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# *N*-Phenyl-*N*′-(2-chloroethyl)ureas (CEUs) as potential antineoplastic agents. Part 3: Role of carbonyl groups in the covalent binding to the colchicine-binding site

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**Abstract**—In the course of the development of *N*-phenyl-*N*′-(2-chloroethyl)ureas (CEUs) as potential antineoplastic agents, we investigated the effect of carbonylated substituting chains of the aromatic ring of CEU on their covalent binding to the colchicine-binding site (C-BS). In this study, we found that CEU, **5e**, **5f**, **8e**, and **8f** substituted by either a methyl ester or a methyl ketyl group at the ω-position exhibited a significant antiproliferative activity on HT-29, M21, and MCF-7 tumor cells. SDS–PAGE assays and cell cycle analysis confirmed that **5e**, **5f**, **8e**, and **8f** covalently bind to the C-BS and arrest the cell division in $G_2$/M phase. Surprisingly, the presence of ω-carboxyl, ω-ethyl esters or ω-amides decreased significantly both the antiproliferative activity and the specificity toward β-tubulin.


## 1. Introduction

For the past decades tubulin has been an attractive target for the development of new anticancer drugs.[1–3] So far, several small-molecule antimitotics have been developed to antagonize three major binding-sites on tubulin, namely, the vinca-, the taxus-, and the colchicine-binding sites (C-BS).[4] While drugs interacting with the vinca- or the taxus-binding sites are involved in clinic for the treatment of several cancers, the therapeutic potential of drugs targeting the C-BS is still under evaluation.[5–7] However, recent results published by Ravelli[8] and coworkers confirmed several assumptions concerning the molecular interactions occurring between colchicinoids and the C-BS that might help to the design of new antimitotics. The binding of colchicinoids into the C-BS seems to follow a bidentate mechanism where the ring A and the ring C bind to their respective subsites on tubulin through thermodynamically independent reaction.[9] The three methoxyl groups on the ring A of colchicinoids act as a complex-stabilizing anchor,[9] which is requisite for the molecule to penetrate and lock itself into a specific conformation, bringing the oxygen atom on ring C (or C′) in contact with key group amino acids in the C-BS through hydrogen bonds or π-bond interactions.[6,10] In the past decades, a large number of natural and synthetic small molecules have been identified as colchicine-binding site antagonists. In order to investigate a common pharmacophore for C-BS antagonists, Nguyen[9] established two classes of C-BS antagonists. First a group of compounds structurally related to colchicine through the presence in their structures of a diaryl system, and a mono to a trimethoxyphenyl

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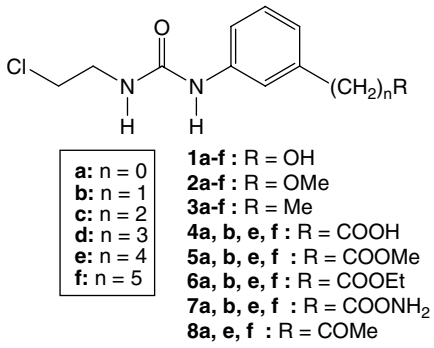



**Figure 1.** General formula of new 1-(2-chloroethyl)-3-phenylurea derivatives.

moiety that gave rise to molecules such as ZD6126,[9] combretastatin,[11] phenstatin,[12] chalcones,[13] A-105972 and A-289099,[14] AVE8062[15], and Oxi4503.[16] Second, a group of structurally unrelated molecules combining miscellaneous compounds such as nocodazole,[17] 2-methoxyestradiol,[18] curacin A,[19] E7010,[20] aryl 2-haloacetamides,[21] and *N*-phenyl-*N*′-(2-chloroethyl) urea (CEUs).[22–27] The latter molecules have been shown to covalently bind to the C-BS and to trigger apoptosis through cytoskeleton disruption. Recently, we published a study showing that the antiproliferative activity of CEUs is significantly improved by substitution of the phenyl ring on position 3 by lower alkyl ω-hydroxylated chains (Fig. 1, **1a**–**f**) while the drugs were still covalently binding to the C-BS.[24,25]

We have recently studied the role of a ω-hydroxyl terminal group on the covalent binding of CEU to β-tubulin (Fig. 1, compounds **2a**–**f** and **3a**–**f**). Moreover, we have demonstrated also that the pharmacophoric moiety *N*-(2-chloroethyl)urea plays an 'anchoring' action similar to the trimethoxy phenyl moiety of colchicinoids.[26] To improve specific steric contacts occurring between the 2-chloroethylamino moiety of CEUs and a key amino acid (either Glu[198] in $\beta_{IV}$-tubulin[23] or Cys[239] in $\beta_{II}$-tubulin[22]) nearby the C-BS, we have prepared four new series of CEU derivatives, namely, ω-carboxyl, ω-esters, ω-amides and ω-ketyl derivatives (Fig. 1, compounds **4**, **5**, **6**, **7**, and **8**).

These various molecular modifications were selected, notably, on the basis that they proved beneficial to the biological affinity of colchicinoids such as ALLO, KAC, MAC, TCB, TKB, and TMB[28,29] (Fig. 2) and that they contain oxygen atoms susceptible to mimic the interactions of the oxygen atom present on the ring C of colchicinoids.

## 2. Results and discussion

### 2.1. Chemistry

Four new series of lower alkyl CEU derivatives functionalized by a ω-terminal group, namely, carboxyl (**4a**, **4b**, **4e**, **4f**), ester (**5a**, **5b**, **5e**, **5f** and **6a**, **6b**, **6e**, **6f**), amidyl (**7a**, **7b**, **7e**, **7f**), and ketyl (**8a**, **8e**, **8f**), were prepared using two different synthetic pathways depicted in Scheme 1.

