(*S*)-**12b**	*tert*-butyl	methyl	35	48	47	12	84	41	0.90
(*R*)-**13a**	*tert*-butyl	ethyl	27	25	28	17	52	28	0.66
(*S*)-**13b**	*tert*-butyl	ethyl	27	24	26	17	60	29	0.63
24	*sec*-butyl	H	5.0	1.8	2.0	2.0	5.2	3.7	1.5
(*R*)-**14a**	*sec*-butyl	methyl	4.2	1.9	2.0	1.7	4.8	3.4	0.76
(*S*)-**14b**	*sec*-butyl	methyl	33	39	34	18	63	35	0.76
(*R*)-**15a**	*sec*-butyl	ethyl	20	20	19	11	38	28	0.58
(*S*)-**15b**	*sec*-butyl	ethyl	27	28	27	12	51	38	0.57
25	isopropyl	H	4.7	2.3	1.9	2.0	4.7	3.6	1.4
(*R*)-**16a**	isopropyl	methyl	2.3	1.0	0.9	0.9	2.9	1.9	0.74
(*S*)-**16b**	isopropyl	methyl	46	65	33	28	>100	54	0.71
(*R*)-**17a**	isopropyl	ethyl	33	28	19	14	55	32	0.65
(*S*)-**17b**	isopropyl	ethyl	46	51	28	24	72	57	0.60
26	iodo	H	16	6.0	6.2	3.6	23	12	1.00
(*R*)-**18a**	iodo	methyl	2.1	1.3	1.0	0.8	3.2	1.8	0.45
(*S*)-**18b**	iodo	methyl	93	76	43	47	93	59	0.51
(*R*)-**19a**	iodo	ethyl	49	34	31	87	52	46	0.11
(*S*)-**19b**	iodo	ethyl	89	79	63	90	80	67	0.13

[a] Dose required to inhibit cell growth by 50%. Values are the mean of at least three independent determinations. [b] Alkylating activity determined by the NBP assay at 540 nm (see Experimental Section).

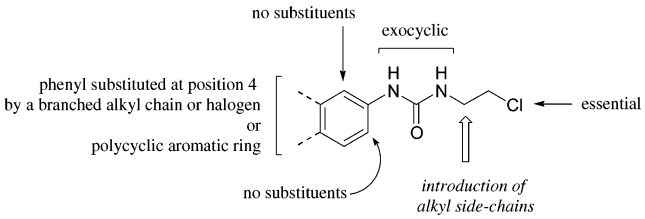


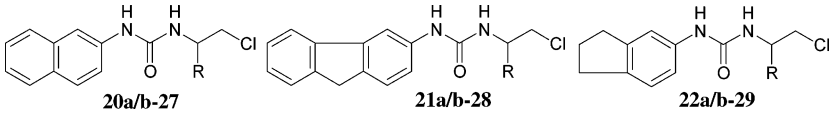
Figure 1. Essential results of structure−activity relationships of CEUs[24] and main modifications further explored in the present study (in italics).

cytotoxicity improved. We also envisaged studying the effects of the concomitant introduction of an asymmetric center in a CEU. This strategy formed the basis for the design and synthesis of a new series of ureas with various branched chloroalkyl groups as anchor chains, presented here. Since Bardos et al. previously showed that alkylating activity was adversely affected by alkyl substitution at the α-position of the chlorine atom,[27] the alkyl side chain was preferentially introduced at the *β*-position (Figure 1). In the present paper, we report the synthesis, the antiproliferative activity, and the *β*-tubulin alkylation kinetics of these second-generation CEUs and their implications for the improvement of tubulin-binding agents.

Chemistry

The *N*-[4-(alkyl/halo)phenyl]-*N*′-(1-alkyl-2-chloro)ethylureas (**12a**,**b**−**19a**,**b**) listed in Table 1 were prepared following the general synthetic pathway presented in Scheme 1. Introduction of the urea function was achieved using the 4-(dimethylamino)pyridine-catalyzed reaction of the relevant 4-alkyl/iodoaniline with di-*tert*-butyl dicarbonate in dichloromethane followed by the trapping of the in situ generated isocyanate with the appropriate (*R*)- or (*S*)-amino alcohol.[28] This procedure ensured a racemate-free synthesis of ureas under mild conditions and circumvented side reactions such as the formation of symmetrical disubstituted urea.[29] Subsequently, chlorination of the chiral 2-hydroxyethylureas (**1a**,**b**−**8a**,**b**) was accomplished by action of triphenylphosphine in a mixture of carbon tetrachloride and dichloromethane, at ambient temperature, affording the final enantiomerically pure (*R*)- or (*S*)-(1-alkyl-2-chloro)ethylurea derivatives (**12a**−**19a** or **12b**−**19b**) with good yield. The isomers exhibited identical spectral properties but opposite optical rotation; the (*R*)-form exhibiting dextro rotation was the (*R*)-(+)-enantiomer, and the antipodal was (*S*)-(−). The *N*-aryl-*N*′-2-chloroalkylureas **20a**,**b**, **21a**,**b**, and **22a**,**b** listed in Table 2 were synthesized

Table 2. Cytotoxic Activity of *N*-Aryl-*N′*-(1-alkyl-2-chloro)ethylureas



20a/b-27 **21a/b-28** **22a/b-29**

		IC_{50} [a] (μM)						
compd	R	B16	HT29	K-562	L1210	MCF-7	T24	alk act.[b] (A_{540}, min^{-1})
27	H	6.3	2.6	2.5	2.9	7.3		1.6
(*R*)-**20a**	methyl	4.5	2.3	2.3	1.7	6.0	4.0	0.83
(*S*)-**20b**	methyl	>100	86	63	20	>100	71	0.78
28	H		13	6.4	6.3			
(*R*)-**21a**	methyl	9.3	6.6	5.1	4.2	10	7.4	
(*S*)-**21b**	methyl	30	28	19	17	29	27	
29	H		10	5.5	5.0			1.4
(*R*)-**22a**	methyl	11	6.4	5.0	4.2	11	7.4	0.74
(*S*)-**22b**	methyl	>100	>100	91	41	>100	92	0.80

[a] Dose required to inhibit cell growth by 50%. Values are the mean of at least three independent determinations. [b] Alkylating activity determined by the NBP assay at 540 nm (see Experimental Section).

according to a similar procedure starting from 2-aminonaphthalene, 2-aminofluorene, and 5-aminoindane, respectively.

Results and Discussion

Antiproliferative Activity. The cytotoxicity of the new ureas (**12a**,**b**−**22a**,**b**) was determined in vitro against six cancer cell lines, namely, murine melanoma (B16), human colon adenocarcinoma (HT29), human chronic myelogenous leukemia (K-562), murine lymphocytotic leukemia (L1210), human estrogen-sensitive breast adenocarcinoma (MCF-7), and human bladder carcinoma (T24). The parent ureas from the first generation (**23**−**29**) were also tested as reference compounds. The results, expressed as IC_{50} values, are reported in Tables 1 and 2.

