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Alkylation potency and protein specificity of aromatic urea derivatives and bioisosteres as potential irreversible antagonists of the colchicine-binding site

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Abstract—A number of *N*-phenyl-*N*′-(2-chloroethyl)ureas (**CEUs**) have been shown to be potent antimitotics through their covalent binding to the colchicine-binding site on intracellular β-tubulin. The present communication aimed to evaluate the role of the electrophilic 2-chloroethyl amino moiety of CEU on cell growth inhibition and the specificity of the drugs as irreversible antagonists of the colchicine-binding site. To that end, several *N*-phenyl-*N*′-(2-ethyl)urea (**EU**), *N*-phenyl-*N*′-(2-chloroethyl)urea (**CEU**), *N*-aryl amino-2-oxazoline (**OXA**), and *N*-phenyl-*N*′-(2-chloroacetyl)urea (**CAU**) derivatives were prepared and tested for their antiproliferative activity, their effect on the cell cycle, and their irreversible binding to β-tubulin. EU derivatives were devoid of antiproliferative activity. CEUs (**2h**–**2i**, **2k**, **2l**, OXA **3e**, **3h**, **3i**, **3k**, **3l**, tBCEU, and ICEU), OXA (**3h**, **3i**, **3k**, **3l**, tBOXA, and IOXA), and CAU (**4a**–**4m**, tBCAU, and ICAU) had GI_{50} between 1.7 and 10 μM on three tumor cell lines. Cytotoxic CEU and OXA arrested the cell cycle in G_2/M phase, while the corresponding CAU were not phase specific. Finally, Western blot analysis clearly showed that only CEUs **2h**, **2k**, **2l**, tBCEU, ICEU and OXA **3h**, **3i**, **3k**, **3l**, tBOXA ,and IOXA were able to bind irreversibly to the colchicine-binding site. Our results suggest that increasing the potency of the electrophilic moiety of the aromatic ureas enhances their antiproliferative activity but decreases significantly their capacity to covalently bind to the colchicine-binding site.

1. Introduction

A major hindrance of chemotherapy origins from the intrinsic and acquired chemoresistance of cancer cells to chemotherapeutic agents.[1–4] To circumvent the lack of tissue selectivity of anticancer agents and to alleviate their deleterious effects, new molecules have to be designed.[5–9] In that context, our ongoing research program focuses on the design and the development of new small-molecule anticancer drugs based on 1-aryl-3-(2-chloroethyl)ureas (**CEUs**).[10–14] CEUs were initially designed as hybrid molecules between aromatic nitrogen mustards and nitrosoureas.[10–18] CEUs are cytotoxic on a large number of cancer cell lines and remain active on most chemoresistant cells.[11] Prototypical CEU such as 4-*tert*-butyl-[3-(2-chloroethyl)ureido]benzene (**tBCEU**) and 4-iodo-3-[(2-chloroethyl)]ureido]benzene (**ICEU**) (Fig. 1) were shown to covalently bind to the colchicine-binding site on human β-tubulin isoform 2.[12,13] Consequently, CEUs disrupt the cytoskeleton, block the cell cycle progression at the G_2/M transition phase, and induce anoikis.[12–14] Interestingly, ICEU exhibited potent antineoplastic activity on mice bearing CT-26 murine colon carcinoma tumors and its [^{125}I]-derivative is biodistributed preferentially into organs of the gastrointestinal tract, notably the colon.

The present work aimed to understand the structure–activity relationships involved in the antimitotic properties of CEU and to improve their biological and biopharmaceutical properties. In that context, we have

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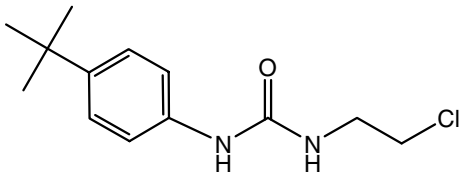
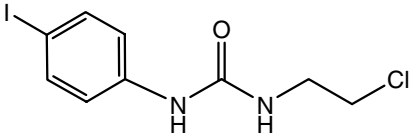


tBCEU



ICEU

Figure 1. Chemical structures of 4-*tert*-butyl-[3-(2-chloroethyl)ureido]benzene (**tBCEU**) and 4-iodo-3-[(2-chloroethyl)]ureido]benzene (**ICEU**).

prepared and assessed the antiproliferative activity and the specificity for the colchicine-binding site on β-tubulin of a series of CEU derivatives and analogues, namely, *N*-phenyl-*N*′-(2-ethyl)ureas (**EU**), *N*-phenyl-*N*′-(2-chloroacetyl)urea derivatives (**CAU**), and *N*-aryl amino-2-oxazoline (4,5-dihydro-*N*-phenyloxazol-2-amines: **OXA**) derivatives. Two main sets of structural modifications were achieved. On one hand, we have varied the nature and the position of the substituents on the aromatic ring. On the other hand, the 2-amino chloroethyl moiety was modified to assess the importance of the electrophilic character on the cell growth inhibition and the specificity toward the colchicine-binding site. To that end, the 2-chloroethyl amino moiety of CEU was converted into a 2-ethylamino group, a 2-amino oxazolinyl group, and a 2-chloroacetylamino group, respectively. EU were prepared as biologically inactive and non-electrophilic derivatives of CEU. OXA derivatives were obtained from the intramolecular cyclization of CEU in presence of KF and SiO_2. They are considered as weak electrophilic entities. CAU are potent electrophilic compounds based on the aryl 2-haloacetamides described by Jiang and known to bind to the colchicine-binding site on β-tubulin.[19–21]

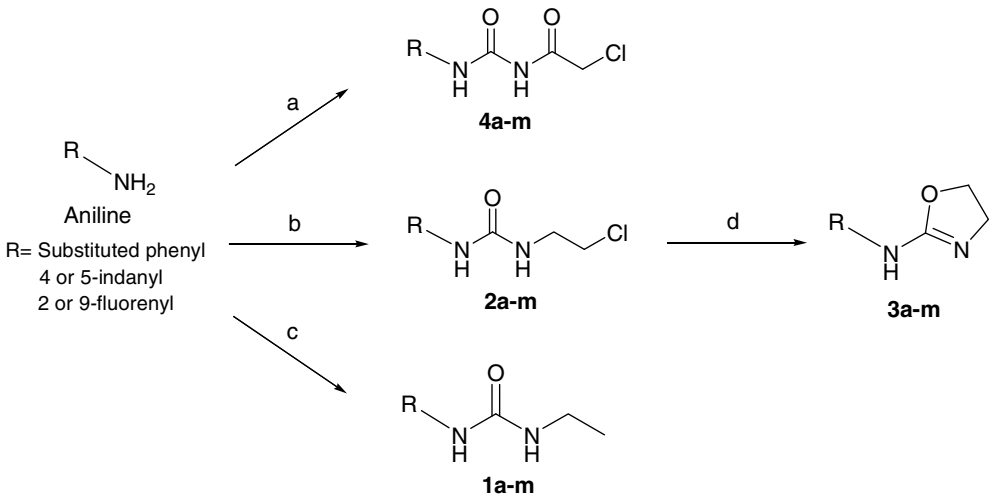
2. Results and discussion

2.1. Chemistry

Scheme 1 illustrates the synthesis of CEU derivatives, EU derivatives, and CAU derivatives performed by nucleophilic addition of either 2-chloroethylisocyanate, ethylisocyanate or 2-chloroacetylisocyanate, respectively, on the corresponding anilines (see Scheme 1).[11,12] OXA derivatives were prepared from the catalytic cyclization of CEU in presence of KF on SiO_2 in acetonitrile at room temperature (Scheme 1).

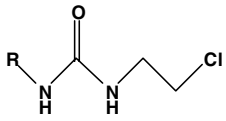
2.1.1. Tumor cell growth inhibition activity. Tumor cell growth inhibition activity of CEU, EU, CAU, and OXA was evaluated on three human cell lines: breast carcinoma MCF-7, skin melanoma M21, and colon carcinoma HT-29 cells. Cell growth inhibition was assessed according to the NCI/NIH Developmental Therapeutics Program.[18] The results are summarized in Tables 1–3 and expressed as the concentration of drug inhibiting cell growth by 50% (GI_{50}).

The results show that the antiproliferative activity of the drugs is related to the presence of an electrophilic group on the urea moiety of the molecules. Accordingly, EU derivatives, which are devoid of electrophilicity, did not inhibit the growth of the cell lines tested in this study at 100 μM (data not shown). However, all CAU derivatives (**4a**–**4m**) bearing the potent electrophilic 2-chloroacetylamino moiety displayed potent antiproliferative activity on all tumor cells tested. Conversely, only CEUs **2h**, **2i**, **2k**, **2l** along with tBCEU and ICEU exhibited a significant proliferation inhibition of tumor cells. The bioisosteric OXA were less active than CAU but generally slightly more potent than their corresponding CEU. It is of interest to point out that the substitution on the 2-position for the OXA derivatives (**3d**–**3g**)



Scheme 1. Reagents: (a) 2-chloroacetylisocyanate, CH_2Cl_2; (b) 2-chloroethylisocyanate, CH_2Cl_2; (c) ethylisocyanate, CH_2Cl_2; (d) SiO_2·KF, CH_3CN.