CEUs bearing lower alkyl chains (n < 3) were prepared using compounds **9a**, **9b**, and **17a** as starting material. Homologous CEU derivatives bearing alkyl chains containing 4 and 5 carbon atoms, respectively, were prepared via a Sonogashira reaction involving the palladium-catalyzed coupling of 1-iodo-3-nitrobenzene **18** to the appropriate alkyne.[24,25]

Ester compounds **10a**, **10b**, **11a**, **11b**, **20e**, **20f**, and **21e**, **21f** were prepared from **9a**, **9b**, **19e**, and **19f** in methanol or ethanol at reflux in presence of catalytic amounts of p-toluenesulfonic acid. Carboxamides **12a**, **12b** and **22e**, **22f** were obtained by conversion of the carboxylated compounds into their corresponding acid chlorides followed by addition of a 25% aqueous ammonium hydroxide solution in toluene. Catalytic reduction of the nitro group or/and the alkyne function using Fe/HCl or $H_2$/Pd afforded derivatives **13a**, **13b**, **13e**, **13f**, **14a**, **14b**, **14e**, **14f**, **15a**, **15b**, **15e**, **15f**, **16a**, **16b**, **16e**, **16f**, and **17e**, **17f**.[24] The latter compounds were finally reacted with 2-chloroethylisocyanate to obtain the corresponding CEU **4a**, **4b**, **4e**, **4f**, **5a**, **5b**, **5e**, **5f**, **6a**, **6b**, **6e**, **6f**, **7a**, **7b**, **7e**, **7f**, and **8a**, **8e**, **8f**.

### 2.2. Tumor cell growth inhibition activity

Tumor cell growth inhibition activity of newly synthesized acids (**4a**, **4b**, **4e**, **4f**), methyl esters (**5a**, **5b**, **5e**, **5f**), ethyl esters (**6a**, **6b**, **6e**, **6f**), amides (**7a**, **7b**, **7e**, **7f**), and ketones (**8a**, **8e**, **8f**) was evaluated on HT-29 human colon carcinoma, M21 human skin melanoma, and MCF-7 human breast carcinoma cell lines. Cell growth inhibition was assessed according to the NCI/NIH Developmental Therapeutics Program.[30]

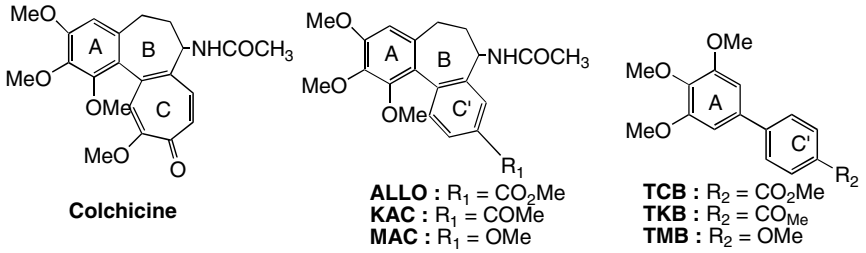
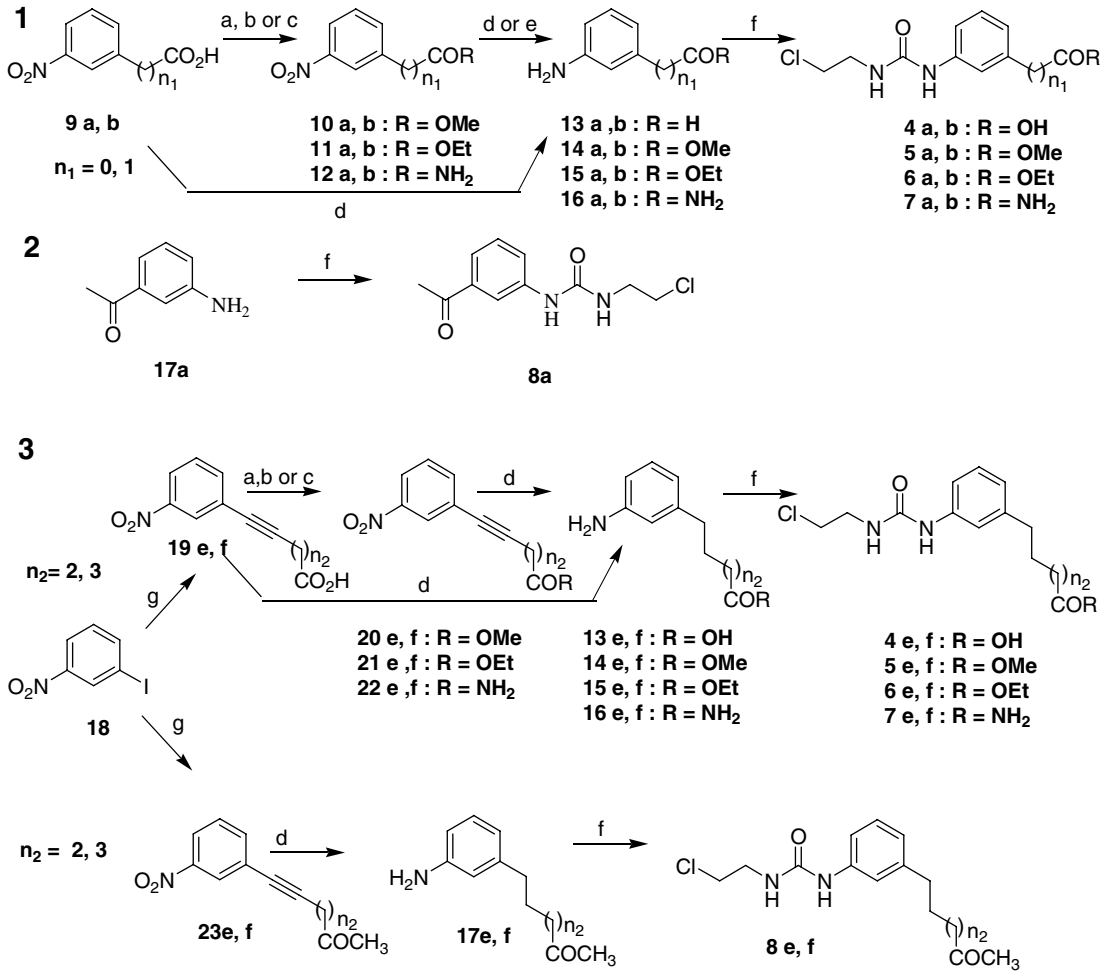



**Figure 2.** Molecular structures of colchicine and colchicinoids. Colchicine is a semi-planar molecule where ring A and ring C are oriented at 54° with respect to each other.[27]



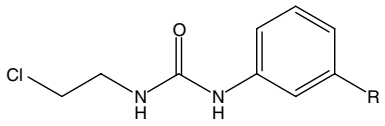

**Scheme 1.** General synthesis of 1-(2-chloroethyl)-3-phenylureas. Reagents and conditions : (a) MeOH, APTS; (b) EtOH, APTS; (c) 1—SOCl2 ; 2—$NH_4OH$/toluene; (d) $H_2$, Pd/C; (e) Fe/HCl; (f) 2-chloroethylisocyanate; (g) (j) $K_2CO_3$, CuI, $PPh_3$, alcyne/1,2-DME/water.