These data clearly showed an enantio dependence of the cytotoxic activity in the *N′*-(1-methyl-2-chloro)ethyl series (**12a**,**b**, **14a**,**b**, **16a**,**b**, **18a**,**b**, and **20a**,**b**−**22a**,**b**), the two isomers invariably differing in their IC_{50} values. The (*R*)-enantiomers (subseries **a**) exhibited a broad spectrum cytotoxicity with IC_{50} values ranging from 0.9 to 13 μM, whereas the corresponding (*S*)-isomers (subseries **b**) showed poor to nonsignificant activity ($IC_{50} \geq 20$ μM). However, this trend was uniformly abolished in the *N′*-(1-ethyl-2-chloro)ethyl series (**13a**,**b**, **15a**,**b**, **17a**,**b**, and **19a**,**b**). A complete loss of potency was observed regardless of the isomer. In addition, the disubstituted homologues bearing an *N′*-(1,1-dimethyl-2-chloro)ethyl group were also found to be inactive (data not shown). Consequently, the acceptable length and degree of ramification of the alkyl side chain were rapidly limited. Severe steric constraints are probably imposed on the alkylating moiety near the active site, and only one methyl group substituent at the β-position of the chlorine atom is tolerated on the chloroalkyl chain. This result is consistent with previous observations of the environment of the cysteine 239 residue in β-tubulin.[22]

Therefore, variations in substitution of the aryl part of CEUs were subsequently examined only in the (*R*)-*N′*-(1-methyl-2-chloro)ethyl subseries. Structurally diverse aryl groups selected on the basis of our previous SAR study,[24] including a phenyl ring substituted at the 4-position with a branched alkyl chain or halogen atom together with naphthyl, fluorenyl, and indanyl groups, were introduced (Tables 1 and 2). 4-Isopropylphenyl (**16a**) and 4-iodophenyl (**18a**) derivatives clearly emerged as the most active compounds of this series. 4-*tert*-Butylphenyl (**12a**), 4-*sec*-butylphenyl (**14a**), and naphthyl (**20a**) congeners were also highly potent, the last demonstrating that replacement of the phenyl ring by bulky polycyclic aromatic systems retained the cytotoxicity. Finally, fluorenyl (**21a**) and indanyl (**22a**) derivatives demonstrated weaker but significant cytotoxic activity.

Overall, compared with the first-generation ureas, the (*R*)-*N′*-(1-methyl-2-chloro)ethyl derivatives of this new series at least conserved (e.g., **12a** vs **23**) but also improved (e.g., **16a** vs **25**) the cytotoxic potency. A 6-fold increase in the activity was noted in particular for compound **18a** compared with **26**. Hence, the introduction of a branched (1-methyl-2-chloro)ethyl group as the alkylating moiety provides a highly stereoselective cytotoxicity-enhancing effect.

Effects on Microtubule Dynamics. From this series, **18a** (the most active) and **12a** (related to the most extensively studied CEU, i.e., **23**) were selected for further biological investigation in order to verify that the new derivatives were antimitotic agents affecting microtubule dynamics, like their first-generation counterparts. The cytotoxicity of **12a**,**b**, **18a**,**b**, **23**, and **26**, as references, was therefore evaluated against CHO (Chinese hamster ovary) cell lines expressing mutated β-tubulin and with differential sensitivity toward antimitotic agents according to their mechanism of action. The CHO-TAX 5-6 cell line is resistant to microtubule stabilizers (e.g., paclitaxel) and hypersensitive to microtubule disrupters (e.g., colchicine) compared with the parent CHO-10001 cells.[30] In contrast, the CHO-VV 3-2 cell line displays opposite properties.[31] As illustrated in Table 3, the (*S*)-isomers (**12b**, **18b**) were devoid of activity regardless of the cell line. Conversely, the (*R*)-isomers (**12a**, **18a**) displayed the same profile as the first-generation compounds **23** and **26**; i.e., their cytotoxicity increased up to 3-fold in the CHO-TAX 5-6 cells (e.g., 0.7 μM for **18a**) and decreased in the CHO-VV 3-2 cells (e.g., 4.7 μM for **18a**) compared with the activity against the parent CHO cells (e.g., 2.3 μM for **18a**). These results confirm that the (*R*)-derivatives of this new series are antimicrotubule agents acting by microtubule disruption.

Table 3. Cytotoxic Activity of *N*-(4-alkyl)Phenyl-*N′*-(1-alkyl-2-chloro)ethylureas on CHO Cell Lines Expressing Mutated Tubulin

		IC_{50} [a] (μM)					
cell lines	nature	**23**	**12a**	**12b**	**26**	**18a**	**18b**
CHO-10001	wild type	7.6	8.1	56	15	2.3	58
CHO-TAX 5-6	paclitaxel-resistant	3.1	3.6	53	6.1	0.7	54
CHO-VV 3-2	colchicine-resistant	13	15	53	23	4.7	51

[a] Dose required to inhibit cell growth by 50%. Values are the mean of at least three independent determinations.

Alkylating Activity. As previously reported, CEUs are weak alkylating agents.[24] The alkylating potential of the new ureas was therefore evaluated with the 4-(*p*-nitrobenzyl)pyridine (NBP) alkylation assay (Tables 1 and 2). This test did not discriminate between the activity of the (*R*)- and (*S*)-enantiomers. However, *N′*-(1-methyl-2-chloro)ethyl derivatives (**12**, **14**, **16**, **18**) generally displayed slightly higher reactivity in the alkylation compared with their *N′*-(1-ethyl-2-chloro)-ethyl homologues (**13**, **15**, **17**, **19**). This tendency was consistent with the cytotoxicity data. In contrast, all the ureas from the new series demonstrated lower alkylating ability than their first-generation analogues (**23**, **26**), whereas they were equally or more cytotoxic. As previously reported by Bardos et al.,[27] the correlation between alkylating activity and cytotoxicty is sometimes poorly satisfactory. Accordingly, this assay is limited to the determination of the intrinsic chemical reactivity (i.e., alkylation of the heterocyclic nitrogen of NBP) and cannot take into account spatial and environmental constraints in the biological active site, which again appears to be determining in this series of ureas.