Table 1. Growth inhibition activity of compounds **2a**–**2m** (CEU), **tBCEU**, **ICEU**, colchicine, vinblastine, and paclitaxel on breast carcinoma MCF-7, skin melanoma M21, and colon carcinoma HT-29 cells and their covalent binding to β-tubulin



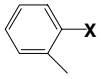
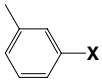
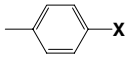
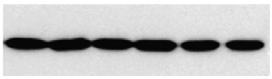
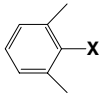
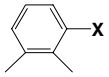
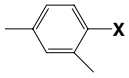
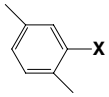
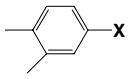
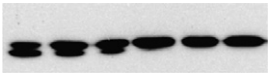
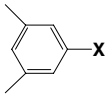
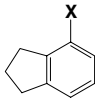
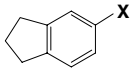
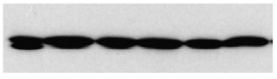
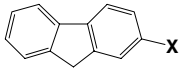
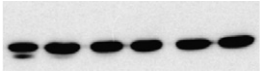
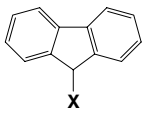
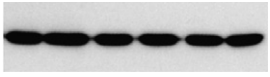
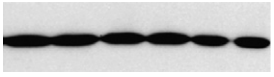
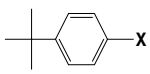
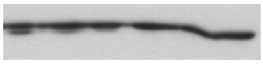
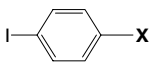
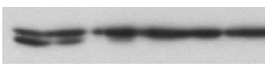
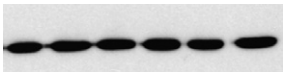
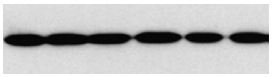
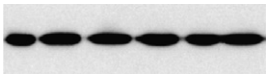
Compound	Substituent	GI_{50} (μM)			β-Tubulin alkylation 48 36 24 16 8 0 h
		HT-29	M21	MCF-7	
2a		n.e.	n.e.	n.e.	
2b		39	81	n.e.	
2c		16	33	44	
2d		n.e.	n.e.	n.e.	
2e		n.e.	n.e.	n.e.	
2f		78	n.e.	n.e.	
2g		67	n.e.	n.e.	
2h		4.2	8.4	12	
2i		8.5	16	31	
2j		95	n.e.	n.e.	
2k		4.7	8.9	17	
2l		2.3	5.1	14.2	
2m		16	38	21	
DMSO		n.e.	n.e.	n.e.	
tBCEU		2.3	4.3	6.2	
ICEU		1.9	3.9	6.0	

Table 1 (*continued*)

Compound	Substituent	GI_{50} (μM)			β-Tubulin alkylation 48 36 24 16 8 0 h
		HT-29	M21	MCF-7	
Colchicine		0.004	0.015	0.009	
Vinblastine		0.002	0.010	0.002	
Paclitaxel		0.015	0.037	0.054	

demonstrated a weak but significant improvement of the antiproliferative activity when compared with the corresponding CEU derivatives.

2.1.2. Cell cycle analysis. Previous studies have shown that prototypical CEU derivatives such as tBCEU and ICEU arrested the cell cycle in G_2/M phase possibly through their covalent binding to the colchicine-binding site.[12] We have evaluated whether the new antiproliferative compounds inhibit the cell cycle in G_2/M phase such as CEUs do. Cell cycle analysis was performed using compounds **2k**, **2l**, **3k**, **3l**, **4k**, and **4l** that were the most potent antiproliferative CEU, OXA, and CAU, respectively. The experiments were conducted on M21 cells at 3, 10, and 30 times the respective GI_{50}.

Figure 2 illustrates that the incubation of M21 cells with compound **4k** or **4l** for 24 h did not induce any significant accumulation of cells in specific phases of the cell cycle. Conversely, CEUs **2k**, **2l** and OXA **3k** and **3l** caused a significant accumulation of the cells in the G_2/M, as reported previously with several CEU derivatives.[12,14]

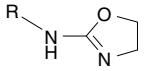
2.1.3. Covalent binding to β-tubulin. β-Tubulin is one of the primary pharmacological targets of tBCEU and ICEU.[12,14] Therefore, we compared the binding of EU, CEU, CAU, and OXA derivatives to β-tubulin using molecules having GI_{50} ranging from 1 to 20 μM.[12,14] Previous studies showed that the covalent binding of CEU to β-tubulin increases significantly the electrophoretic mobility of the protein in SDS–PAGE conditions.[12,14] The alkylation of β-tubulin by the various molecules was assessed on human intracellular β-tubulin present in living cells not on purified β-tubulin.

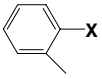
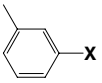
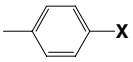
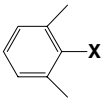
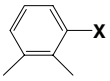
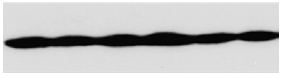
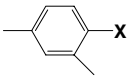
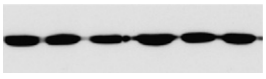
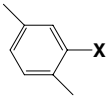
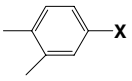
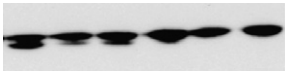
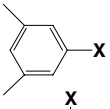
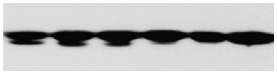
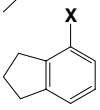
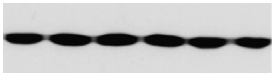
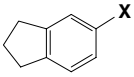
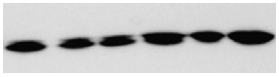
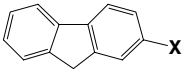
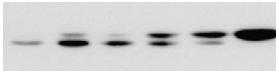
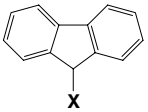
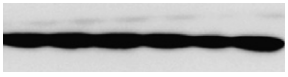
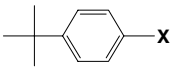
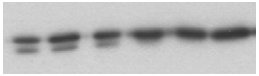
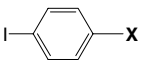
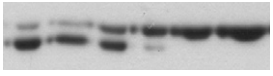
As previously reported, the covalent binding of active CEU on the colchicine-binding site of β-tubulin was identified by the appearance of an additional immunoreactive band exhibiting an apparent lower molecular weight of β-tubulin. In addition, the appearance of the β-tubulin by-product was time-dependent.[12,14] We showed also that compounds **2h**, **2k**, tBCEU, and ICEU, and their corresponding OXA **3h**, **3k**, tBOXA, and IOXA were induced the production of the alkylated band of β-tubulin after 16 to 24 h, while other CEUs such as **2l** needed 48 h of incubation. In contrast, CAU (**4a**–**4m**) and some OXA (**3e** and **3f**) did not alkylate β-tubulin but they were cytotoxic on all tumor cell lines suggesting that their covalent binding to the colchicine-binding site is not involved in the mechanism of action of these compounds.

3. Conclusion

This study aimed to understand the importance of the electrophilic moiety of CEU derivatives on the antiproliferative activity, the specificity of the covalent binding of the drug to the colchicine-binding site, and the effect on cell cycle blockage specificity. Our results show that, as expected, the non-electrophilic EU derivatives were pharmacologically inactive. Interestingly, most OXA and CAU derivatives (**4a**–**4m**) were antiproliferative, while only CEUs **2h**–**2l**, tBCEU, and ICEU have shown significant activity on the three tumor cell lines tested. Experiments conducted to compare the effect of the active prototypical indanyl substituted **2k**, **3k**, **4k** and fluorenyl substituted **2l**, **3l**, and **4l** on the cell cycle showed that CEU and OXA derivatives were accumulating the cells in G_2/M phase, while the equivalent CAU did not exhibit arrest of the cell division in any specific phases, suggesting that CAU exhibit antiproliferative activity through a different mechanism of action. Interestingly, several derivatives were tested using SDS–PAGE on β-tubulin. The results displayed that compounds **2h**, **2k**, **2l**, **3h**, **3i**, **3k**, **3l**, tBCEU, ICEU, tBOXA, and IOXA were able to covalently bind to β-tubulin. All other molecules seemed cytotoxic through mechanism(s) of action unrelated to colchicine-binding site antagonism. Our results suggest that the nature of the electrophilic moiety bonded to similar biofunctional groups is of utmost importance for the specificity of the molecule toward the colchicine-binding site. Furthermore, compounds having the same biofunctional groups, bearing equivalent electrophilic moieties and exhibiting similar antiproliferative activity, might kill the cells through different mechanism of action.

Table 2. Growth inhibition activity of compounds **3a**–**3m** (OXA), **tBOXA**, and **IOXA** on breast carcinoma MCF-7, skin melanoma M21, and colon carcinoma HT-29 cells and their covalent binding to β-tubulin



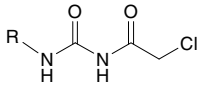
Compound	Substituent	GI_{50} (μM)			β-Tubulin alkylation 48 36 24 16 8 0 h
		HT-29	M21	MCF-7	
3a		13	18	21	
3b		33	61	52	
3c		13	22	27	
3d		15	27	23	
3e		9.0	17	15	
3f		15	26	23	
3g		44	73	72	
3h		2.7	5.1	5.7	
3i		7.0	12	19	
3j		16	30	26	
3k		4.9	8.1	9.1	
3l		1.9	3.2	3.7	
3m		15	17	11	
tBOXA		1.5	2.4	3.0	
IOXA		1.3	2.0	2.9	

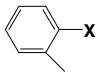
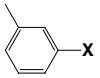
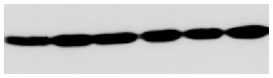
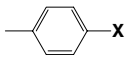
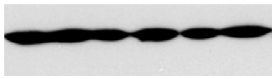
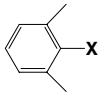
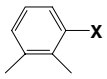
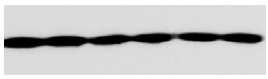
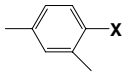
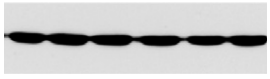
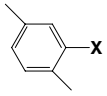
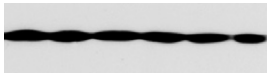
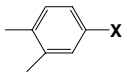
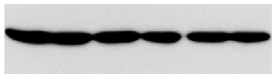
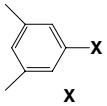
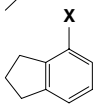
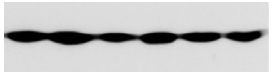
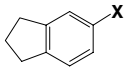
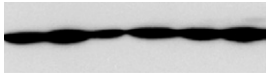
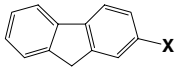
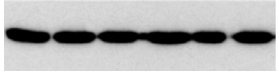
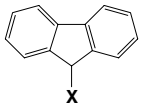
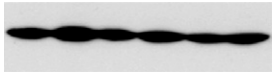
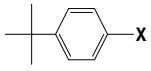
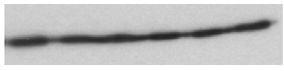
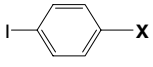
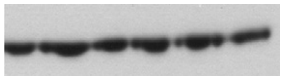
4. Experimental