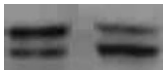
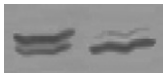
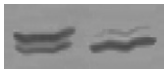
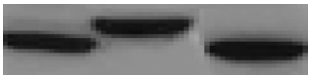
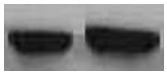
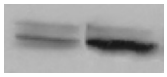
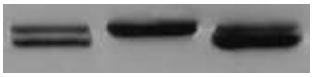
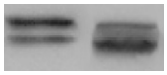
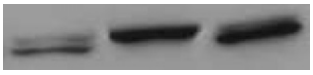
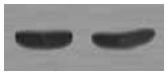
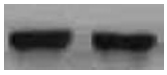
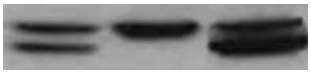
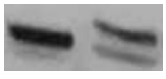
CEUs were tested at sequential dilutions ranging from 0.1 to 100 μM. The antiproliferative activities of CEU are listed in Table 1 and expressed as the concentration of drug inhibiting cell growth by 50% ($GI_{50}$). The parent urea derivatives **1f**, **2f**, and **3e** were also tested as references.[25] Structure-activity relationship studies focused on the effect of carbonyl terminal groups present on the side chain substituting the phenyl ring of *N*-phenyl-*N*′-(2-chloroethyl)ureas. As shown in Table 1, compounds **4a**, **4b**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**, and **8a** carrying a lower alkyl chain ($n < 3$) were inactive. These results confirm our previous observations on ω-hydroxylated and methoxylated CEU.[25] Compounds **4f**, **5e**, **5f**, **6e**, **6f**, **7e**, **7f**, and **8e**, **8f** showed $GI_{50}$ values ranging from 0.6 to 47.5 μM. However, compound **4e** did not show any significant antiproliferative activity. Three interesting effects were observed: First, the presence of an alkyl chain having more than 3 carbon atoms seems to be prerequisite for significant $GI_{50}$. Second, the substitution of the side chain by a carboxylic group (**4**–**7**) led to a dramatic decrease of the $GI_{50}$ when compared to compound **1f**. Finally, the ketyl group gave rise to molecules as active as compound **1f**. Antiproliferative activity of CEU substituted by a lower alkyl chain seems to be dependent on the nature of the ω-terminal group. In summary, the antiproliferative activity of CEU increases according to the following order: $OH > OCH_3 \approx CH_3 \approx COCH_3 \ggg CO_2CH_3 > CONH_2 > CO_2\ C_2H_5 > CO_2H$.

### 2.3. β-Tubulin alkylation

Concerning the alkylation of the $\beta_{II}$-tubulin, we chose to evaluate the most potent compounds of each series. Therefore, the potency of compounds **4f**, **5e**, **5f**, **6e**, **7e,** and **8e**, **8f** was assessed by SDS–PAGE followed by Western blotting as reported previously.[22] The appearance of a second band exhibiting an apparent faster $\beta_{II}$-tubulin mobility indicates the presence of $\beta_{II}$-tubulin alkylated by CEU (see Table 1).[22,31] Interestingly, compounds **6e** and **7e** were cytotoxic without any covalent binding to the C-BS. However, the methyl ester **5e**, **5f** and the ketyl homologues **8e** and 8**f** exhibited a significantly higher antiproliferative effect and a low affinity for C-BS. This suggests that the CEUs' inhibitory activity and affinity for $\beta_{II}$-tubulin are certainly related but cannot account as the only parameters of structure-activity relationships describing that class of compounds. In that context, small changes in the molecular structure of CEU may result in a

**Table 1.** $GI_{50}$ values, electrophoretic mobility assays, and competition assays of 1-(2-chloroethyl)-3-phenylurea derivatives




| Compound | $GI_{50}$ (μM) | | | Alkylated β-tubulin 24 h 48 h | Competition (48 h) with CTRL Col (5 μM) Vinb (5 μM) |
|---|---|---|---|---|---|
| | HT-29 | M21 | MCF-7 | | |
| DMSO | | | |  |  |
| **1f** R = –$(CH_2)_5$–OH | 0.16 | 0.27 | 0.48 |  |  |
| **2f** R = –$(CH_2)_5$–OMe | 0.84 | 0.94 | 1.9 |  |  |
| **3e** R = –$(CH_2)_4$–$CH_3$ | 0.96 | 0.87 | 1.6 |  |  |
| **4a** R = –COOH | 68.1 | 67.9 | 82.9 | | |
| **4b** R = –$CH_2$–COOH | >100 | >100 | >100 | | |
| **4e** R = –$(CH_2)_4$–COOH | >100 | >100 | >100 |  | |
| **4f** R = –$(CH_2)_5$–COOH | 35.9 | 47.5 | >100 | | |
| **5a** R = –COOMe | 80.2 | 86.2 | >100 | | |
| **5b** R = –$CH_2$–COOMe | 82.7 | >100 | >100 | | |
| **5e** R = –$(CH_2)_4$–COOMe | 8.1 | 7.3 | 15.1 |  |  |
| **5f** R = –$(CH_2)_5$–COOMe | 7.2 | 7.1 | 10.2 |  |  |
| **6a** R = –COOEt | >100 | 96.4 | >100 | | |
| **6b** R = –$CH_2$–COOEt | >100 | >100 | >100 | | |
| **6e** R = –$(CH_2)_4$–COOEt | 8.2 | 11.0 | 8.7 |  | |
| **6f** R = –$(CH_2)_5$–COOEt | 19.8 | 17.7 | 22.5 | | |
| **7a** R = –$CONH_2$ | >100 | >100 | >100 | | |
| **7b** R = –$CH_2$–$CONH_2$ | >100 | >100 | >100 | | |
| **7e** R = –$(CH_2)_4$–$CONH_2$ | 3.6 | 5.7 | 11.2 |  | |
| **7f** R = –$(CH_2)_5$–$CONH_2$ | 5.7 | 8.9 | 21.8 | | |
| **8a** R = –$COCH_3$ | >100 | >100 | >100 | | |
| **8e** R = –$(CH_2)_4$–$COCH_3$ | 0.6 | 1.1 | 2.0 |  |  |
| **8f** R = –$(CH_2)_5$–$COCH_3$ | 0.9 | 2.1 | 3.9 |  |  |