***β*-Tubulin Alkylation.** Additional biological experiments were then performed to verify that the cytotoxicity of these new derivatives resulted from *β*-tubulin alkylation and to study the kinetics of this process. After incubation with the drug (**12a**,**b**, **18a**,**b**, **23**, and **26** as references), total cellular proteins were separated by SDS−PAGE and *β*-tubulin was revealed by Western blot using an anti-*β*-tubulin antibody. As shown in Figure 2A, *β*-tubulin and alkylated *β*-tubulin appeared as two immunoreactive bands, the latter migrating more quickly.[25] Ureas **12b** and **18b** from the (*S*)-*N′*-(1-methyl-2-chloro)ethyl series, devoid of cytotoxicity, did not display such a pattern, and the second band corresponding to the alkylated *β*-tubulin was not observed. A comparable result was obtained for both (*S*)- and (*R*)-*N′*-(1-ethyl-2-chloro)ethyl derivatives (**13a**,**b**−**19a**,**b**) (data not shown). In contrast, the cytotoxic ureas **12a** and **18a** induced the formation of the second band associated with alkylated *β*-tubulin in a time-dependent process. Moreover, Figure 2B clearly shows that both the kinetic profile and the rate of alkylated *β*-tubulin formation varied greatly with the series of ureas. The time course of the *β*-tubulin alkylation by 4-*tert*-butylphenyl derivatives **12a** and **23** was characterized by an increase starting at 12 h until a steady state was reached at 40 h, whereas a constant progression was observed over 48 h for 4-iodo derivatives **18a** and **26**. The new derivatives **12a** and **18a** displayed a faster alkylation of the *β*-tubulin compared with those of the first generation. Accordingly, the detection of the second band occurred earlier for the urea **18a** (12 h) than for **26** (32 h). Furthermore, the maximum extent of alkylation rose to 90% and 60% of the total *β*-tubulin at 48 h for compounds **18a** and **12a** vs 24% and 50% for compounds **26** and **23**. The reaction was therefore much more complete with the new ureas. All these data show that the *β*-tubulin alkylation can be accelerated and enhanced by the second-generation ureas. As shown for compound **18a**, the effectiveness of CEUs as antimitotic and antiproliferative agents appears to be primarily related to the rapidity of their binding to *β*-tubulin.

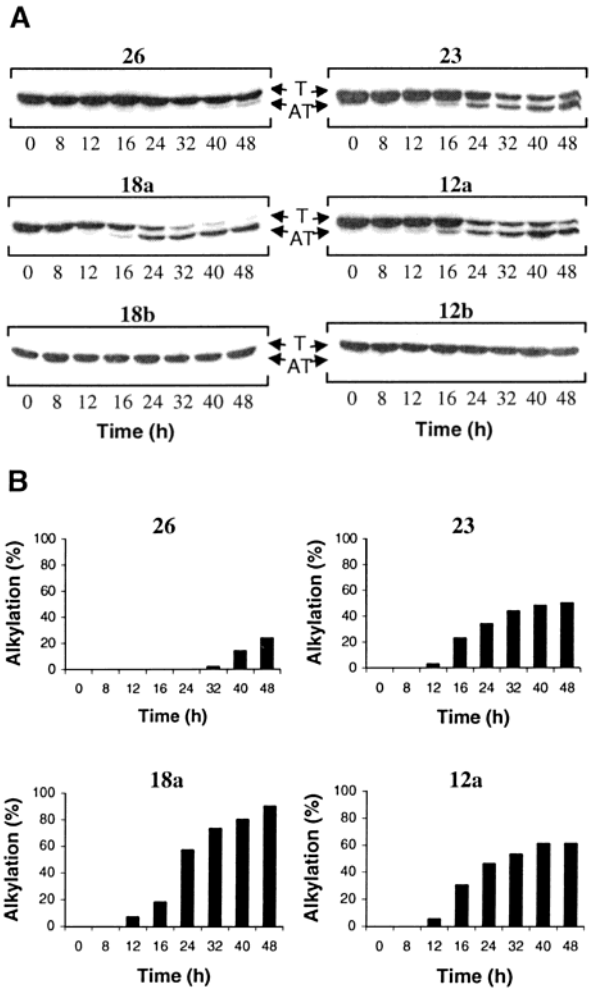


Figure 2. Kinetics of *β*-tubulin alkylation induced by the ureas **12a**,**b**, **18a**,**b**, **23**, and **26** as references. MDA-MB-231 cells were incubated with each drug (30 μM) at 37 °C for the indicated time and analyzed by Western blot with *β*-tubulin antibody. The gel picture is shown at the top (A), and the related quantitative results are plotted at the bottom (B). T and AT refer to tubulin and alkylated tubulin, respectively.

Conclusion

The present study confirms that the SARs for discrete regions of the urea's core structure are not independent and mainly emphasizes the fundamental role played by the alkylating moiety in the cytotoxic activity. The presence of a urea function borne by (i) a phenyl ring substituted at the 4-position by a branched alkyl chain or a halogen atom or (ii) a polycyclic aryl group was still essential to maintain the cytotoxicity. Concerning the alkylating moiety, our initial work established that linear elongation of the chloroalkyl chain greatly diminished the cytotoxicity and that a 2-chloroethyl group

was largely optimal.[24] However, our present results demonstrate that the introduction of a branched chloroalkyl chain as the alkylating moiety is fully tolerated, although limited to a (1-methyl-2-chloro)ethyl group, and lead to ureas with enhanced cytotoxicity (e.g., **16a** and **18a**). Because of an induced asymmetric center in the molecule, an enantio-dependent activity was noted. The cytotoxicity resided exclusively in the (*R*)-isomers. Further investigations carried out with **12a** and **18a** showed that they acted through β-tubulin alkylation and displayed a microtubule disrupter profile. Moreover, when compared with the first-generation analogues, these novel ureas showed a faster and more extensive alkylation of the β-tubulin that can be correlated, for **18a**, to its enhanced cytotoxicity, despite a lower intrinsic alkylating activity.

All these findings indicate that (i) the active site of the β-tubulin is stereoselective and (ii) the alkylation rate is not dependent only on the reactivity toward sulfhydryl groups. Spatial environment and steric factors are therefore also strongly determining for access to the active site and interaction with the cysteine residue. Since enantiomers have identical physicochemical properties, the divergence in their cytotoxicity should be determined by the difference in their orientation in the active site of the β-tubulin. It is speculated that the side chain of the branched chloroethyl group of the (*R*)-isomer interacts with a narrow hydrophobic region in the active site,[22,25] accommodating only a methyl group. This adjustment of the side chain would allow a sterically favored orientation of the alkylating moiety, promoting the approach of the chlorine atom toward the sulfhydryl group of the cysteine 239 residue and thereby the β-tubulin alkylation.

Finally, we have established that chemical modifications at a given site of the CEU's alkylating chain can lead to accelerated β-tubulin alkylation and thereby an improved antiproliferative potency. From these second-generation ureas, **18a** showing a particular cytotoxicity-enhancing effect was selected for further preclinical evaluation as a potential anticancer drug.

Experimental Section

Chemistry. Chemical Methods. Proton NMR spectra were recorded on a Brucker AM-400 spectrometer (Bruker, Germany). Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard. IR spectra were recorded on an Unicam spectrometer. Uncorrected melting points were determined on an Electrothermal melting point apparatus. Optical rotations were measured on a Jasco DIP-360 polarimeter. Elemental analyses were performed by the Microanalytical Laboratory of the Chemistry Department, University of Montréal (Canada), and were within $\pm$0.4% of theory for the formulas given unless otherwise indicated.

All reactions were conducted under a rigorously dried argon atmosphere, using oven-dried glassware. Chemicals were supplied by Aldrich Chemical Co. (Milwaukee, WI). Dichloromethane was stored over 4 Å molecular sieves. Liquid flash chromatography was performed on silica gel 60 A (American Chemicals Ltd., Montréal, Canada), using the indicated solvent mixture expressed as volume/volume ratios.