4.1. Chemistry and chemical methods

Proton NMR spectra were recorded on a Bruker AM-300 spectrometer (Bruker, Germany). Chemical shifts (δ) are reported in parts per million, relative to the internal tetramethylsilane standard. IR spectra were recorded on a Unicam spectrometer. Uncorrected melting points were determined on an electrothermal melting point apparatus. Mass spectra were recorded on the best antiproliferative compounds, by the proteo-

Table 3. Growth inhibition activity of compounds **4a**–**4m** (CAU), **tBCAU**, and **ICAU** on breast carcinoma MCF-7, skin melanoma M21, and colon carcinoma HT-29 cells and their covalent binding to β-tubulin



Compound	Substituent	GI_{50} (μM)			β-Tubulin alkylation 48 36 24 16 8 0 h
		HT-29	M21	MCF-7	
4a		9.8	6.9	10.6	
4b		9.0	4.0	6.1	
4c		10	4.9	7.5	
4d		6.6	6.3	7.4	
4e		6.0	4.7	6.5	
4f		11	7.4	8.6	
4g		6.7	3.8	5.8	
4h		6.9	4.3	5.5	
4i		6.1	3.7	4.3	
4j		3.2	4.9	2.2	
4k		7.0	1.7	4.0	
4l		13	4.8	5.0	
4m		8.6	3.4	4.8	
tBCAU		3.3	1.1	4.8	
ICAU		6.9	4.2	6.8	

mic and mass spectrometry centre, University of Toronto. All reactions were conducted under a dried nitrogen atmosphere. Chemicals were supplied by Aldrich Chemicals (Milwaukee, WI). Liquid flash chromatography was performed on silica gel 60 A (American Chemicals Ltd, Montreal, Canada), using the indicated solvent mixture expressed as volume/volume ratios. Solvents and reagents were used without purification unless specified otherwise. The progress of all reactions was monitored using TLC on precoated silica gel plates (Merck Silica Gel 60 F_{254}). The chromatograms were viewed under UV light at 254 nm.

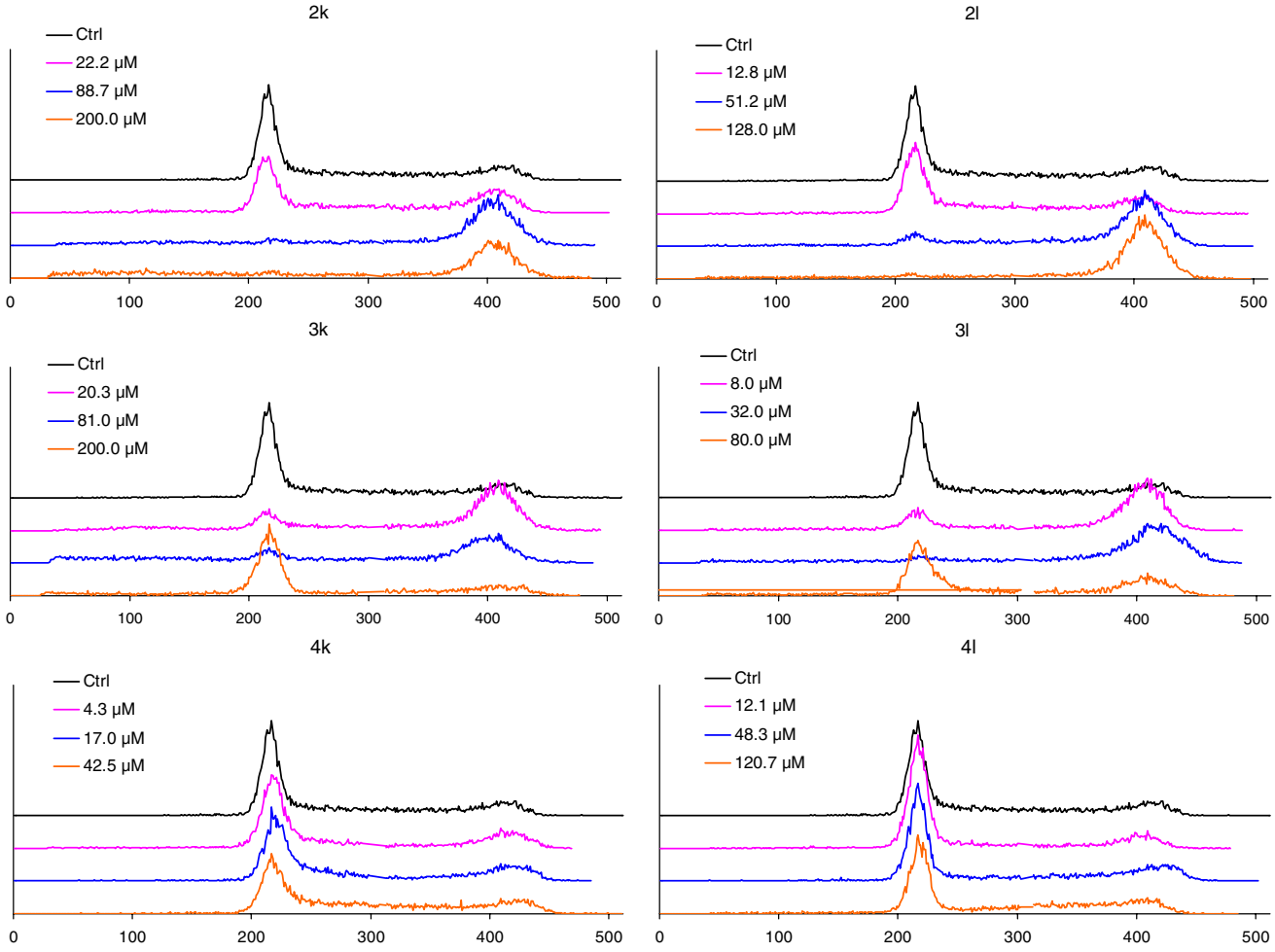


Figure 2. Effect of **2k**, **3k**, **4k**, **2l**, **3l**, and **4l** (CEU, OXA, CAU derivatives) on the cell cycle. Exponentially growing M21 cells were incubated in the absence or presence of the drug for 24 h at 37 °C. The cell cycle was evaluated using propidium iodide staining and flow cytometry analysis.

4.2. General preparation of compounds 1a–1m

Ethylisocyanate (3.6 mmol) was added dropwise to a stirred solution of the relevant aniline (3 mmol) in dichloromethane (15 mL). The reaction mixture was stirred overnight at ambient temperature. The resulting crude precipitate was filtered, washed with cold ether, and purified by recrystallization from ethanol and water.

4.2.1. 1-Ethyl-3-*o*-tolylurea (1a). Compound **1a** was synthesized from the nucleophilic addition of *o*-toluidine to ethylisocyanate. Yield: 82%; mp: 166–168 °C; IR (KBr) *v*: 3326 (NH), 1631 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.85 (d, 1H, J = 8.0 Hz, Ar), 7.60 (s, 1H, NH), 7.11 (m, 2H, Ar), 6.88 (t, 1H, J = 7.3 Hz, Ar), 6.52 (br s, 1H, NH), 3.14 (m, 2H, CH_2), 2.20 (s, 3H, CH_3), 1.09 (t, 3H, J = 7.3 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.4, 138.3, 130.0, 126.7, 126.1, 121.8, 120.5, 34.0, 17.9, 15.4.

4.2.2. 1-Ethyl-3-*m*-tolylurea (1b). Compound **1b** was synthesized from the nucleophilic addition of *m*-toluidine to ethylisocyanate. Yield: 100%; mp: 96–98 °C; IR (KBr) *v*: 3328 (NH), 1706 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.79 (s, 1H, NH), 7.09 (m, 3H, Ar), 6.79 (m, 1H, Ar), 5.93 (s, 1H, NH), 3.18 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 1.06 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ: 151.7, 133.9, 133.6, 123.6, 118.5, 115.7, 112.1, 29.7, 16.2, 10.2.

4.2.3. 1-Ethyl-3-*p*-tolylurea (1c). Compound **1c** was synthesized from the nucleophilic addition of *p*-toluidine to ethylisocyanate. Yield: 10%; mp: 145–148 °C; IR (KBr) *v*: 3324 (NH), 1716 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.28 (s, 1H, NH), 7.28 (d, 2H, J = 8.3 Hz, Ar), 7.02 (d, 2H, J = 8.2 Hz, Ar), 6.03 (br s, 1H, NH), 3.10 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 1.05 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.3, 138.1, 129.6, 129.0, 117.8, 34.0, 20.3, 15.5.

4.2.4. 1-Ethyl-3-(2,6-dimethylphenyl)urea (1d). Compound **1d** was synthesized from the nucleophilic addition of 2,6-dimethylaniline to ethylisocyanate. Yield: 93%; mp: 236–238 °C; IR (KBr) *v*: 3317 (NH), 1628 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.41 (s, 1H, NH), 7.04 (s, 3H, Ar), 5.97 (br s, 1H, NH), 3.09 (m, 2H, CH_2), 2.17 (s, 6H, $2 \times CH_3$), 1.05 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 156.0, 136.2, 135.6, 127.7, 125.6, 34.3, 18.3, 15.7.

4.2.5. 1-Ethyl-3-(2,3-dimethylphenyl)urea (1e). Compound **1e** was synthesized from the nucleophilic addition

of 2,3-dimethylaniline to ethylisocyanate. Yield: 94%; mp: 181–184 °C; IR (KBr) *v*: 3317 (NH), 1633 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.61 (s, 1H, NH), 7.53 (d, 1H, $J = 8.0$ Hz, Ar), 6.98 (t, 1H, $J = 7.71$ Hz, Ar), 6.83 (d, 1H, $J = 7.4$ Hz, Ar), 6.38 (br s, 1H, NH), 3.12 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 1.08 (t, 3H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.6, 137.9, 136.3, 126.8, 125.1, 124.2, 34.1, 20.4, 15.5, 13.5.