$GI_{50}$ = concentration of drug inhibiting cell growth by 50%. Colchicine (Col), Vinblastine (Vinb). HT-29 = human colon carcinoma, M21 = human skin melanoma and MCF-7 = human breast carcinoma.

lower affinity to the C-BS without affecting the $GI_{50}$, this suggesting that the antiproliferative activity may involve the alkylation and the inactivation or the activation of other important cellular proteins. To confirm our hypothesis that CEUs **5e**, **5f** and **8e**, **8f** are binding covalently to the C-BS on $\beta_{II}$-tubulin, we have assessed the irreversible binding of those CEUs using competition assays with drugs having a high affinity to $\beta_{II}$-tubulin such as colchicine and vinblastine. Compounds **1f**, **2f** and **3e** were also

**Table 2.** Effect of compounds **1f**, **2f**, **3e**, **5e**, **5f**, **8e**, and **8f** on tumor cell apoptosis and cell cycle progression

| CEU | Drug conc (μM) | Apoptotic cells (%) | $G_0/G_1$ (%) | S (%) | $G_2/M$ (%) |
|---|---|---|---|---|---|
| DMSO | 0.50% | 2 | 50 | 34 | 14 |
| **1f** R = $(CH_2)_5$–OH | 1.2 | 12 | 50 | 11 | 22 |
| | 4 | 27 | 6 | 7 | 53 |
| **2f** R = $(CH_2)_5$–OMe | 3 | 14 | 29 | 14 | 37 |
| | 10 | 24 | 7 | 9 | 52 |
| **3e** R = $(CH_2)_4$–$CH_3$ | 3 | 18 | 14 | 11 | 50 |
| | 10 | 31 | 6 | 7 | 49 |
| **5e** R = $(CH_2)_4$–$COOCH_3$ | 7 | 4 | 46 | 36 | 14 |
| | 21 | 6 | 40 | 34 | 20 |
| | 175 | 19 | 18 | 1 | 62 |
| **5f** R = $(CH_2)_5$–$COOCH_3$ | 7 | 4 | 45 | 37 | 14 |
| | 21 | 5 | 49 | 31 | 15 |
| | 175 | 19 | 8 | 1 | 72 |
| **8e** R = $(CH_2)_4$–$COCH_3$ | 5 | 11 | 44 | 13 | 25 |
| | 15 | 23 | 6 | 10 | 53 |
| **8f** R = $(CH_2)_5$–$COCH_3$ | 5 | 21 | 10 | 18 | 48 |
| | 15 | 16 | 11 | 18 | 53 |

tested as references.[24,25] Afterward, the cells were harvested and the proteins were quantified and analyzed by Western blot. As shown in Table 1, cells treated with CEUs (10-fold the $GI_{50}$) exhibited a second immunoreactive band of $\beta_{II}$-tubulin. The competition assay between colchicine and the CEUs showed the disappearance of the band corresponding to the CEU-tubulin complex, suggesting that compounds **5e**, **5f** and **8e**, **8f** were covalently bound into the colchicine-binding site. The competition assay between vinblastine and the selected CEUs showed no changes in the alkylation pattern. These latter results highlighted the affinity of the CEU compounds (**5e**, **5f**, **8e**, and **8f**) to the C-BS.

### 2.4. DNA cell cycle analysis

Antimicrotubule agents such as colchicine and vinblastine are known to arrest the cell cycle progression in $G_2/M$ phase through microtubule disruption. Analogous action between CEUs and these microtubule-disrupting agents on the cytoskeleton prompted us to examine the effects of compounds **5e**, **5f**, **8e**, and **8f** on cell cycle progression. Exponentially growing M21 cells were treated with compounds **1f**, **2f**, **3e**, all used as references, and compounds **5e**, **5f** and **8e**, **8f** and DMSO. Flow cytometry analysis data showed that compounds **5e**, **5f**, **8e**, and **8f** caused a significant accumulation of cells in $G_2/M$ phase (Table 2). This suggests that compounds **5e**, **5f, 8e**, and **8f** may induce microtubule disruption through mechanisms of action similar to those of colchicine and vinblastine.

## 3. Conclusion

In summary, we report here our efforts to determine the structure-activity relationships related to the antimicrotubule activity of CEUs by varying the ω-functionality of the substituting moiety of the aromatic ring of CEUs. In our experiments the ω-functionalities were –COOH, –COOR, –CONHR, and –C(O)R, respectively. Following examination of these four series of synthetic derivatives CEUs substituted on the side chain by electron-withdrawing groups showed that methyl ester and ketyl groups give potent cell growth inhibitory CEU. However, the presence of a free carboxyl group inhibits the antiproliferative activity of the drugs presumably through the presence of a negative charge preventing the binding of alkyl side chain into a putative hydrophobic pocket. The presence of lower alkyl saturated chains bearing terminal groups such as OH, $CH_3$, $OCH_3$, $COOCH_3$, and $COCH_3$, and *N*-(2-chloroethyl) pharmacophore seems essential for both antiproliferative and antitubulin activities. Most CEU, tested showed cell growth inhibition as previously demonstrated[24,32,33] through the alkylation of the C-BS leading to microtubule depolymerization and arrest of the cell cycle progression in $G_2/M$ phase. Of interest, some CEUs were cytotoxic without covalently binding to C-BS suggesting that the molecular structure of the CEU pharmacophore could be modulated to inhibit selectively the activity of other key-proteins involved in cell life and death.

## 4. Experimental

### 4.1. Biological assays and reagents

Biochemicals, drugs, and the monoclonal anti-β-tubulin antibody (clone TUB 2.1) were purchased from Sigma Chemical (St.-Louis, MO). Defined iron-supplemented bovine calf serum, high glucose DMEM with pyruvate, nitrocellulose membrane, and the ECL Western blotting detection reagent kit were provided by Hyclone (Road Logan, UT), Invitrogen, Bio-Rad (Mississauga, Canada), and GE Healthcare (Oakville, Canada), respectively. All drugs were dissolved in DMSO. The

concentration of DMSO in the culture medium was maintained under 0.5% (v/v) to avoid the toxicity of the vehicle.