The parent CEUs (**23**−**26**) were synthesized according to the general procedure previously described.[24]

General Procedure for *N*-(4-Alkylphenyl)-*N*′-(1-alkyl-2-hydroxy)ethylureas. Preparation of 1a,b−11a,b. To a stirred solution of di-*tert*-butyl dicarbonate (3.9 mmol) and 4-(dimethylamino)pyridine (0.4 mmol) in anhydrous dichloromethane (20 mL) was added dropwise the relevant aniline (3.7 mmol). The reaction mixture was stirred for 30 min at ambient temperature, and the required (*R*)- or (*S*)-amino alcohol was added dropwise. The mixture was then stirred overnight at ambient temperature. After evaporation of the solvent, the crude product was purified by flash chromatography on silica gel (dichloromethane/ethyl acetate, 20/80) to afford the hydroxyalkylurea as a colorless oil or solid. The latter can be recrystallized from ethanol and ether.

(*R*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-methyl-2-hydroxy)-ethylurea (1a): yield, 53%; mp 89−91°C; $[\alpha]^{25}_{D}$ +14.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.13 (d, J = 6.7 Hz, 3H, NCHCH_3), 1.28 (s, 9H,CH_3), 3.13 (broad s, 1H, OH, exchanges with D_2O), 3.40−3.75 (m, 2H, CH_2OH), 3.90−3.94 (m, 1H, CH), 4.95 (broad s, 1H, N*H*CH, exchanges with D_2O), 6.68 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.15−7.34 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (1b): yield, 72%; mp 89−91°C; $[\alpha]^{25}_{D}$ −14.6 (*c* 0.70, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.11 (d, J = 6.7 Hz, 3H, NCHCH_3), 1.26 (s, 9H, CH_3), 3.43−3.75 (m, 2H, CH_2OH), 3.91−3.94 (m, 1H, CH), 5.22 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.03 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.15−7.27 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (2a): yield, 75%; mp 98−100 °C; $[\alpha]^{25}_{D}$ +25.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.17 (s, 9H, CH_3), 1.21−1.48 (m, 2H, NCHCH_2-CH_3), 3.31−3.62 (m, 3H, NCH, CH_2OH), 4.28 (s, 1H, OH, exchanges with D_2O), 5.77 (d, J = 7.9 Hz, 1H, N*H*CH, exchanges with D_2O), 7.09−7.17 (m, 4H, Ar−H), 7.87 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (2b): yield, 86%; mp 100−102 °C; $[\alpha]^{25}_{D}$ −27.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.27 (s, 9H, CH_3), 1.34−1.55 (m, 2H, NCHCH_2-CH_3), 3.40−3.70 (m, 4H, NCH, CH_2OH, OH, exchanges with D_2O), 5.13 (d, J = 7.4 Hz, 1H, N*H*CH, exchanges with D_2O), 6.93 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.15−7.27 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (3a): yield, 70%; mp 84−86 °C; $[\alpha]^{25}_{D}$ +13.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77 (t, J = 7.4 Hz, 3H, CH_3CH$_2$), 1.06 (d, J = 6.7 Hz, 3H, NCHCH_3), 1.15 (d, J = 6.9 Hz, 3H, CHCH_3), 1.46−1.56 (m, 4H, CH_2CH$_3$), 2.46−2.53 (m, 1H, CH), 3.40−3.45, 3.55−3.60 (2 m, 2H, CH_2OH), 3.85−3.90 (m, 1H, NCH), 3.99 (broad s, 1H, OH, exchanges with D_2O), 5.48 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.01−7.16 (m, 4H, Ar−H), 7.39 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (3b): yield, 70%; mp 84−86 °C; $[\alpha]^{25}_{D}$ −13.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.75 (t, J = 7.5 Hz, 3H, CH_3CH$_2$), 1.03 (d, J = 6.0 Hz, 3H, NCHCH_3), 1.13 (d, J = 7.0 Hz, 3H, CHCH_3), 1.46−1.53 (m, 4H, CH_2CH$_3$), 2.40−2.52 (m, 1H, CH), 3.35−3.43, 3.52−3.57 (2 m, 2H, CH_2OH), 3.82−3.86 (m, 1H, NCH), 4.46 (broad s, 1H, OH, exchanges with D_2O), 5.77 (broad s, 1H, N*H*CH, exchanges with D_2O), 6.97−7.17 (m, 4H, Ar−H), 7.73 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (4a): yield, 60%; $[\alpha]^{25}_{D}$ +24.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76 (t, J = 7.3 Hz, 3H, NCHCH$_2$CH_3), 0.86 (t, J = 7.4 Hz, 3H, CH$_2$CH_3), 1.15 (d, J = 6.8 Hz, 3H, CHCH_3), 1.29−1.37 (m, 4H, NCHCH_2CH$_3$), 1.46−1.55 (m, 2H, CH_2CH$_3$), 2.44−2.51 (m, 1H, CH), 3.46−3.65 (m, 3H, CH_2OH, NCH), 4.18 (broad s, 1H, OH, exchanges with D_2O), 5.61 (d, J = 7.6 Hz, 1H, N*H*CH, exchanges with D_2O), 6.99−7.25 (m, 4H, Ar−H), 7.62 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (4b): yield, 76%; $[\alpha]^{25}_{D}$ −23.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 7.3 Hz, 3H, NCHCH$_2$CH_3), 0.86 (t, J = 7.4 Hz, 3H, CH$_2$CH_3), 1.22 (d, J = 6.8 Hz, 3H, CHCH_3), 1.29−