4.2.6. 1-Ethyl-3-(2,4-dimethylphenyl)urea (1f). Compound **1f** was synthesized from the nucleophilic addition of 2,4-dimethylaniline to ethylisocyanate. Yield: 97%; mp: 185–192 °C; IR (KBr) *v*: 3328 (NH), 1633 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.64 (d, 1H, $J = 8.1$ Hz, Ar), 7.51 (s, 1H, NH), 6.91 (m, 2H, Ar), 6.39 (br s, 1H, NH), 3.12 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 1.07 (t, 3H, $J = 7.1$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.5, 135.7, 130.7, 130.6, 127.2, 126.5, 121.1, 34.0, 20.3, 17.8, 15.5.

4.2.7. 1-Ethyl-3-(2,5-dimethylphenyl)urea (1g). Compound **1g** was synthesized from the nucleophilic addition of 2,5-dimethylaniline to ethylisocyanate. Yield: 100%; mp: 176–180 °C; IR (KBr) *v*: 3329 (NH), 1634 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.66 (s, 1H, NH), 7.52 (s, 1H, Ar), 7.00 (d, 1H, $J = 7.5$ Hz, Ar), 6.70 (d, 1H, $J = 7.3$ Hz, Ar), 6.49 (br s, 1H, NH), 3.12 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 1.08 (t, 3H, $J = 7.1$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.4, 138.1, 134.9, 129.9, 123.7, 122.5, 121.2, 34.0, 21.0, 17.5, 15.4.

4.2.8. 1-Ethyl-3-(3,4-dimethylphenyl)urea (1h). Compound **1h** was synthesized from the nucleophilic addition of 3,4-dimethylaniline to ethylisocyanate. Yield: 21%; mp: 145–146 °C; IR (KBr) *v*: 3332 (NH), 1635 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.20 (s, 1H, NH), 7.13 (m, 2H, Ar), 6.95 (d, 1H, $J = 8.1$ Hz, Ar), 6.01 (br s, 1H, NH), 3.10 (m, 2H, CH_2), 2.14 (d, 6H, $J = 8.6$ Hz, $2 \times CH_3$), 1.05 (t, 3H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.3, 138.3, 136.1, 129.5, 128.4, 119.1, 115.3, 33.9, 19.6, 18.6, 15.5.

4.2.9. 1-Ethyl-3-(3,5-dimethylphenyl)urea (1i). Compound **1i** was synthesized from the nucleophilic addition of 3,5-dimethylaniline to ethylisocyanate. Yield: 77%; mp: 149–151 °C; IR (KBr) *v*: 3342 (NH), 1656 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.24 (s, 1H, NH), 7.03 (s, 2H, Ar), 6.53 (s, 1H, Ar), 6.07 (br s, 1H, NH), 3.12 (m, 2H, CH_2), 2.21 (s, 6H, $2 \times CH_3$), 1.06 (t, 3H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.2, 140.5, 137.5, 122.6, 115.5, 34.0, 21.1, 15.5.

4.2.10. 4,5-Dihydro-*N*-(2,3-dihydro-1H-inden-4-yl)oxazol-2-amine (1j). Compound **1j** was synthesized from the nucleophilic addition of 4-aminoindan to ethylisocyanate. Yield: 78%; mp: 168–170 °C; IR (KBr) *v*: 3321 (NH), 1636 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.75 (d, 1H, $J = 8.1$ Hz, Ar), 7.66 (s, 1H, NH), 6.99 (t, 1H, $J = 7.6$ Hz, Ar), 6.81 (d, 1H, $J = 7.3$ Hz, Ar), 6.47 (s, 1H, NH), 3.12 (m, 2H, CH_2), 2.86 (t, 2H, $J = 7.4$ Hz, CH_2), 2.75 (t, 2H, $J = 7.3$ Hz, CH_2), 2.02 (m, 2H, CH_2), 1.08 (t, 3H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.2, 144.2, 136.5, 131.4, 126.6, 117.3, 116.1, 33.9, 32.8, 29.9, 24.4, 15.4.

4.2.11. 1-Ethyl-3-(2,3-dihydro-1H-inden-5-yl)urea (1k). Compound **1k** was synthesized from the nucleophilic addition of 5-aminoindan to ethylisocyanate. Yield: 47%; mp: 136–139 °C; IR (KBr) *v*: 3323 (NH), 1631 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.26 (s, 1H, NH), 7.33 (s, 1H, Ar), 7.08 (m, 2H, Ar), 6.04 (br s, 1H, NH), 3.12 (m, 2H, CH_2), 2.78 (m, 4H, $2 \times CH_2$), 1.98 (m, 2H, CH_2), 1.06 (t, 3H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.4, 144.0, 138.8, 136.0, 124.0, 116.0, 114.1, 34.0, 32.6, 31.7, 25.2, 15.5.

4.2.12. 1-Ethyl-3-(9H-fluoren-2-yl)urea (1l). Compound **1l** was synthesized from the nucleophilic addition of 2-aminofluorene to ethylisocyanate. Yield: 64%; mp: 239–242 °C; IR (KBr) *v*: 3329 (NH), 1638 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8,55 (s, 1H, NH), 7.75 (t, 3H, $J = 8.1$ Hz, Ar), 7.52 (d, 1H, $J = 7.4$ Hz, Ar), 7.35 (m, 2H, Ar), 7.23 (t, 1H, $J = 7.4$ Hz, Ar), 6.17 (br s, 1H, NH), 3.87 (s, 2H, CH_2), 3.17 (m, 2H, CH_2), 1.10 (t, 3H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.3, 143.9, 142.5, 141.4, 139.9, 134.4, 126.7, 125.6, 124.9, 120.1, 119.0, 116.6, 114.5, 36.5, 34.1, 15.5.

4.2.13. 1-Ethyl-3-(9H-fluoren-9-yl)urea (1m). Compound **1m** was synthesized from the nucleophilic addition of 9-aminofluorene to ethylisocyanate. Yield: 80%; IR (KBr) *v*: 3336 (NH), 1655 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.85 (d, 2H, $J = 7.4$ Hz, Ar), 7.55 (d, 2H, $J = 7.3$ Hz, Ar), 7.42 (t, 2H, $J = 7.2$ Hz, Ar), 7.34 (t, 2H, $J = 7,3$ Hz, Ar), 6.42 (br s, 1H, NH), 5.84 (d, 2H, $J = 8.2$ Hz, CH, NH), 3.16 (t, 2H, $J = 6.3$ Hz, CH_2), 1.07 (t, 3H, $J = 7.1$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 158.6, 146.0, 139.8, 128.2, 127.6, 124.9, 120.1, 55.2, 34.4, 15.7.

4.2.14. 1-(4-*tert*-Butylphenyl)-3-ethylurea (tBEU). tBEU was synthesized from the nucleophilic addition of 4-tert-butylaniline to ethylisocyanate. Yield: 65%; mp: 133–137 °C; IR (KBr) *v*: 3318 (NH), 1640 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.30 (s, 1H, NH), 7.30 (d, 2H, $J = 8.6$ Hz, Ar), 7.23 (d, 2H, $J = 8.5$ Hz, Ar), 6.03 (br s, 1H, NH), 3.11 (m, 2H, CH_2), 1.25 (s, 9H, $3 \times CH_3$), 1.09 (t, 3H, $J = 7.0$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 156.6, 145.8, 132.8, 125.3, 122.2, 40.7, 36.3, 31.4, 15.7.

4.2.15. 1-Ethyl-3-(4-iodophenyl)urea (IEU). IEU was synthesized from the nucleophilic addition of 4-iodoaniline to ethylisocyanate. Yield: 58%; mp: 238–240 °C; IR (KBr) *v*: 3319 (NH), 1634 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.55 (s, 1H, NH), 7.53 (d, 2H, $J = 8.6$ Hz, Ar), 7.25 (d, 2H, $J = 8.6$ Hz, Ar), 6.16 (br s, 1H, NH), 3.10 (m, 2H, CH_2), 1.05 (t, 3H, $J = 7.1$ Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 154.1, 140.5, 137.1, 119.9, 83.4, 34.5, 15.5.

4.3. General preparation of compounds 2a–2m

CEUs **2a**–**2I**, **2k**–**2l** and tBCEU and ICEU were prepared as published previously.[15,16] 2-chloroethylisocyanate (3.6 mmol) was added dropwise to a stirred

solution of the relevant aniline (3 mmol) in dichloromethane (15 mL). The reaction mixture was stirred overnight at ambient temperature. The resulting precipitate was filtered, washed with cold ether, and purified by recrystallization from ethanol and water.

4.3.1. 1-(2-Chloroethyl)-3-*o*-tolylurea (2a). Compound **2a** was synthesized from the nucleophilic addition of *o*-toluidine to 2-chloroethylisocyanate. Yield: 66%; mp: 148–151 °C; IR (KBr) *v*: 3336 (NH), 1632 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.84 (m, 2H, Ar, NH), 7.12 (m, 2H, Ar), 6.91 (m, 2H, Ar, NH), 3.69 (t, 2H, $J = 6.2$ Hz, CH_2), 3.45 (m, 2H, CH_2), 2.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.4, 138.0, 130.1, 127.1, 126.1, 122.2, 120.8, 44.6, 41.3, 18.0.

4.3.2. 1-(2-Chloroethyl)-3-*m*-tolylurea (2b). Compound **2b** was synthesized from the nucleophilic addition of *m*-toluidine to 2-chloroethylisocyanate. Yield: 26%; mp: 228–229 °C; IR (KBr) *v*: 3320 (NH), 1636 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.60 (s, 1H, NH), 7.25 (s, 1H, Ar), 7.20 (d, 1H, $J = 8.4$ Hz, Ar), 7.12 (t, 1H, $J = 7.9$ Hz, Ar), 6.74 (d, 1H, $J = 7.2$ Hz, Ar), 6.42 (br s, 1H, NH), 3.67 (t, 2H, $J = 6.2$ Hz, CH_2), 3.44 (t, 2H, $J = 5.9$ Hz, CH_2), 2.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.1, 140.3, 137.8, 128.5, 122.0, 118.3, 115.0, 44.5, 41.3, 21.3.