**4.1.1. Cell culture and 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.[19] 96-well tissue culture plates were seeded with 100 μL of tumor cell lines suspended in high glucose DMEM containing 5% (v/v) defined iron-supplemented bovine calf serum. Plates were incubated at 37 °C, 5% $CO_2$ for 24 h. Drugs, freshly solubilized in DMSO, were diluted in fresh medium and aliquots of 100 μL containing sequential dilution were added. Final drug concentrations ranged from 100 to 0.1 μM. Plates were incubated for 48 h. Assays were stopped by addition of cold trichloroacetic acid to the wells (final concentration was 10%), followed by incubation for 60 min at 4 °C. Plates were washed five times with tap water. Sulforhodamine B solution (50 μL) at 0.1% (w/v) in 1% (v/v) 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 5 times with 1% acetic acid. Bonded stain was solubilized with 150 μL of 20 mM Tris base, and the absorbance was read using a μQuant Universal Microplate Spectrophotometer (Biotek, Winooski, VT) at 585 nm. The results were compared with those of a control reference plate fixed on the treatment day and the growth inhibition percentage was calculated for each drug. The experiments were performed at least twice in triplicate.

**4.1.2. Electrophoretic mobility shift assay to evaluate β-tubulin alkylation.** Exponentially growing M21 cells ($2.2 \times 10^5$) were plated in 12-well plates and incubated overnight at 37 °C. The cells were treated with 5 μM either of colchicine or vinblastine and a concentration of CEU equivalent to 10× the $GI_{50}$. The cells were incubated with the drugs for 24 and 48 h, respectively, and then harvested using a rubber policeman and centrifuged for 3 min at 8000 rpm. The pellets were washed with 500 μL of cold PBS, solubilized using Laemmli buffer. Samples ($5 \times 10^4$ cells) were analyzed by 10% SDS–PAGE. After electrophoresis, proteins were transferred onto nitrocellulose membranes which were then incubated in PBS, pH 7.4, containing 5% fat-free dried milk and 0.1% Tween 20™ (PBSMT) for 2 h at room temperature and then with 1/500 monoclonal anti-β-tubulin for 2 h. Membranes were washed with PBSMT and incubated with 1/2500 peroxidase-conjugated antimouse immunoglobulin in PBSMT for 1 h. Detection of the immunoblot was carried out with the ECL Western blotting detection reagent kit.

**4.1.3. Cell cycle analysis.** M21 cells were incubated with the different drugs for 24 h, harvested, resuspended in 1 mL of PBS, and fixed by the addition of 2.4 ml of ice-cold anhydrous ethanol. Then, $5 \times 10^5$ cells from each sample were centrifuged for 3 min at 8000 rpm. Cell pellets were resuspended in PBS containing 50 μg/mL of propidium iodide and 40 U/mL of RNase A (Boehringer Mannheim, Laval, Canada). Cell suspensions were incubated at room temperature for 30 min, and cell cycle distribution was analyzed by flow cytometry (Coulter Corporation, Miami, FL). Quantification of the FACS analyses was carried out with the software developed by Scripps Research Institute (http://www.scripps.edu/e_index.html).

### 4.2. Experimental procedures

**4.2.1. Chemistry and chemical methods.** Proton NMR spectra were recorded on a Brucker AM-300 spectrometer (Bruker, Germany). Chemical shifts ($\delta$) are reported in parts per million relative to the internal standard tetramethylsilane. IR spectra were recorded on a Unicam spectrometer. Uncorrected melting points were determined on an Electrothermal melting point apparatus. ESIMS spectra were carried out in the Mass Spectroscopy Laboratory of Molecular Medicine Research Centre, Medical Sciences Bldg, University of Toronto (http://www.medresearch.utoronto.ca/pmsc_home.html). All reactions were conducted under rigorously dried nitrogen atmosphere. All chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI). Compounds **1f**, **2f**, and **3e** were prepared as described previously.[24,25] Purification of compounds was performed by liquid flash chromatography on silica gel 60 A (American Chemicals Ltd., Montreal, Canada). 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 $F_{254}$).

### 4.3. General preparation of compounds 4a–f, 5a–f, 6a–f, 7a–f, 8a, 8e, and 8f

2-Chloroethylisocyanate (1.640 mmol) was added dropwise to a cold solution (ice bath) of the required aniline (1.370 mmol) in dry dichloromethane (15 mL per g of aniline). The ice bath was then removed and the reaction mixture was stirred at room temperature for 20 h. After completion of the reaction, the solvent was evaporated under reduced pressure to give an off-white solid, which was purified by flash chromatography.

**4.3.1. 3-[3-(2-Chloroethyl)ureido]benzoic acid (4a).** Compound **4a** was synthesized from **13a**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 26%; mp 208–210 °C; IR (KBr): $\nu$ 3353,1693, 1642, 1243 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 12.87 (brs, 1H, OH), 8.91 (brs, 1H, NH), 8.08 ( s, 1H, Ar), 7.68 (d, 1H, $J = 7.0$, Ar), 7.61 (d, 1H, $J = 7.5$, Ar), 7.36 (m, 1H, Ar), 6.48 (brs, 1H, NH), 3.68 (m, 2H, $CH_2$), 3,45 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 167.4, 155.0, 140.6, 131.3, 128.9, 122.1, 121.9, 118.5, 44.3, 41.3. ESIMS: ($m/z$): 265.0 $[M+Na]^+$, 243.0 $[M+1]^+$.

**4.3.2. 3-[3-(2-Chloroethyl)ureido]phenyl acetic acid (4b).** Compound **4b** was synthesized from **13b**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 25%; mp 214–215 °C; IR (KBr): $\nu$ 3311, 1658, 1632, 1245 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.95 (brs, 1H, NH), 7.89 (s, 1H, Ar), 7,61 (d, 1H, $J = 8.0$, Ar), 7.41 (d, 1H, $J = 8.0$, Ar),

7.29 (m, 1H, Ar), 6.61 (brs, 1H, NH), 3.68 (m, 4H, $CH_2$), 3.34 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 168.8, 155.7, 148.6, 135.2, 128.5, 116.5, 114.7, 113.2, 47.3, 44.8, 38.2. ESIMS: (*m*/*z*): 279.0 $[M+Na]^+$, 257.0 $[M+1]^+$.