1.37 (m, 4H, NCHCH_2CH$_3$), 1.46−1.55 (m, 2H, CH_2CH$_3$), 2.54−2.65 (m, 1H, CH), 3.65−3.75 (m, 3H, CH_2OH, NCH), 4.18 (broad s, 1H, OH, exchanges with D_2O), 5.41 (d, J = 7.6 Hz, 1H, N*H*CH, exchanges with D_2O), 7.13−7.22 (m, 4H, Ar−H), 7.40 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (5a): yield, 50%; mp 89−91 °C; $[\alpha]^{25}_D$ +12.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, J = 6.7 Hz, 3H, NCHCH_3), 1.22 (d, J = 6.7 Hz, 6H, CH$_3$), 2.84−2.89 (m, 1H, NC*H*CH$_3$), 3.13 (broad s, 1H, OH, exchanges with D_2O), 3.50−3.53, 3.64−3.68 (2 m, 2H, CH_2OH), 3.95−3.98 (m, 1H, NCH), 4.90 (broad s, 1H, N*H*CH, exchanges with D_2O), 6.52 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.13−7.16 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (5b): yield, 40%; mp 89−91 °C; $[\alpha]^{25}_D$ −13.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.12 (d, J = 6.7 Hz, 3H, NCHCH_3), 1.21 (d, J = 6.7 Hz, 6H, CH$_3$), 2.81−2.90 (m, 1H, NC*H*CH$_3$), 3.30 (broad s, 1H, OH, exchanges with D_2O), 3.38−3.40, 3.60−3.64 (2 m, 2H, CH_2OH), 3.93−3.98 (m, 1H, NCH), 5.07 (broad s, 1H, N*H*CH, exchanges with D_2O), 6.81 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.12−7.16 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (6a): yield, 60%; $[\alpha]^{25}_D$ +22.5 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.21 (d, J = 6.7 Hz, 6H, CH$_3$), 1.33−1.60 (m, 2H, NCHCH_2CH$_3$), 2.18 (broad s, 1H, OH, exchanges with D_2O), 2.80−2.90 (m, 1H, CH), 3.45−3.50 (m, 1H, NCH), 3.63−3.71 (m, 2H, CH_2OH), 6.60 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.05−7.17 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (6b): yield, 79%;$[\alpha]^{25}_D$ −21.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.21 (d, J = 6.7 Hz, 6H, CH$_3$), 1.36−1.56 (m, 2H, NCHCH_2CH$_3$), 2.21 (broad s, 1H, OH, exchanges with D_2O), 2.81−2.90 (m, 1H, CH), 3.48−3.54 (m, 1H, NCH), 3.66−3.71 (m, 2H, CH_2OH), 6.90 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.13−7.20 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(4-Iodophenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (7a): yield, 30%; mp 106−108 °C; $[\alpha]^{25}_D$ +21.0 (*c* 0.10, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.15 (d, J = 6.9 Hz, 3H, NCHCH_3), 3.29−3.40 (m, 2H, CH_2OH), 4.00−4.10 (m, 1H, NC*H*CH$_3$), 6.03 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.18−7.50 (m, 4H, Ar−H), 8.57 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1635 (C=O) cm^{-1}.

(*S*)-*N*-(4-Iodophenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (7b): yield, 60%; mp 105−107 °C; $[\alpha]^{25}_D$ −18.2 (*c* 0.20, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.15 (d, J = 7.0 Hz, 3H, NCHCH_3), 3.11−3.35 (m, 2H, CH_2OH), 3.66−3.90 (m, 1H, NC*H*CH$_3$), 6.31 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.21−7.52 (m, 4H, Ar−H), 8.82 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1635 (C=O) cm^{-1}.

(*R*)-*N*-(4-Iodophenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (8a): yield, 55%; mp 88−90 °C; $[\alpha]^{25}_D$ −27.3 (*c* 0.05, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.88 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.30−1.49 (m, 2H, NCHCH_2CH$_3$), 3.30−3.50 (m, 3H, CHCH_2-OH), 4.63 (broad s, 1H, OH, exchanges with D_2O), 6.30 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.20−7.50 (m, 4H, Ar−H), 8.03 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(4-Iodophenyl-*N*′-(1-ethyl-2-hydroxy)ethylurea (8b): yield, 67%; mp 84−86 °C; $[\alpha]^{25}_D$ −28.0 (*c* 0.05, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.31−1.51 (m, 2H, NCHCH_2CH$_3$), 3.29−3.49 (m, 3H, CHCH_2-OH), 4.71 (broad s, 1H, OH, exchanges with D_2O), 6.00 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.20−7.52 (m, 4H, Ar−H), 8.56 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}.

(*R*)-*N*-(2-Naphthyl-*N*′-(1-methyl-2-hydroxy)ethylurea (9a): yield, 35%; mp 108−110 °C; $[\alpha]^{25}_D$ +13.6 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.21 (d, J = 7.0 Hz, 3H, NCHCH_3), 3.55−3.75 (m, 2H, CH_2OH), 3.90−4.03 (m, 1H, NC*H*CH$_3$), 7.30−7.95 (m, 7H, Ar−H), 8.20 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3310 (NH), 1630 (C=O) cm^{-1}.

(*S*)-*N*-(2-Naphthyl-*N*′-(1-methyl-2-hydroxy)ethylurea (9b): yield, 44%; mp 107−109 °C; $[\alpha]^{25}_D$ −15.0 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.22 (d, J = 7.0 Hz, 3H, NCHCH_3), 3.54−3.75 (m, 2H, CH_2OH), 3.88−4.02 (m, 1H, NC*H*CH$_3$), 7.25−7.92 (m, 7H, Ar−H), 8.23 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1630 (C=O) cm^{-1}.

(*R*)-*N*-(2-Fluorenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (10a): yield, 43%; mp 132−134 °C; $[\alpha]^{25}_D$ +23.3 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.20 (d, J = 7.0 Hz, 3H, NCHCH_3), 3.50−3.85 (m, 3H, CH_2OH, CH$_2$ fluorenyl), 3.90−4.05 (m, 1H, NC*H*CH$_3$), 7.15−7.65 (m, 7H, Ar−H); IR (KBr) ν 3300 (NH), 1630 (C=O) cm^{-1}.

(*S*)-*N*-(2-Fluorenyl-*N*′-(1-methyl-2-hydroxy)ethylurea (10b): yield, 54%; mp 132−134 °C; $[\alpha]^{25}_D$ −22.5 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.23 (d, J = 7.0 Hz, 3H, NCHCH_3), 3.50−3.85 (m, 3H, CH_2OH, CH$_2$ fluorenyl), 3.94−4.03 (m, 1H, NC*H*CH$_3$), 7.12−7.64 (m, 7H, Ar−H); IR (KBr) ν 3300 (NH), 1630 (C=O) cm^{-1}.

(*R*)-*N*-(5-Indanyl-*N*′-(1-methyl-2-hydroxy)ethylurea (11a): yield, 54%; mp 98−100 °C; $[\alpha]^{25}_D$ +19.8 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.15 (d, J = 7.0 Hz, 3H, NCHCH_3), 2.00−2.15, 2.80−2.90 (2m, 6H, CH$_2$ indanyl), 3.40−3.68 (m, 2H, CH_2-OH), 3.83−3.98 (m, 1H, NC*H*CH$_3$), 6.90−7.15 (m, 7H, Ar−H); IR (KBr) ν 3300 (NH), 1640 (C=O) cm^{-1}.

(*S*)-*N*-(5-Indanyl-*N*′-(1-methyl-2-hydroxy)ethylurea (11b): yield, 56%; mp 99−101°C; $[\alpha]^{25}_D$ −19.5 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.23 (d, J = 7.0 Hz, 3H, NCHCH_3), 2.00−2.15, 2.80−2.90 (2m, 6H, CH$_2$ indanyl), 3.50−3.79 (m, 2H, CH_2-OH), 3.88−4.00 (m, 1H, NC*H*CH$_3$), 6.90−7.18 (m, 7H, Ar−H); IR (KBr) ν 3310 (NH), 1640 (C=O) cm^{-1}.