4.3.3. 1-(2-Chloroethyl)-3-*p*-tolylurea (2c). Compound **2c** was synthesized from the nucleophilic addition of *p*-toluidine to 2-chloroethylisocyanate. Yield: 48%; mp: 178–183 °C; IR (KBr) *v*: 3326 (NH), 1641 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.58 (s, 1H, NH), 7.32 (d, 2H, $J = 8.4$ Hz, Ar), 7.04 (d, 2H, $J = 8.3$ Hz, Ar), 6.41 (br s, 1H, NH), 3.67 (t, 2H, $J = 6.2$ Hz, CH_2), 3.46 (t, 2H, $J = 5.9$ Hz, CH_2), 2.23 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.2, 137.8, 130.0, 129.3, 129.1, 118.0, 44.5, 41.3, 20.3. MS (ESI) *m*/*z*: 213.1 (M^++1).

4.3.4. 1-(2-Chloroethyl)-3-(2,6-dimethylphenyl)urea (2d). Compound **2d** was synthesized from the nucleophilic addition of 2,6-dimethylaniline to 2-chloroethylisocyanate. Yield: 44%; mp: 165–168 °C; IR (KBr) *v*: 3303 (NH), 1634 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.74 (s, 1H, NH), 7.04 (s, 3H, Ar), 6.39 (s, 1H, NH), 3.64 (t, 2H, $J = 6.3$ Hz, CH_2), 3.40 (m, 2H, CH_2), 2.18 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.9, 135.9, 135.7, 127.7, 125.8, 44.5, 41.5, 18.2.

4.3.5. 1-(2-Chloroethyl)-3-(2,3-dimethylphenyl)urea (2e). Compound **2e** was synthesized from the nucleophilic addition of 2,3-dimethylaniline to 2-chloroethylisocyanate. Yield: 62%; mp: 148–151 °C; IR (KBr) *v*: 3321 (NH), 1634 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.92 (s, 1H, NH), 7.52 (d, 1H, $J = 8.0$ Hz, Ar), 7.00 (t, 1H, $J = 8.2$ Hz, Ar), 6.86 (d, 1H, $J = 7.4$ Hz, Ar), 6.78 (br s, 1H, NH), 3.68 (t, 2H, $J = 6.1$ Hz, CH_2), 3.44 (m, 2H, CH_2), 2.31 (s, 3H, CH_3), 2.11 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.6, 137.6, 136.5, 127.2, 125.2, 124.5, 120.3, 44.6, 41.4, 20.4, 13.6.

4.3.6. 1-(2-Chloroethyl)-3-(2,4-dimethylphenyl)urea (2f). Compound **2f** was synthesized from the nucleophilic addition of 2,4-dimethylaniline to 2-chloroethylisocyanate. Yield: 76%; mp: 170–172 °C; IR (KBr) *v*: 3309 (NH), 1634 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.80 (s, 1H, NH), 7.63 (d, 1H, $J = 8.1$ Hz, Ar), 6.89 (m, 2H, Ar), 6.81 (br s, 1H, NH), 3.67 (t, 2H, $J = 6.1$ Hz, CH_2), 3.44 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.5, 135.4, 131.1, 130.7, 127.6, 126.5, 121.4, 44.6, 41.4, 20.3, 17.9.

4.3.7. 1-(2-Chloroethyl)-3-(2,5-dimethylphenyl)urea (2g). Compound **2g** was synthesized from the nucleophilic addition of 2,5-dimethylaniline to 2-chloroethylisocyanate. Yield: 74%; mp: 170–175 °C; IR (KBr) *v*: 3353 (NH), 1638 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.80 (s, 1H, NH), 7.66 (s, 1H, Ar), 7.00 (d, 1H, $J = 7.6$ Hz, Ar), 6.89 (br s, 1H, NH), 6.72 (d, 1H, $J = 7.6$ Hz, Ar), 3.68 (t, 2H, $J = 6.2$ Hz, CH_2), 3.43 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 2.15 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 155.4, 137.8, 134.9, 129.9, 124.0, 122.8, 121.5, 44.6, 41.3, 20.9, 17.5.

4.3.8. 1-(2-Chloroethyl)-3-(3,4-dimethylphenyl)urea (2h). Compound **2h** was synthesized from the nucleophilic addition of 3,4-dimethylaniline to 2-chloroethylisocyanate. Yield: 32%; mp: 149–151 °C; IR (KBr) *v*: 3329 (NH), 1642 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.49 (s, 1H, NH), 7.17 (t, 2H, $J = 7.9$ Hz, Ar), 6.98 (d, 1H, $J = 8.1$ Hz, Ar), 6.40 (br s, 1H, NH), 3.67 (t, 2H, $J = 6.1$ Hz, CH_2), 3.44 (m, 2H, CH_2), 2.16 (d, 6H, $J = 9.5$ Hz, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ: 155.2, 138.0, 136.2, 129.6, 128.8, 119.3, 115.5, 44.5, 41.3, 19.6, 18.6. MS (ESI) *m*/*z*: 227.2 (M^+ + 1).

4.3.9. 1-(2-Chloroethyl)-3-(3,5-dimethylphenyl)urea (2i). Compound **2i** was synthesized from the nucleophilic addition of 3,5-dimethylaniline to 2-chloroethylisocyanate. Yield: 54%; mp: 152–154 °C; IR (KBr) *v*: 3325 (NH), 1640 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 8.54 (s, 1H, NH), 7.05 (s, 2H, Ar), 6.56 (s, 1H, Ar), 6.42 (br s, 1H, NH), 3.67 (t, 2H, $J = 6.1$ Hz, CH_2), 3.45 (t, 2H, $J = 5.8$ Hz, CH_2), 2.22 (s, 6H, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ: 155.1, 140.2, 137.6, 122.9, 115.6, 44.4, 41.3, 21.1. MS (ESI) *m*/*z*: 227.2 (M^++1).

4.3.10. 1-(2-Chloroethyl)-3-(2,3-dihydro-1H-inden-4-yl)urea (2j). Compound **2j** was synthesized from the nucleophilic addition of 4-aminoindan to 2-chloroethylisocyanate. Yield: 72%; mp: 147–149 °C; IR (KBr) *v*: 3327 (NH), 1632 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.91 (s, 1H, NH), 7.75 (d, 1H, $J = 8.1$ Hz, Ar), 7.03 (t, 1H, $J = 7.6$ Hz, Ar), 7.76 (d, 2H, $J = 6.7$ Hz, Ar, NH), 3.69 (t, 2H, $J = 6.0$ Hz, CH_2), 3.45 (m, 2H, CH_2), 2.87 (t, 2H, $J = 7.4$ Hz, CH_2), 2.77 (t, 2H, $J = 7.3$ Hz, CH_2), 2.03 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 155.1, 144.3, 136.2, 131.7, 126.7, 117.7, 116.3, 44.7, 41.3, 32.8, 29.9, 24.4.

4.3.11. 1-(2-Chloroethyl)-3-(2,3-dihydro-1H-inden-5-yl)urea (2k). Compound **2k** was synthesized from the nucleophilic addition of 5-aminoindan to 2-chloroethylisocyanate. Yield: 47%; mp: 155–157 °C; IR (KBr) *v*: 3330 (NH), 1637 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6)

δ: 8.56 (s, 1H, NH), 7.35 (s, 1H, Ar), 7.12 (m, 2H, Ar), 6.40 (br s, 1H, NH), 3.66 (t, 2H, J = 5.9 Hz, CH_2), 3.41 (t, 2H, J = 5.9 Hz, CH_2), 2.81 (m, 4H, $2 \times CH_2$), 2.00 (t, 2H, J = 7.3 Hz, CH_2); ^{13}C NMR (DMSO-d_6) δ: 155.3, 144.1, 138.5, 136.4, 124.1, 116.2, 114.2, 44.5, 41.3, 31.8, 25.4. MS (ESI) m/z: 239.2 (M^++1).

4.3.12. 1-(2-Chloroethyl)-3-(9H-fluoren-2-yl)urea (2l). Compound **2l** was synthesized from the nucleophilic addition of 2-aminofluorene to 2-chloroethylisocyanate. Yield: 75%; mp: 203–206 °C; IR (KBr) v: 3330 (NH), 1635 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 8.77 (s, 1H, NH), 7.76 (t, 3H, J = 7.7 Hz, Ar), 7.53 (d, 1H, J = 7.4 Hz, Ar), 7.34 (t, 2H, J = 7.1 Hz, Ar), 7.24 (t, 1H, J = 7.3 Hz, Ar), 6.46 (br s, 1H, NH), 3.88 (s, 2H, CH_2), 3.70 (t, 2H, J = 6.2 Hz, CH_2), 3.46 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 155.2, 143.9, 142.5, 141.4, 139.5, 134.7, 136.7, 125.7, 125.0, 120.2, 119.2, 116.7, 114.6, 44.5, 41.3, 36.5. MS (ESI) m/z: 287.2 (M^++1).

4.3.13. 1-(2-Chloroethyl)-3-(9H-fluoren-9-yl)urea (2m). Compound **2m** was synthesized from the nucleophilic addition of 9-aminofluorene to 2-chloroethylisocyanate. Yield: 45%; mp: 228–230 °C; IR (KBr) v: 3353 (NH), 1622 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 7.85 (d, 2H, J = 7.5 Hz, Ar), 7.56 (d, 2H, J = 7.3 Hz, Ar), 7.43 (t, 2H, J = 7.2 Hz, Ar), 7.34 (t, 2H, J = 7,3 Hz, Ar), 6.70 (br s, 1H, NH), 6.21 (br s, 1H, NH), 5.86 (d, 1H, J = 8.6 Hz, CH), 3.70 (t, 2H, J = 6.3 Hz, CH_2), 3.48 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 158.5, 145.7, 139.8, 128.3, 127.6, 124.9, 120.1, 55.2, 44.6, 41.7. MS (ESI) m/z: 287.2 (M^++1).

4.3.14. 1-(4-*tert*-Butylphenyl)-3-(2-chloroethyl)urea (tBCEU).[16] tBCEU was synthesized from the nucleophilic addition of 4-tertbutylaniline to 2-chloroethylisocyanate. Yield: 60%; mp: 130–134 °C; IR (KBr) v: 3376 (NH), 1649 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 8.56 (s, 1H, NH), 7.30 (m, 4H, Ar), 6.37 (br s, 1H, NH), 3.67 (t, 2H, J = 6.2 Hz, CH_2), 3.43 (m, 3H, CH_3), 1.26 (s, 9H, $3 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ: 156.7, 146.8, 135.6, 126.0, 120.8, 44.2, 42.0, 34.3, 31.4.