**4.3.3. 5-{3-[3-(2-Chloroethyl)ureido]phenyl}pentanoic acid (4e).** Compound **4e** was synthesized from **13e**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 37%; IR (NaCl): *v* 3274, 1651, 1593, 1244 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$): $\delta$ 7.57 (brs, 1H, NH), 7.36 (m, 2H, Ar), 7.07 (m, 1H, Ar), 6.87 (d, 1H, $J = 7.0$, Ar), 6.74 (brs, 1H, NH), 3.66 (m, 4H, $CH_2$), 3.46 (m, 4H, $CH_2$), 2.59 (m, 2H, $CH_2$), 1.63 (m, 2H, $CH_2$); $^{13}C$ NMR (acetone-$d_6$): $\delta$ 174.6, 155.1, 143.7, 129.2, 123.9, 122.2, 118.9, 116.2, 49.7, 44.6, 41.2, 36.1, 30.2, 29.8. ESIMS: (*m*/*z*): 297.1 $[M-1]^+$.

**4.3.4. 6-{3-[3-(2-Chloroethyl)ureido]phenyl}hexanoic acid (4f).** Compound **4f** was synthesized from **13f**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (90/10)). Yield: 31%; mp 128–130 °C; IR (KBr): *v* 3306, 1690, 1633, 1241 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$): $\delta$ 8.17 (brs, 1H, NH), 7.31 (m, 1H Ar), 7.12 (m, 2H, Ar), 6.79 (d, 1H, $J = 7{,}5$, Ar), 6.23 (brs, 1H, NH), 3.64 (m, 2H, $CH_2$), 3.54 (m, 2H, $CH_2$), 2.55 (m, 2H, $CH_2$), 2.20 (m, 2H, $CH_2$), 1.71 (m, 4H, $CH_2$), 1.22 (m, 2H, $CH_2$); $^{13}C$ NMR (acetone-$d_6$): $\delta$ 174.1, 156.1, 143.9, 141.1, 129.2, 122.6, 119.2, 116.8, 44.9, 42.5, 36.3, 34.1, 31.8, 30.6, 25.4. ESIMS: (*m*/*z*): 335.1 $[M+Na]^+$, 313.1 $[M+1]^+$.

**4.3.5. 3-[3-(2-Chloroethyl)ureido]benzoic acid methyl ester (5a).** Compound **5a** was synthesized from **14a**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 77%; mp 103–105 °C; IR (KBr): *v* 3309, 1709,1638, 1235 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 8.21 (brs, 1H, NH), 7.87 (s, 1H, Ar), 7.61 (d, 1H, $J = 7{,}0$, Ar), 7.58 (d, 1H, $J = 7{,}0$, Ar), 7.21 (m, 1H, Ar), 5.89 (brs, 1H, NH), 3.76 (s, 3H, $CH_3$), 3.57 (m, 4H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 167.1, 156.2, 139.3, 131.1, 129.0, 125.0, 123.9, 120.4, 52.2, 46.4, 42.0. ESIMS: (*m*/*z*): 257.0 $[M+1]^+$.

**4.3.6. 3-[3-(2-Chloroethyl)ureido]phenyl acetic acid methyl ester (5b).** Compound **5b** was synthesized from **14b**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 61%; mp 96–97 °C; IR (KBr): *v* 3330, 1732, 1650, 1234 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 7.57 (brs, 1H, NH), 7.21 (m, 3H, Ar), 6.79 (m, 1H, Ar), 5.91 (brs, 1H, NH), 3.57 (m, 9H, $CH_2$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 172.5, 156.0, 139.1, 134.7, 129.2, 124.0, 120.9, 118.8, 52.1, 44.4, 40.9, 40.3. ESIMS: (*m*/*z*): 293.0 $[M+Na]^+$, 271.0 $[M+1]^+$.

**4.3.7. 5-{3-[3-(2-Chloroethyl)ureido]phenyl}pentanoic acid methyl ester (5e).** Compound **5e** was synthesized from **14e**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (97/3)). Yield: 19%; IR (NaCl): *v* 3326, 1702,1629, 1212 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 8.21 (brs, 1 H, NH), 7.07 (m, 1H Ar), 6.57 (m, 3H, Ar), 6.17 (brs, 1H, NH), 3.66, (s, 3H, $CH_3$), 3.51 (m, 4H, $CH_2$), 2.58 (m, 2H, $CH_2$), 2.33 (t, 2H, $J = 7.0$, $CH_2$), 1.65 (m, 2H, $CH_2$), 1.25 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 174.1, 155.3, 146.3, 143.4, 129.2, 118.8, 115.3, 112.8, 51.3, 44.7, 41.8, 35.5, 34.2, 30.4, 24.6. ESIMS: (*m*/*z*): 337.1 $[M+2+Na]^+$, 336.1 $[M+1+Na]^+$, 335.1 $[M+Na]^+$, 315.1 $[M+3]^+$, 314.1 $[M+2]^+$, 213.1 $[M+1]^+$.

**4.3.8. 6-{3-[3-(2-Chloroethyl)ureido]phenyl}hexanoic acid methyl ester (5f).** Compound **5f** was synthesized from **14f**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (97/3)). Yield: 32%; IR (NaCl): *v* 3323, 1702, 1631, 1251 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 8.11 (brs, 1H, NH), 7,48 (m, 2H, Ar), 6.51 (m, 2H, Ar), 6.38 (brs, 1H, NH), 3.81 (s, 3H, $CH_3$), 3.57 (m, 6H, $CH_2$), 2.51 (m, 2H, $CH_2$), 2.17 (m, 4H, $CH_2$), 1.78 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 174.1, 155.3, 138.3, 131.7, 129.3, 124.2, 121.1, 116.8, 51.4, 44.6, 41.9, 35.9, 34.1, 32.6, 30.8, 23.6. ESIMS: (*m*/*z*): 351.1 $[M+2+Na]^+$, 350.1 $[M+1+Na]^+$, 349.1 $[M+Na]^+$, 329.1 $[M+3]^+$, 328.2 $[M+2]^+$, 227.1 $[M+1]^+$.

**4.3.9. 3-[3-(2-Chloroethyl)ureido]benzoic acid ethyl ester (6a).** Compound **6a** was synthesized from **15a**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 88%; mp 128–130 °C; IR (KBr): *v* 3335, 1730, 1642, 1238 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 8.22 (brs, 1H, NH), 7.93 (s, 1H, Ar), 7.63 (d, 1H, $J = 8.0$, Ar), 7.57 (d, 1H, $J = 7.0$, Ar), 7.30 (m, 1H, Ar), 6.35 (brs, 1H, NH), 4.31 (q, 2H, $J = 7.0$, $CH_2$), 3.57 (m, 4H, $CH_2$), 1.34 (t, 3H, $J = 7.0$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 166.7, 156.0, 139.1, 131.0, 129.1, 124.3, 124.1, 120.5, 61.2, 44.5, 42.0, 14.2. ESIMS: (*m*/*z*): 293.0 $[M+Na]^+$, 271.0 $[M+1]^+$.