General Procedure for *N*-(4-Alkylphenyl)-*N*′-(1-alkyl-2-chloro)ethylureas. Preparation of 12a,b−22a,b. A solution of the relevant hydroxyalkylurea **1a**,**b**−**11a**,**b** (2.4 mmol) and triphenylphosphine (3.7 mmol) in a mixture of dichloromethane (20 mL) and carbon tetrachloride (6 mL) was stirred overnight at room temperature. The solvents were evaporated under reduced pressure, and the crude product was purified by flash chromatography on silica gel (ethyl ether/petroleum ether, 50/50) to give the chloroethylurea as a colorless oil or white solid. The latter can be recrystallized from ethanol and ether.

(*R*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-methyl-2-chloro)ethylurea (12a): yield, 80%; mp 114−116 °C; $[\alpha]^{25}_D$ +11.8 (*c* 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 6.7 Hz, 3H, CH$_3$), 1.28 (s, 9H, CH$_3$), 3.54−3.75 (2 m, 2H, CH$_2$Cl), 4.27−4.30 (m, 1H, CH), 5.14 (broad s, 1H, N*H*CH$_2$, exchanges with D_2O), 6.65 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.17−7.33 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{14}H_{21}ClN_2O$) C, H, N.

(*S*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-methyl-2-chloro)ethylurea (12b): yield, 86%; mp 114−116 °C; $[\alpha]^{25}_D$ −15.1 (*c* 1.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.17 (d, J = 6.7 Hz, 3H, CH$_3$), 1.25 (s, 9H, CH$_3$), 3.52−3.58 (2 m, 2H, CH$_2$Cl), 4.15−4.20 (m, 1H, CH), 5.92 (broad s, 1H, N*H*CH$_2$, exchanges with D_2O), 7.14−7.25 (m, 4H, Ar−H), 7.69 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{14}H_{21}ClN_2O$) C, H, N.

(*R*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-ethyl-2-chloro)ethylurea (13a): yield, 65%; $[\alpha]^{25}_D$ +27.6 (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.4 Hz, 3H, NCHCH$_2$CH_3), 1.24 (s, 9H, CH$_3$), 1.31−1.63 (m, 2H, NCHCH_2CH$_3$), 3.53−3.66 (m, 2H, CH$_2$Cl), 3.95−3.98 (m, 1H, NCH), 5.66 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.15−7.25 (m, 4H, Ar−H), 7.48 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{15}H_{23}ClN_2O$) C, H, N.

(*S*)-*N*-(4-*tert*-Butyl)phenyl-*N*′-(1-ethyl-2-chloro)ethylurea (13b): yield, 86%; $[\alpha]^{25}_D$ −26.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H, NCHCH$_2$CH_3), 1.23 (s, 9H, CH$_3$), 1.36−1.60 (m, 2H, NCHCH_2CH$_3$), 3.50−3.61 (m, 2H, CH$_2$Cl), 3.95−3.98 (m, 1H, NCH), 5.88 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.15−7.24 (m, 4H, Ar−H), 7.74 (broad

s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{15}H_{23}ClN_2O$) C, H, N.

(*R*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-methyl-2-chloro)ethylurea (14a): yield, 88%; mp 127−129 °C; $[\alpha]^{25}_D$ +13.3 (*c* 1.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.80 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 1.20 (d, *J* = 6.8 Hz, 3H, NCHC*H*$_3$), 1.24 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$), 1.51−1.61 (m, 4H, C*H*$_2$CH$_3$), 2.53−2.60 (m, 1H, CH), 3.55−3.60, 3.72−3.78 (2 m, 2H, CH$_2$Cl), 4.10−4.12 (m, 1H, NCH), 4.84 (broad s, 1H, N*H*CH$_2$, exchanges with D_2O), 6.21 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.11−7.19 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{14}H_{21}ClN_2O$) C, H, N.

(*S*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-methyl-2-chloro)ethylurea (14b): yield, 81%; mp 124−126 °C; $[\alpha]^{25}_D$ −16.3 (*c* 2.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.80 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 1.17−1.25 (2d, *J* = 6.7, 6.8 Hz, 6H, CHC*H*$_3$, NCHC*H*$_3$), 1.51−1.61 (m, 2H, C*H*$_2$CH$_3$), 2.53−2.60 (m, 1H, CH), 3.46−3.60, 3.73−3.78 (2 m, 2H, CH$_2$Cl), 4.27−4.29 (m, 1H, NCH), 5.96 (broad s, 1H, N*H*CH$_2$, exchanges with D_2O), 6.21 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.12−7.19 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{14}H_{21}ClN_2O$) C, H, N.

(*R*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-ethyl-2-chloro)ethylurea (15a): yield, 50%; mp 135−137 °C; $[\alpha]^{25}_D$ +30.8 (*c* 1.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.78 (t, *J* = 7.3 Hz, 3H, NCHCH$_2$C*H*$_3$), 0.90 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 1.18 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$), 1.47−1.62 (m, 4H, C*H*$_2$CH$_3$, NCHC*H*$_2$CH$_3$), 2.48−2.56 (m, 1H, C*H*CH$_3$), 3.56−3.71 (m, 2H, CH$_2$Cl), 4.00−4.06 (m, 1H, NCH), 5.45 (d, *J* = 8.5 Hz, 1H, N*H*CH, exchanges with D_2O), 7.08−7.25 (m, 4H, Ar−H), 7.65 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{15}H_{23}ClN_2O$) C, H, N.

(*S*)-*N*-(4-*sec*-Butyl)phenyl-*N*′-(1-ethyl-2-chloro)ethylurea (15b): yield, 67%; mp 134−136 °C; $[\alpha]^{25}_D$ −31.5 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.82 (t, *J* = 7.3 Hz, 3H, NCHCH$_2$C*H*$_3$), 0.95 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 1.21 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$), 1.52−1.68 (m, 4H, C*H*$_2$CH$_3$, NCHC*H*$_2$CH$_3$), 2.50−2.60 (m, 1H, C*H*CH$_3$), 3.73−3.78 (m, 2H, CH$_2$Cl), 4.05−4.09 (m, 1H, NCH), 4.90 (d, *J* = 8.5 Hz, 1H, N*H*CH, exchanges with D_2O), 7.11−7.20 (m, 4H, Ar−H), 7.32 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{15}H_{23}ClN_2O$) C, H, N.

(*R*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-methyl-2-chloro)ethylurea (16a): yield, 70%; mp 125−127 °C; $[\alpha]^{25}_D$ +15.7 (*c* 1.40, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.23 (d, *J* = 6.7 Hz, 3H, NCHC*H*$_3$), 1.24 (d, *J* = 6.6 Hz, 6H, CH$_3$), 2.85−2.91 (m, 1H, C*H*CH$_3$), 3.56−3.60, 3.73−3.78 (2 m, 2H, CH$_2$Cl), 4.28−4.30 (m, 1H, NCH), 4.84 (broad s, 1H, N*H*CH$_2$, exchanges with D_2O), 6.20 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.16−7.18 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{13}H_{19}ClN_2O$) C, H, N.