4.3.15. 1-(2-Chloroethyl)-3-(4-iodophenyl)urea (ICEU).[16] ICEU was synthesized from the nucleophilic addition of 4-iodoaniline to 2-chloroethylisocyanate. Yield: 81%; mp: 195–198 °C; IR (KBr) v: 3324 (NH), 1632 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 8.79 (s, 1H, NH), 7.55 (d, 2H, J = 8.5 Hz, Ar), 7.27 (d, 2H, J = 8.5 Hz, Ar), 6.46 (br s, 1H, NH), 3.67 (t, 2H, J = 6.1 Hz, CH_2), 3.46 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 155.4, 140.2, 137.4, 120.1, 83.9, 44.2, 42.0.

4.4. General preparation of compounds 3a–3m

To a stirred solution of the appropriate N-phenyl-N'-(2-chloroethyl)urea derivatives (CEUs) (0.35 mmol) in acetonitrile (10 mL) was added a mixture of SiO_2·KF (60:40%) (3.5 mmol). The suspension was refluxed overnight. After cooling, the mixture was filtered and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (dichloromethane/methanol 95:5).

4.4.1. 4,5-Dihydro-*N*-*o*-tolyloxazol-2-amine (3a). Compound **3a** was synthesized from **2a** and SiO_2·KF. Yield: 79%; IR (KBr) v: 3356 (NH), 1648 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.36 (d, 1H, Ar), 7.17 (m, 2H, Ar), 6.95 (t, 1H, J = 7.3 Hz, Ar), 6.02 (s, 1H, NH), 4.34 (t, 2H, J = 8.0 Hz, CH_2), 3.70 (t, 2H, J = 8.0 Hz, CH_2), 2.22 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 157.1, 142.2, 130.3, 129.1, 126.5, 122.9, 121.7, 67.1, 47.3, 17.9. MS (ESI) m/z: 177.2 (M^++1).

4.4.2. 4,5-Dihydro-*N*-*m*-tolyloxazol-2-amine (3b). Compound **3b** was synthesized from **2b** and SiO_2·KF. Yield: 29%; mp: 92–99 °C; IR (KBr) v: 3343 (NH), 1687 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.14 (m, 3H, Ar), 6.83 (d, 1H, J = 7.1 Hz, Ar), 5.24 (s, 1H, NH), 4.38 (t, 2H, J = 8.2 Hz, CH_2), 3.86 (t, 2H, J = 8.3 Hz, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 157.7, 141.3, 138.8, 128.7, 123.4, 120.3, 116.7, 67.3, 49.8, 21.5.

4.4.3. 4,5-Dihydro-*N*-*p*-tolyloxazol-2-amine (3c). Compound **3c** was synthesized from **2c** and SiO_2·KF. Yield: 51%; mp: 138–142 °C; IR (KBr) v: 3257 (NH), 1652 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.13 (d, 2H, J = 7.9 Hz, Ar), 7.06 (d, 2H, J = 8.3 Hz, Ar), 6.20 (br s, 1H, NH), 4.35 (t, 2H, J = 8.3 Hz, CH_2), 3.81 (t, 2H, J = 8.4 Hz, CH_2), 2.30 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 157.9, 139.1, 131.8, 129.1, 119.8, 67.3, 49.63, 21.0. MS (ESI) m/z: 177.1 (M^++1).

4.4.4. 4,5-Dihydro-*N*-(2,6-dimethylphenyl)oxazol-2-amine (3d). Compound **3d** was synthesized from **2d** and SiO_2·KF. Yield: 64%; IR (KBr) v: 3285 (NH), 1702 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.02 (d, 2H, J = 7.4 Hz, Ar), 6.91 (t, 1H, J = 7.1 Hz, Ar), 6.02 (s, 1H, NH), 4.35 (t, 2H, J = 7.5 Hz, CH_2), 3.57 (t, 2H, J = 7.7 Hz, CH_2), 2.17 (s, 6H, $2 \times CH_3$); ^{13}C NMR ($CDCl_3$) δ: 156.1, 143.4, 131.5, 127.8, 123.3, 67.1, 44.1, 18.3.

4.4.5. 4,5-Dihydro-*N*-(2,3-dimethylphenyl)oxazol-2-amine (3e). Compound **3e** was synthesized from **2e** and SiO_2·KF. Yield: 56%; IR (KBr) v: 3357 (NH), 1648 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.12 (d, 1H, J = 7.8 Hz, Ar), 7.04 (t, 1H, J = 7.53 Hz, Ar), 6.87 (d, 1H, J = 7.3 Hz, Ar), 5.80 (s, 1H, NH), 4.36 (t, 2H, J = 8.0 Hz, CH_2), 3.69 (t, 2H, J = 8.0 Hz, CH_2), 2.28 (s, 3H, CH_3), 2.14 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 157.2, 142,5, 137.3, 128.5, 125.7, 125.0, 120.2, 67.1, 46.6, 20.6, 13.7. MS (ESI) m/z: 191.3 (M^++1).

4.4.6. 4,5-Dihydro-*N*-(2,4-dimethylphenyl)oxazol-2-amine (3f). Compound **3f** was synthesized from **2f** and SiO_2·KF. Yield: 54%; IR (KBr) v: 3378 (NH), 1694 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.23 (m, 1H, Ar), 6.97 (m, 2H, Ar), 6.03 (s, 1H, NH), 4.34 (t, 2H, J = 8.0 Hz, CH_2), 3.70 (t, 2H, J = 8.0 Hz, CH_2), 2.28 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 157.5, 139.2, 132.5, 131.1, 129.3, 127.0, 122.0, 67.2, 47.5, 20.8, 17.8. MS (ESI) m/z: 191.3 (M^+ + 1).

4.4.7. 4,5-Dihydro-*N*-(2,5-dimethylphenyl)oxazol-2-amine (3g). Compound **3g** was synthesized from **2g** and SiO_2·KF. Yield: 75%; mp: 105–110 °C; IR (KBr) v:

3179 (NH), 1682 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.19 (s, 1H, Ar), 7.03 (d, 1H, $J = 7.6$ Hz, Ar), 6.78 (d, 1H, $J = 7.4$ Hz, Ar), 6.36 (s, 1H, NH), 4.34 (t, 2H, $J = 8.0$ Hz, CH_2), 3.71 (t, 2H, $J = 8.0$ Hz, CH_2), 2.31 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 157.2, 141.9, 136.1, 130.1, 126.0, 123.8, 122.4, 67.1, 47.4, 21.2, 17.5.

4.4.8. 4,5-Dihydro-*N*-(3,4-dimethylphenyl)oxazol-2-amine (3h). Compound **3h** was synthesized from **2h** and SiO_2·KF. Yield: 95%; mp: 135–140 °C; IR (KBr) *v*: 3198 (NH), 1691 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.02 (m, 3H, Ar), 6.59 (s, 1H, NH), 4.36 (t, 2H, $J = 8.3$ Hz, CH_2), 3.80 (t, 2H, $J = 8.4$ Hz, CH_2), 2.21 (d, 6H, $J = 5.6$ Hz, $2 \times CH_3$); ^{13}C NMR ($CDCl_3$) δ: 158.0, 139.3, 137.0, 130.5, 130.0, 121.1, 117.2, 67.3, 49.9, 19.9, 19.0. MS (ESI) *m*/*z*: 191.2 (M^++1).

4.4.9. 4,5-Dihydro-*N*-(3,5-dimethylphenyl)oxazol-2-amine (3i). Compound **3i** was synthesized from **2i** and SiO_2·KF. Yield: 77%; mp: 118–125 °C; IR (KBr) *v*: 3246 (NH), 1712 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.92 (s, 2H, Ar), 6.86 (s, 1H, Ar), 6.65 (s, 1H, NH), 4.36 (t, 2H, $J = 8.3$ Hz, CH_2), 3.85 (t, 2H, $J = 8.4$ Hz, CH_2), 2.28 (s, 6H, $2 \times CH_3$); ^{13}C NMR ($CDCl_3$) δ: 157.9, 141.3, 138.5, 124.2, 117.3, 67.3, 50.1, 21.4. MS (ESI) *m*/*z*: 191.2 (M^++1).

4.4.10. 4,5-Dihydro-*N*-(2,3-dihydro-1H-inden-4-yl)oxazol-2-amine (3j). Compound **3j** was synthesized from **2j** and SiO_2·KF. Yield: 78%; mp: 72–79 °C; IR (KBr) *v*: 3360 (NH), 1701 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.37 (d, 1H, $J = 7.8$ Hz, Ar), 7.11 (t, 1H, $J = 7.6$ Hz, Ar), 6.91 (d, 2H, $J = 7.3$ Hz, Ar), 5.91 (s, 1H, NH), 4.35 (t, 2H, $J = 8.2$ Hz, CH_2), 3.80 (t, 2H, $J = 8.2$ Hz, CH_2), 2.93. (t, 2H, $J = 7.4$ Hz, CH_2), 2.79 (t, 2H, $J = 7.3$ Hz, CH_2), 1.27 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ: 157.3, 145.1, 138.4, 133.9, 127.1, 118.9, 117.7, 67.2, 49.3, 33.3, 30.1, 24.8. MS (ESI) *m*/*z*: 203.2 (M^++1).

4.4.11. 4,5-Dihydro-*N*-(2,3-dihydro-1H-inden-5-yl)oxazol-2-amine (3k). Compound **3k** was synthesized from **2k** and SiO_2·KF. Yield: 68%; mp: 130–134 °C; IR (KBr) *v*: 3352 (NH), 1679 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.15 (s, 1H, Ar), 7.10 (d, 1H, $J = 7.5$ Hz, Ar), 6.99 (d, 1H, $J = 7.9$ Hz, Ar), 4.36 (t, 2H, $J = 8.3$ Hz, CH_2), 3.82 (t, 2H, $J = 8.4$ Hz, CH_2), 2.85 (m, 4H, $2 \times CH_2$), 2.05 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ: 158.1, 145.1, 139.8, 138.2, 124.4, 118.0, 116.1, 67.3, 49.8, 33.1, 32.3, 25.6. MS (ESI) *m*/*z*: 203.2 (M^++1).