**4.3.10. 3-[3-(2-Chloroethyl)ureido]phenyl acetic acid ethyl ester (6b).** Compound **6b** was synthesized from **15b**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 52%; mp 95–97 °C; IR (KBr): *v* 3341, 1727, 1595, 1230 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 7.89 (brs, NH, 1H), 7.17 (s, 1H, Ar), 7.08 (m, 2H, Ar), 6.81 (d, 1H, $J = 8.0$, Ar), 6.54 (brs, 1H, NH), 4.09 (q, 2H, $J = 7{,}0$, $CH_2$), 3.46 (m, 6H, $CH_2$), 1.21 (t, 3H, $J = 7{,}0$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 172.1, 156.2, 139.2, 134.9, 129.7, 123.8, 120.8, 118.6, 61.1, 44.4, 42.0, 41.8, 14.1. ESIMS: (*m*/*z*): 307.0 $[M+Na]^+$, 285.0 $[M+1]^+$.

**4.3.11. 5-{3-[3-(2-Chloroethyl)ureido]phenyl}pentanoic acid ethyl ester (6e).** Compound **6e** was synthesized from **15e**. The crude product was purified by flash chromatography (hexanes/ethyl acetate (6/4)). Yield: 87%; mp > 310 °C; IR (KBr): *v* 3311, 1728, 1636, 1184 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 7.23 (m, 4H, Ar, NH), 6.89 (m, 1H, Ar), 5.63 (brs, 1H, NH), 4.31 (q, 2H, $J = 7.0$, $CH_2$), 3.63 (m, 4H, $CH_2$), 2.56 (m, 2H, $CH_2$), 2.39 (m, 2H, $CH_2$), 1.81 (m, 4H, $CH_2$), 1.23 (t, 3H, $J = 7.0$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 175.1, 155.8, 143.6, 138.3, 129.3, 124.2, 121.1, 118.6, 60.3, 44.8, 42.0, 35.5, 34.2, 30.9, 25.3, 14.2. ESIMS: (*m*/*z*): 349.1 $[M+Na]^+$, 327.1 $[M+1]^+$.

**4.3.12. 6-{3-[3-(2-Chloroethyl)ureido]phenyl}hexanoic acid ethyl ester (6f).** Compound **6f** was synthesized from **15f**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 81%; mp 72–74 °C;

IR (KBr): $\nu$ 3323, 1725, 1634, 1247 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 7.14 (m, 4H, Ar, NH), 6.86 (d, 1H, $J = 7.0$, Ar), 5.66 (brs, 1H, NH), 4.10 (q, 2H, $J = 7.0$, $CH_2$), 3.59 (m, 4H, $CH_2$), 2.54 (m, 2H, $CH_2$), 2.27 (m, 2H, $CH_2$), 1.62 (m, 4H, $CH_2$), 1.35 (m, 5H, $CH_2$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 174.9, 155.9, 143.9, 138.4, 129.1, 124.0, 120.9, 118.3, 60.3, 53.4, 44.8, 42.0, 35.6, 34.3, 30.9, 24.8, 14.2. ESIMS: (*m*/*z*): 363.1 $[M+Na]^+$, 341.1 $[M+1]^+$.

**4.3.13. 3-[3-(2-Chloroethyl)ureido]benzoic acid amide (7a).** Compound **7a** was synthesized from **16a**. The crude product was purified by flash chromatography (ethyl acetate/EtOH (95/5)). Yield: 68%; mp 212–214 °C; IR (KBr): $\nu$ 3320, 1660, 1635 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.92 (brs, 1H, NH), 7.83 (m, 2H, Ar), 7.40 (d, 1H, $J = 8.0$, Ar), 7.32 (m, 3H, Ar, $NH_2$), 6.57 (brs, 1H, NH), 3.69 (m, 2H, $CH_2$), 3.44 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 168.1, 155.1, 140.4, 135.1, 128.5, 120.5, 120.1, 117.3, 44.4, 41.3. ESIMS: (*m*/*z*): 264.0 $[M+Na]^+$, 242.0 $[M+1]^+$.

**4.3.14. 3-[3-(2-Chloroethyl)ureido]phenyl acetic acid amide (7b).** Compound **7b** was synthesized from **16b**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (90/10)). Yield: 57%; mp 119–121 °C; IR (KBr): $\nu$ 3324, 1664, 1627 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.17 (brs, 1H, NH), 8.12 (d, 1H, $J = 8.0$, Ar), 7.65 (m, 5H, Ar, $NH_2$), 7.03 (brs, 1H, NH), 3.68 (s, 2H, $CH_2$), 3.45 (m, 4H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.3, 152.5, 147.7, 138.7, 136.1, 129.6, 123.8, 121.4, 58.1, 41.3, 40.1. ESIMS: (*m*/*z*): 278.0 $[M+Na]^+$.

**4.3.15. 5-{3-[3-(2-Chloroethyl)ureido]phenyl}pentanoic acid amide (7e).** Compound **7e** was synthesized from **16e**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (90/10)). Yield: 23%; IR (NaCl): $\nu$ 3328, 1670, 1657 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.03 (brs, 1H, NH), 7.11 (m, 5H, Ar, $NH_2$), 6.69 (d, 1H, $J = 7.0$, Ar), 6.21 (brs, 1H, NH), 3.47 (m, 4H, $CH_2$), 3.34 (m, 2H, $CH_2$), 2.67 (t, 2H, $J = 7.0$, $CH_2$), 2.53 (m, 2H, $CH_2$), 2.39 (t, 2H, $J = 7.0$, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 172.3, 155.8, 147.9, 137.5, 130.3, 125.6, 124.7, 122.9, 58.3, 44.9, 41.2, 33.9, 29.3, 15.0. ESIMS: (*m*/*z*): 320.1 $[M+Na]^+$, 298.1 $[M+1]^+$.