(*S*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-methyl-2-chloro)ethylurea (16b): yield, 82%; mp 128−130 °C; $[\alpha]^{25}_D$ −12.6 (*c* 0.90, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.23 (d, *J* = 6.7 Hz, 3H, NCHC*H*$_3$), 1.24 (d, *J* = 6.6 Hz, 6H, CH$_3$), 2.83−2.92 (m, 1H, C*H*CH$_3$), 3.55−3.60, 3.72−3.77 (2 m, 2H, CH$_2$Cl), 4.29−4.31 (m, 1H, NCH), 4.90 (broad s, 1H, N*H*CH$_2$, exchanges with D_2O), 6.29 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.15−7.18 (m, 4H, Ar−H); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{13}H_{19}ClN_2O$) C, H, N.

(*R*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-ethyl-2-chloro)ethylurea (17a): yield, 60%; mp 145−147 °C; $[\alpha]^{25}_D$ +33.5 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86 (t, *J* = 7.4 Hz, 3H, NCHCH$_2$C*H*$_3$), 1.14 (d, *J* = 6.7 Hz, 6H, CH$_3$), 1.48−1.54 (m, 2H, NCHC*H*$_2$CH$_3$), 2.76−2.80 (m, 1H, CH), 3.56−3.63 (2 m, 2H, CH$_2$Cl), 3.95−3.98 (m, 1H, NC*H*CH$_2$CH$_3$), 5.26 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.08−7.14 (m, 4H, Ar−H), 6.92 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{14}H_{21}ClN_2O$) C, H, N.

(*S*)-*N*-(4-Isopropyl)phenyl-*N*′-(1-ethyl-2-chloro)ethylurea (17b): yield, 60%; mp 146−148 °C; $[\alpha]^{25}_D$ (*c* 1.00, $CHCl_3$) −36.6; ^1H NMR ($CDCl_3$) δ 0.94 (t, *J* = 7.4 Hz, 3H, NCHCH$_2$C*H*$_3$), 1.23 (d, *J* = 6.7 Hz, 6H, CH$_3$), 1.41−1.61 (m, 2H, NCHC*H*$_2$CH$_3$), 2.83−2.92 (m, 1H, CH isopropyl), 3.62−3.77 (2 m, 2H, CH$_2$Cl), 4.03−4.10 (m, 1H, NC*H*CH$_2$CH$_3$), 4.84 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.18−7.24 (m, 4H, Ar−H), 6.25 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{14}H_{21}ClN_2O$) C, H, N.

(*R*)-*N*-(4-Iodophenyl-*N*′-(1-methyl-2-chloro)ethylurea (18a): yield, 40%; mp 118−120°C; $[\alpha]^{25}_D$ +22.5 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.15 (d, *J* = 6.9 Hz, 3H, NCHC*H*$_3$), 3.51−3.68 (m, 2H, C*H*$_2$Cl), 3.96−4.03 (m, 1H, NC*H*CH$_3$), 6.29 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.21−7.54 (m, 4H, Ar−H), 8.62 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1635 (C=O) cm^{-1}. Anal. ($C_{10}H_{12}ClIN_2O$) C, H, N.

(*S*)-*N*-(4-Iodophenyl-*N*′-(1-methyl-2-chloro)ethylurea (18b): yield, 20%; mp 118−120 °C; $[\alpha]^{25}_D$ −25.8 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.15 (d, *J* = 6.8 Hz, 3H, NCHC*H*$_3$, 3.31−3.67 (m, 2H, C*H*$_2$Cl), 3.96−4.03 (m, 1H, NC*H*CH$_3$), 6.25 (broad s, 1H, N*H*CH, exchanges with D_2O), 7.21−7.54 (m, 4H, Ar−H), 8.60 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1635 (C=O) cm^{-1}. Anal. ($C_{10}H_{12}ClIN_2O$) C, H, N.

(*R*)-*N*-(4-Iodophenyl-*N*′-(1-ethyl-2-chloro)ethylurea (19a): yield, 15%; mp 144−146 °C; $[\alpha]^{25}_D$ +23.8 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 0.88 (t, *J* = 7.3 Hz, 3H, NCHCH$_2$C*H*$_3$), 1.49−1.60 (m, 2H, NCHC*H*$_2$CH$_3$), 3.63−3.78 (m, 3H, CHCH$_2$-Cl), 5.80 (d, *J* = 8.1 Hz, 1H, N*H*CH, exchanges with D_2O), 7.18−7.42 (m, 4H, Ar−H), 8.54 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3300 (NH), 1635 (C=O) cm^{-1}. Anal. ($C_{11}H_{14}ClIN_2O$) C, H, N.

(*S*)-*N*-(4-Iodophenyl-*N*′-(1-ethyl-2-chloro)ethylurea (19b): yield, 20%; mp 144−146 °C; $[\alpha]^{25}_D$ +23.3 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 0.89 (t, *J* = 7.4 Hz, 3H, NCHCH$_2$C*H*$_3$), 1.41−1.64 (m, 2H, NCHC*H*$_2$CH$_3$), 3.68−3.81 (m, 3H, CHCH$_2$-Cl), 6.20 (d, *J* = 8.1 Hz, 1H, N*H*CH, exchanges with D_2O), 7.22−7.55 (m, 4H, Ar−H), 8.62 (broad s, 1H, ArN*H*, exchanges with D_2O); IR (KBr) ν 3320 (NH), 1635 (C=O) cm^{-1}. Anal. ($C_{11}H_{14}ClIN_2O$) C, H, N.

(*R*)-*N*-(2-Naphthyl-*N*′-(1-methyl-2-chloro)ethylurea (20a): yield, 52%; mp 136−138 °C; $[\alpha]^{25}_D$ +29.2 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.23 (d, *J* = 6.6 Hz, 3H, NCHC*H*$_3$), 3.54−3.80 (m, 2H, C*H*$_2$Cl), 4.31−4.35 (m, 1H, NC*H*CH$_3$), 7.25−7.83 (m, 7H, Ar−H); IR (KBr) ν 3320 (NH), 1630 (C=O) cm^{-1}. Anal. ($C_{14}H_{15}ClN_2O$) C, H, N.

(*S*)-*N*-(2-Naphthyl-*N*′-(1-methyl-2-chloro)ethylurea (20b): yield, 59%; mp 138−140 °C; $[\alpha]^{25}_D$ −32.5 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.23 (d, *J* = 7.0 Hz, 3H, NCHC*H*$_3$), 3.54−3.78 (m, 2H, C*H*$_2$Cl), 4.31−4.34 (m, 1H, NC*H*CH$_3$), 7.25−7.82 (m, 7H, Ar−H); IR (KBr) ν 3310 (NH), 1630 (C=O) cm^{-1}. Anal. ($C_{14}H_{15}ClN_2O$) C, H, N.