4.4.12. *N*-(9H-Fluoren-2-yl)-4,5-dihydrooxazol-2-amine (3l). Compound **3l** was synthesized from **2l** and SiO_2·KF. Yield: 74%; mp: 168–172 °C; IR (KBr) *v*: 3208 (NH), 1687 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.68 (m, 3H, Ar), 7.50 (d, 1H, $J = 7.4$ Hz, Ar), 7.35 (t, 1H, $J = 7.4$ Hz, Ar), 7.23 (m, 2H, Ar), 5.24 (br s, 1H, NH), 4.41 (t, 2H, $J = 9.0$ Hz, CH_2), 3.89 (t, 4H, $J = 9.0$ Hz, $2 \times CH_2$); ^{13}C NMR ($CDCl_3$) δ: 157.5, 144.5, 143.0, 141.6, 140.3, 136.6, 126.7, 125.9, 124.9, 120.2, 119.2, 118.5, 116.3, 67.3, 49.9, 37.0. MS (ESI) *m*/*z*: 251.2 (M^++1).

4.4.13. *N*-(9H-Fluoren-9-yl)-4,5-dihydrooxazol-2-amine (3m). Compound **3m** was synthesized from **2m** and SiO_2·KF. Yield: 48%; mp: 155–158 °C; IR (KBr) *v*: 3175 (NH), 1648 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.64 (m, 4H, Ar), 7.31 (m, 4H, Ar), 5.77 (s, 1H, NH), 4.62 (s, 1H, CH), 4.28 (t, 2H, $J = 8.4$ Hz, CH_2), 3.78 (t, 2H, $J = 8.5$ Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ: 161.9, 144.9, 140.2, 128.6, 127.6, 125.3, 119.8, 68.3, 58.4, 52.4. MS (ESI) *m*/*z*: 251.2 (M^++1).

4.4.14. *N*-(4-*tert*-Butylphenyl)-4,5-dihydrooxazol-2-amine (tBOXA). tBOXA was synthesized from **tBCEU** and SiO_2·KF. Yield: 47%; mp: 150–154 °C; IR (KBr) *v*: 3186 (NH), 1698 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.28 (d, 2H, $J = 8.7$ Hz, Ar), 7.19 (d, 2H, $J = 8.7$ Hz, Ar), 5.52 (s, 1H, NH), 4.38 (t, 2H, $J = 8.3$ Hz, CH_2), 3.82 (t, 2H, $J = 8.3$ Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ: 156.7, 143.4, 138.7, 125.1, 117.6, 65.9, 51.3, 38.4, 31.3.

4.4.15. 4,5-Dihydro-*N*-(4-iodophenyl)oxazol-2-amine (IOXA). IOXA was synthesized from **ICEU** and SiO_2·KF. Yield: 56%; mp: 126–131 °C; IR (KBr) *v*: 3185 (NH), 1679 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.48 (d, 2H, $J = 8.6$ Hz, Ar), 6.94 (m, 2H, $J = 8.7$ Hz, Ar), 4.35 (t, 2H, $J = 8.2$ Hz, CH_2), 3.69 (t, 2H, $J = 8.3$ Hz, CH_2), 3.65 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 157.1, 140.5, 137.1, 128.8, 87.5, 65.5, 51.4.

4.5. General preparation of compounds 4a–4m

2-Chloroacetylisocyanate (3.6 mmol) was added dropwise to a stirred solution of the relevant aniline (3 mmol) in dichloromethane (15 mL). The reaction mixture was stirred overnight at ambient temperature. The resulting crude precipitate was filtered, washed with cold ether, and purified by recrystallization from ethanol and water.

4.5.1. 1-(2-Chloroacetyl)-3-*o*-tolylurea (4a). Compound **4a** was synthesized from the nucleophilic addition of *o*-toluidine to 2-chloroacetylisocyanate. Yield: 63%; mp: 145–146 °C; IR (KBr) *v*: 3235 (NH), 1709 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 11.06 (s, 1H, NH), 10.18 (s, 1H, NH), 7.94 (d, 1H, $J = 8.0$ Hz, Ar), 7.23 (m, 2H, Ar), 7.06 (t, 1H, $J = 7.3$ Hz, Ar), 4.42 (s, 2H, CH_2), 2.27 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 169.1, 150.3, 135.8, 130.4, 127.7, 126.5, 124.1, 121.0, 43.2, 17.5. MS (ESI) *m*/*z*: 227.0 (M^++1).

4.5.2. 1-(2-Chloroacetyl)-3-*m*-tolylurea (4b). Compound **4b** was synthesized from the addition of *m*-toluidine to 2-chloroacetylisocyanate. Yield: 95%; mp: 227–228 °C; IR (KBr) *v*: 3249 (NH), 1706 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.91 (s, 1H, NH), 10.13 (s, 1H, NH), 7.35 (m, 2H, Ar), 7.23 (t, 1H, $J = 7.8$ Hz, Ar), 6.93 (d, 1H, $J = 7.4$ Hz, Ar), 4.41 (s, 2H, CH_2), 2.30 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 168.7, 150.2, 138.4, 137.4, 128.8, 124.6, 120.2, 116.9, 43.2, 21.1. MS (ESI) *m*/*z*: 227.0 (M^++1).

4.5.3. 1-(2-Chloroacetyl)-3-*p*-tolylurea (4c). Compound **4c** was synthesized from the nucleophilic addition of *p*-toluidine to 2-chloroacetylisocyanate. Yield: 59%; mp: 189–192 °C; IR (KBr) *v*: 3238 (NH), 1720 (C=O)

cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.89 (s, 1H, NH), 10.11 (s, 1H, NH), 7.42 (d, 2H, $J = 8.2$ Hz, Ar), 7.15 (d, 2H, $J = 8.1$ Hz, Ar), 4.40 (s, 2H, CH_2), 2.27 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 168.7, 150.2, 134.9, 132.9, 129.4, 119.8, 43.2, 20.4. MS (ESI) *m/z*: 227.0 (M^++1).

4.5.4. 1-(2-Chloroacetyl)-3-(2,6-dimethylphenyl)urea (4d). Compound **4d** was synthesized from the nucleophilic addition of 2,6-dimethylaniline to 2-chloroacetylisocyanate. Yield: 100%; mp: 203–205 °C; IR (KBr) *v*: 3285 (NH), 1730 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.90 (s, 1H, NH), 9.52 (s, 1H, NH), 7.12 (s, 3H, Ar), 4.41 (s, 2H, CH_2), 2.20 (s, 6H, $2 \times CH_3$) ; ^{13}C NMR (DMSO-d_6) δ: 168.6, 150.7, 135.1, 133.9, 127.8, 126.8, 43.2, 18.1. MS (ESI) *m/z*: 241.1 (M^++1).

4.5.5. 1-(2-Chloroacetyl)-3-(2,3-dimethylphenyl)urea (4e). Compound **4e** was synthesized from the nucleophilic addition of 2,3-dimethylaniline to 2-chloroacetylisocyanate. Yield: 69%; mp: 168–170 °C; IR (KBr) *v*: 3245 (NH), 1716 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.98 (s, 1H, NH), 10.10 (s, 1H, NH), 7.68 (d, 1H, $J = 8.0$ Hz, Ar), 7.09 (t, 1H, $J = 7.7$ Hz, Ar), 6.99 (d, 1H, $J = 7.4$ Hz, Ar), 4.41 (s, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.14 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 169.0, 150.5, 136.9, 135.5, 127.3, 126.1, 125.6, 120.0, 43.2, 20.3, 13.3. MS (ESI) *m/z*: 241.1 (M^++1).

4.5.6. 1-(2-Chloroacetyl)-3-(2,4-dimethylphenyl)urea (4f). Compound **4f** was synthesized from the nucleophilic addition of 2,4-dimethylaniline to 2-chloroacetylisocyanate. Yield: 20%; mp: 171–173 °C; IR (KBr) *v*: 3235 (NH), 1698 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 11.00 (s, 1H, NH), 10.08 (s, 1H, NH), 7.77 (d, 1H, $J = 8.2$ Hz, Ar), 7.03 (m, 2H, Ar), 4.40 (s, 2H, CH_2), 2.24 (d, 6H, $J = 11.7$ Hz, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ: 169.0, 150.3, 133.20, 133.17, 130.9, 127.8, 126.9, 121.2, 43.1, 20.4, 17.4. MS (ESI) *m/z*: 241.1 (M^++1).

4.5.7. 1-(2-Chloroacetyl)-3-(2,5-dimethylphenyl)urea (4g). Compound **4g** was synthesized from the nucleophilic addition of 2,5-dimethylaniline to 2-chloroacetylisocyanate. Yield: 90%; mp: 178–179 °C; IR (KBr) *v*: 3241 (NH), 1711 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 11.04 (s, 1H, NH), 10.14 (s, 1H, NH), 7.79 (s, 1H, Ar), 7.11 (d, 1H, $J = 7.6$ Hz, Ar), 6.86 (d, 1H, $J = 7.4$ Hz, Ar), 4.41 (s, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-V) δ: 169.1, 150.2, 135.6, 135.5, 130.1, 124.6, 124.5, 121.4, 43.1, 20.9, 17.1. MS (ESI) *m/z*: 241.1 (M^++1).

4.5.8. 1-(2-Chloroacetyl)-3-(3,4-dimethylphenyl)urea (4h). Compound **4h** was synthesized from the nucleophilic addition of 3,4-dimethylaniline to 2-chloroacetylisocyanate. Yield: 39%; mp: 148–150 °C; IR (KBr) *v*: 3239 (NH), 1700 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.88 (s, 1H, NH), 10.07 (s, 1H, NH), 7.27 (m, 2H, Ar), 7.08 (d, 1H, $J = 7.8$ Hz, Ar), 4.39 (s, 2H, CH_2), 2.19 (d, 6H, $J = 8.1$ Hz, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ: 168.7, 150.2, 136.7, 135.1, 131.7, 129.8, 120.9, 117.2, 43.2, 19.5, 18.7. MS (ESI) *m/z*: 241.1 (M^++1).