(*R*)-*N*-(2-Fluorenyl-*N*′-(1-methyl-2-chloro)ethylurea (21a): yield, 57%; mp 194−196 °C; $[\alpha]^{25}_D$ +20.1 (*c* 0.70, MeOH); ^1H NMR (DMSO-d_6) δ 1.25 (d, *J* = 7.0 Hz, 3H, NCHC*H*$_3$), 3.50−3.85 (m, 3H, C*H*$_2$Cl, CH$_2$ fluorenyl), 4.20−4.40 (m, 1H, NC*H*CH$_3$), 4.90 (d, *J* = 8.1 Hz, 1H, N*H*CH, exchanges with D_2O), 6.35 (broad s, 1H, ArN*H*, exchanges with D_2O), 7.10−7.75 (m, 7H, Ar−H); IR (KBr) ν 3320 (NH), 1630 (C=O) cm^{-1}. Anal. ($C_{17}H_{17}ClN_2O$) C, H, N.

(*S*)-*N*-(2-Fluorenyl-*N*′-(1-methyl-2-chloro)ethylurea (21b): yield, 55%; mp 194−196 °C; $[\alpha]^{25}_D$ −23.3 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.26 (d, *J* = 6.7 Hz, 3H, NCHC*H*$_3$), 3.55−3.83 (m, 3H, C*H*$_2$Cl, CH$_2$ fluorenyl), 4.29−4.32 (m, 1H, NC*H*CH$_3$), 5.29 (d, *J* = 8.1 Hz, 1H, N*H*CH, exchanges with D_2O), 7.18−7.70 (m, 7H, Ar−H); IR (KBr) ν 3320 (NH), 1630 (C=O) cm^{-1}. Anal. ($C_{17}H_{17}ClN_2O$) C, H, N.

(*R*)-*N*-(5-Indanyl-*N*′-(1-methyl-2-chloro)ethylurea (22a): yield, 50%; mp 146−148 °C; $[\alpha]^{25}_D$ +14.8 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.23 (d, *J* = 7.0 Hz, 3H, NCHC*H*$_3$), 2.02−2.17, 2.83−2.90 (2m, 6H, CH$_2$ indanyl), 3.46−3.76 (m, 2H, C*H*$_2$-Cl), 4.25−4.30 (m, 1H, NC*H*CH$_3$), 6.95−7.17 (m, 7H, Ar−H); IR (KBr) ν 3300 (NH), 1640 (C=O) cm^{-1}. Anal. ($C_{13}H_{17}ClN_2O$) C, H, N.

(*S*)-*N*-(5-Indanyl-*N*′-(1-methyl-2-chloro)ethylurea (22b): yield, 55%; mp 148−150 °C; $[\alpha]^{25}_D$ −14.8 (*c* 1.00, MeOH); ^1H NMR (DMSO-d_6) δ 1.23 (d, *J* = 7.0 Hz, 3H, NCHC*H*$_3$), 2.03−2.16, 2.83−2.90 (2m, 6H, CH$_2$ indanyl), 3.53−3.78 (m, 2H, C*H*$_2$-

Cl), 4.26−4.30 (m, 1H, NC*H*CH_3), 6.94−7.18 (m, 7H, Ar−H); IR (KBr) ν 3300 (NH), 1640 (C═O) cm^{-1}. Anal. ($C_{13}H_{17}ClN_2O$) C, H, N.

Biological Assays. Reagents. Biochemicals, drugs, and the monoclonal antibody anti-β-tubulin (clone TUB 2.1) were obtained from Sigma Chemical (St. Louis, MO), and bovine calf serum was obtained from Hyclone (Road Logan, UT). The ECL Western blotting detection reagent kit was purchased from Amersham Canada (Oakville, Canada). Mixtures are expressed as volume/volume ratios unless otherwise indicated. All drugs were dissolved in DMSO, and the final concentration of DMSO in the culture medium was maintained at 0.5%.

Cell Culture. Cells were grown in RPMI 1640 medium supplemented with 10% bovine calf serum. Wild-type Chinese hamster ovary cells (CHO-10001), colchicine- and vinblastine-resistant CHO-VV 3-2 cells and paclitaxel-resistant CHO-TAX 5-6 cells were generously provided by Dr. Fernando Cabral (University of Texas Medical School, Houston, TX).[30,31]

Cytotoxicity Assay. Cytotoxicity was assessed using the Alamar Blue assay as described by O'Brien et al.[32] Briefly, (1−5) × 10^3 cells in 100 μL were seeded in 96-well plates and preincubated for 24 h. After addition of 100 μL of fresh medium containing increasing concentrations of the test drug, cells were incubated at 37 °C for 72 h. The culture medium was replaced by 50 μL of PBS containing 20% Alamar Blue in RPMI-1640. Cell survival was calculated from fluorescence (excitation, 485 nm; emission, 590 nm) measured with a FL 600 reader (Bio-Tek Instruments). Cytotoxicity was expressed as the dose of drug required to inhibit cell growth by 50% (IC_{50}). Each value is the mean of at least three independent determinations.

Determination of Alkylating Activity. The alkylation rate of the test drug was evaluated by the 4-(4-nitrobenzyl)-pyridine (NBP) colorimetric assay described by Bardos et al.[27] Briefly, 90 μL of a 5% (w/v) solution of NBP in 1-propanol was distributed in 96-well plates. Then, 10 μL of a 40 mM test derivatives solution in 1-propanol was added and the mixture was shaken. The plates were sealed with a plastic membrane and heated to 70 °C for 60, 75, 90, or 120 min. The reaction was stopped by adding 150 μL of 3-aminopropanol and *tert*-butyl alcohol (90/10). The plates were shaken for 10 s, and the absorbance was measured at 540 nm with a Multiskan MS reader (Labsystem). Blank samples without drug gave the background values. The absorbance readings were plotted against time, and the linear regression of the respective curves generated for each drug gave the alkylation rate directly. Data are given as mean values from at least three independent experiments.

Analysis of Alkylated β-Tubulin. Exponentially growing MDA-MB-231 cells (∼2.2 × 10^5) were plated in 12-well plates and incubated overnight at 37 °C. The cells were then treated with a 30 μM test compound solution, except for the control, for 0, 1, 2, 4, 8, 12, 24, and 48 h and were harvested directly in culture medium using a rubber policeman. Cells were centrifuged, and the pellets were washed with 500 μL of cold PBS. After centrifugation, the pellets were lysed using 1 × Laemmli sample buffer.[33] Samples (5 × 10^4 cells) were analyzed by 10% SDS−PAGE using the Laemmli system.[33] Membranes were then incubated with PBSMT (PBS, pH 7.4, 5% fatfree dry milk and 0.1% Tween-20) for 1 h at room temperature and then with 1/500 monoclonal anti-β-tubulin (clone TUB 2.1) for 1 h. Membranes were washed with PBSMT and incubated with 1/2500 peroxidase-conjugated antimouse immunoglobulin in PBSMT for 30 min. Detection of the immunoblot was carried out with the ECL Western blotting detection reagent kit. The optical densities of normal and alkylated β-tubulin bands were integrated using an NIH imager (Scion Corporation, Frederick, MD), and the percentage of alkylated β-tubulin was calculated. Data are representative of three separate experiments.

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