4.5.9. 1-(2-Chloroacetyl)-3-(3,5-dimethylphenyl)urea (4i). Compound **4i** was synthesized from the nucleophilic addition of 3,5-dimethylaniline to 2-chloroacetylisocyanate. Yield: 75%; mp: 178–183 °C; IR (KBr) *v*: 3256 (NH), 1703 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.89 (s, 1H, NH), 10.09 (s, 1H, NH), 7.16 (s, 2H, Ar), 6.76 (s, 1H, Ar), 4.40 (s, 2H, CH_2), 2.26 (s, 6H, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ: 168.8, 150.1, 138.2, 137.3, 125.4, 117.4, 43.2, 21.0. MS (ESI) *m/z*: 241.1 (M^++1).

4.5.10. 1-(2-Chloroacetyl)-3-(2,3-dihydro-1H-inden-4-yl)-urea (4j). Compound **4j** was synthesized from the nucleophilic addition of 4-aminoindan to 2-chloroacetylisocyanate. Yield: 74%; mp: 203–205 °C; IR (KBr) *v*: 3238 (NH), 1716 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 11.04 (s, 1H, NH), 10.16 (s, 1H, NH), 7.79 (d, 1H, $J = 8.0$ Hz, Ar), 7.14 (t, 1H, $J = 7.6$ Hz, Ar), 7.00 (d, 1H, $J = 7.9$ Hz, Ar), 4.41 (s, 2H, CH_2), 2.91 (t, 2H, $J = 7.3$ Hz, CH_2), 2.81 (t, 2H, $J = 7.2$ Hz, CH_2), 2.07 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 169.1, 150.1, 144.8, 133.7, 133.0, 127.1, 119.9, 117.1, 43.2, 32.8, 29.6, 24.3. MS (ESI) *m/z*: 253.1 (M^++1).

4.5.11. 1-(2-Chloroacetyl)-3-(2,3-dihydro-1H-inden-5-yl)-urea (4k). Compound **4k** was synthesized from the nucleophilic addition of 5-aminoindan to 2-chloroacetylisocyanate. Yield: 24%; mp: 170–174°C; IR (KBr) *v*: 3257 (NH), 1704 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.89 (s, 1H, NH), 10.11 (s, 1H, NH), 7.44 (s, 1H, Ar), 7.18 (m, 2H, Ar), 4.39 (s, 2H, CH_2), 2.85 (m, 4H, $2 \times CH_2$), 2.01 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 168.7, 150.2, 144.5, 139.2, 135.5, 124.4, 117.9, 116.0, 43.2, 32.5, 31.8, 25.2. MS (ESI) *m/z*: 253.1 (M^++1).

4.5.12. 1-(2-Chloroacetyl)-3-(9H-fluoren-2-yl)urea (4l). Compound **4l** was synthesized from the nucleophilic addition of 2-aminofluorene to 2-chloroacetylisocyanate. Yield: 78%; mp: 235–236 °C; IR (KBr) *v*: 3234 (NH), 1712 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.97 (s, 1H, NH), 10.29 (s, 1H, NH), 7.85 (d, 3H, $J = 8.8$ Hz, Ar), 7.57 (d, 1H, $J = 7.29$ Hz, Ar), 7.49 (d, 1H, $J = 8.1$ Hz, Ar), 7.37 (t, 1H, $J = 7,3$ Hz, Ar), 7.29 (t, 1H, $J = 7.3$ Hz, Ar), 4.43 (s, 2H, CH_2), 3.92 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 155.3, 136.8, 130.6, 129.4, 127.4, 123.6, 122.9, 113.3, 112.9, 111.6, 106.9, 106.2, 105.2, 103.2, 29.8, 23.1. MS (ESI) *m/z*: 301.1 (M^++1).

4.5.13. 1-(2-Chloroacetyl)-3-(9H-fluoren-9-yl)urea (4m). Compound **4m** was synthesized from the nucleophilic addition of 9-aminofluorene to 2-chloroacetylisocyanate. Yield: 48%; mp: 205–208 °C; IR (KBr) *v*: 3324 (NH), 1729 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.91 (s, 1H, NH), 8.44 (s, 1H, NH), 7.88 (d, 2H, $J = 7.4$ Hz, Ar), 7.60 (d, 2H, $J = 7.3$ Hz, Ar), 7.46 (t, 2H, $J = 7.3$ Hz, Ar), 7.36 (t, 2H, $J = 7,3$ Hz, Ar), 5.98 (d, 1H, $J = 8.0$ Hz, CH), 4.36 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 168.5, 153.5, 144.2, 140.0, 128.8, 127.9, 124.8, 120.4, 54.7, 43.1. MS (ESI) *m/z*: 301.1 (M^++1).

4.5.14. 1-(4-*tert*-Butylphenyl)-3-(2-chloroacetyl)urea (tBCAU). tBCAU was synthesized from the nucleophilic addition of 4-tertbutylaniline to 2-chloroacetylisocyanate.

Yield: 60%; mp: 170–173 °C; IR (KBr) *v*: 3240 (NH), 1714 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.22 (s, 1H, NH), 9.63 (s, 1H, NH), 7.35 (m, 4H, Ar), 4.18 (s, 2H, CH_2), 1.32 (s, 9H, 3 X CH_3); ^{13}C NMR (DMSO-d_6) δ: 168.6, 150.2, 146.3, 134.8, 125.6, 119.7, 43.2, 34.1, 31.2.

4.5.15. 1-(2-Chloroacetyl)-3-(4-iodophenyl)urea (ICAU). **ICAU** was synthesized from the nucleophilic addition of 4-iodoaniline to 2-chloroacetylisocyanate. Yield: 81%; mp: 235–240 °C; IR (KBr) *v*: 3237 (NH), 1718 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.93 (s, 1H, NH), 10.16 (s, 1H, NH), 7.66 (d, 2H, $J = 8.7$ Hz, Ar), 7.37 (d, 2H, $J = 8.7$ Hz, Ar), 4.38 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 168.7, 156.3, 137.0, 128.0, 83.6, 66.0.

4.6. Biological assays

4.6.1. Materials and reagents. Vinblastine sulfate, colchicine, paclitaxel, and the monoclonal antibody anti-β-tubulin (clone TUB 2.1) were obtained from Sigma Chemicals (St. Louis, MO). The peroxidase-conjugated anti-mouse immunoglobulin and ECL Western blotting detection reagent kit were purchased from Amersham Canada (Oakville, Canada). Propidium iodide and RNase were purchased from (Boehringer Mannheim, Laval, Canada).

4.6.2. Cell lines and tissue culture. HT-29 human colon carcinoma, M21 human skin melanoma, and MCF-7 human breast carcinoma cells were purchased from the American Type Culture Collection. The cells were cultured in calf serum iron supplemented (Hyclone) medium containing $NaHCO_3$ (2.2 g/L), glucose (4.5 g/L), and glutamine (292 μg/mL). The cells were maintained at 37 °C in a moisture-saturated atmosphere containing 5% CO_2.

4.6.3. Tumor cell growth inhibition activity. The growth inhibition potency of CEUs was assessed using the procedure described by the National Cancer Institute for its drug screening program.[22] 96-well microtiter plates were seeded with 100 μL of tumor cell lines in calf serum iron supplemented (Hyclone) medium. Plates were incubated at 37 °C, 5% CO_2 for 24 h. Freshly solubilized drugs in DMSO were diluted in fresh medium and aliquots of 100 μL containing sequential dilution of drugs were added. Final drug concentrations ranged from 10 to 0.3 μM. DMSO concentration was maintained lower than 0.5% to avoid growth inhibition. Plates were incubated for 48 h. Assays were stopped by addition of cold trichloroacetic acid to the wells (10% final concentration), followed by incubation for 1 h at 4 °C. Plates were washed five times with water. Sulforhodamine B solution (50 μL) at 0.1% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 15 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid. Bonded dye was solubilized with 10 mM Tris base, and the absorbance was read using a μQuant Universal Microplate Spectrophotometer (Biotek, Winooski, VT) at 585 nm. A background OD from a control reference plate fixed on the day of treatment was subtracted from the OD obtained with the 48-h growth period. The growth inhibition percentage was calculated in reference to the control DMSO-treated cells for each drug concentration. The experiments were performed at least in triplicate. The GI_{50} assay was considered valid when the variability among data for a given set of conditions, within the same experiment, was less than 10% with respect to the mean value.

4.6.4. Cell cycle analysis. After incubation of 3.5×10^5 M21 cells with compounds (CAU, CEU or OXA) at three concentrations (3, 10, and $30 \times GI_{50}$) or DMSO for 24 h, the cells were harvested by trypsinization, washed with PBS, resuspended in 1 mL of PBS, and fixed by the addition of 2.4 mL of ice-cold ethanol (anhydrous). Then, cells were centrifuged for 5 min at 1000*g*. Cell pellets were resuspended in PBS containing 50 μg/mL of propidium iodide and 200 μg/mL of RNase. Mixtures were incubated at room temperature for 30 min, and cell cycle distribution was analyzed using an Epics Elite ESP flow cytometer (Coulter Corporation, Miami, FL).

4.6.5. Western blot analysis of β-tubulin monomer. Prior to drug exposure, approximately 5×10^5 M21 human melanoma cells were seeded into six-well plates and incubated for 24 h. Exponentially growing M21 human melanoma cells were incubated in the presence of 25 μM test compound, except for controls, for 0, 6, 12, and 24 h at 37 °C. The control consisted of the final concentration of DMSO in the culture medium (maintained under 0.5% (v/v)). Vinblastine sulfate, colchicine, and paclitaxel were tested at a concentration of 50 nM. After the treatments, floating and adherent M21 cells were pooled, washed in ice-cold PBS, and then lysed in 100 μL Laemmli sample buffer 1×. The cell extracts were boiled for 5 min, separated on 10% SDS–PAGE electrophoresis gel, and transferred onto nitrocellulose membrane. The membranes were incubated with TBSMT (TBS, pH 7.4, 5% fat-free dried milk, and 0.1% Tween 20) for 1 h at 37 °C, and then with 1:500 monoclonal anti-β-tubulin (clone TUB 2.1) for 1 h at room temperature. Membranes were washed with TBST and incubated with 1:2500 peroxidase-conjugated anti-mouse immunoglobulin in TBSMT for 1 h at room temperature. After washing the membranes with TBST, detection of the immunoblot was carried out with an enhanced chemiluminescence (ECL) detection reagent kit.

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