**4.3.16. 6-{3-[3-(2-Chloroethyl)ureido]phenyl}hexanoic acid amide (7f).** Compound **7f** was synthesized from **16f**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (90/10)). Yield: 27%; IR (NaCl): $\nu$ 3326, 1669, 1633 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.21 (brs, 1H, NH), 7.38 (m, 4H, Ar, $NH_2$), 6.55 (m, 2H, Ar), 6.38 (brs, 1H, NH), 3.45 (m, 6H, $CH_2$), 2.56 (m, 4H, $CH_2$), 2.23 (m, 4H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 172.3, 146.2, 143.8, 137.4, 129.2, 123.7, 121.1, 117.2, 60.1, 44.7, 42.1, 35.9, 32.4, 30.9, 23.8. ESIMS: (*m*/*z*): 334.1 $[M+Na]^+$, 312.1 $[M+1]^+$.

**4.3.17. 1-(3-Acetylphenyl)-3-(2-chloroethyl)urea (8a).** Compound **8a** was synthesized from **17a**. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (90/10)). Yield: 94% (3.329 g); IR (NaCl): $\nu$ 3356, 1664, 1320; $^1H$ RMN (DMSO-$d_6$): $\delta$ 8.97 (brs, 1H, NH), 8.03 (s, 1H, Ar), 7.66 (d, 1H, $J = 8.0$, Ar), 7.53 (d, 1H, $J = 7.5$, Ar), 7.39 (m, 1H, Ar), 6.51 (brs, 1H, NH), 3.68 (t, 2H, $J = 6.0$, $CH_2$), 3.46 (t, 2H, $J = 6.0$, $CH_2$); $^{13}C$ RMN (DMSO-$d_6$): $\delta$ 197.8, 155.1, 140.8, 137.4, 129.1, 122.3, 121.3, 117.0, 44.3, 41.3, 26.7. ESIMS: (*m*/*z*): 263.0 $[M+Na]^+$, 241.0 $[M+1]^+$.

**4.3.18. 1-[3-(5-Oxohexyl)phenyl]-3-(2-chloroethyl)urea (8e).** Compound **8e** was synthesized from **17e**. The crude product was purified by flash chromatography (hexane/ethyl acetate (65/35)). Yield: 73%; IR (NaCl): $\nu$ 3346 (NH), 1711, 1316; $^1H$ RMN (acetone-$d_6$) $\delta$: 7.17 (m, 4H, Ar, NH), 6.87 (m, 1H, Ar,), 5.70 (brs, 1H, NH), 3.59 (m, 4H, $CH_2$), 2.54 (m, 2H, $CH_2$), 2.42 (m, 2H, $CH_2$), 2.12 (s, 3H, $CH_3$), 1.57 (m, 4H, $CH_2$); $^{13}C$ RMN (acetone-$d_6$): $\delta$ 209.5, 155.9, 143.5, 138.4, 129.2, 124.1, 120.9, 118.4, 44.7, 43.5, 42.0, 35.6, 30.7, 30.0, 23.4. ESIMS: (*m*/*z*): 335.2 $[M+K]^+$, 321.2 $[M+2+Na]^+$, 319.2 $[M+Na]^+$, 299.3 $[M+3]^+$; 297.2 $[M+1]^+$.

**4.3.19. 1-[3-(5-Oxoheptyl)phenyl]-3-(2-chloroethyl)urea (8f).** Compound **8f** was synthesized from **17f**. The crude product was purified by flash chromatography (hexane/ethyl acetate (65/35)). Yield: 72%; IR (NaCl): $\nu$ 3324, 1710, 1306; $^1H$ NMR ($CDCl_3$): $\delta$ 7.75 (brs, 1H, NH), 7.07 (m, 3H, Ar), 6.79 (d, 1H, $J = 5.0$, Ar) 5.89 (brs, 1H, NH), 3.49 (m, 4H, $CH_2$), 2.43 (m, 4H, $CH_2$), 2.12 (s, 3H, $CH_3$), 1.54 (m, 4H, $CH_2$), 1.25 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 210.2, 156.5, 143.6, 138.84, 128.9, 123.2, 120.0, 117.4, 44.3, 43.7, 42.0, 35.7, 31.0, 29.9, 28.7, 23.6. ESIMS (*m*/*z*): 349.2 $[M+K]^+$, 335.3 $[M+2+Na]^+$, 333.2 $[M+Na]^+$, 313.3 $[M+3]^+$; 311.3 $[M+1]^+$.

### 4.4. General preparation of compounds 13a, 13b, 13e, 13f, 14a, 14b, 14e, 14f, 15a, 15b, 15e, 15f, 16a, 16b, 16e, 16f, and 17e, 17f

**Method A:** The appropriate nitro compound (1.00 mmol) was dissolved in a mixture of ethanol and water (10:1, 22 mL). Powdered iron (7.28 mmol) and five drops of concentrated hydrochloric acid were added. The mixture was refluxed for 4 h. After cooling, the mixture was evaporated to dryness. A saturated solution of $Na_2CO_3$ (20 mL) was added, and the mixture was extracted with dichloromethane ($3 \times 15$ mL). The organic portions were combined, dried over $Na_2SO_4$, and concentrated under reduced pressure. The solid residue was then purified by flash chromatography on silica gel to afford **14a**, **14b** or **15a**, **15b**.

**Method B:** A mixture of the appropriate nitro compound (0.43 mmol), Pd/C 10% in ethanol (30 mL) was reduced under hydrogen atmosphere (38 psi) overnight. The catalyst was removed by filtration on Celite and the filtrate was evaporated to dryness. The residue was purified by flash chromatography on silica gel to afford **13a**, **13b**, **13e**, **13f** and **14e**, **14f** and **15e**, **15f** and **16a**, **16b**, **16e**, **16f** and **17e**, **17f**.

**4.4.1. 3-Aminobenzoic acid (13a).** Compound **13a** was synthesized from **9a** using Method B. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 57%; mp 246–250 °C; IR (KBr): $\nu$ 3427, 1638, 1390 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.23 (s, 1H, Ar), 7.08 (d, 1H, $J$ = 8.0, Ar), 7.03 (m, 1H, Ar), 6.63 (d, 1H, $J$ = 7.5, Ar), 5.85 (brs, 2H, $NH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 168.9, 148.2, 135.5, 128.2, 117.0, 116.4, 114.8.

**4.4.2. 2-(3-Aminophenyl)acetic acid (13b).** Compound **13b** was synthesized from **9b** using Method B. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 74%; mp 166–169 °C; IR (KBr) $\nu$ 3390, 1639, 1402 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.19 (m, 2H, Ar), 6.69 (m, 2H, Ar), 5.17 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 168.7, 148.5, 135.1, 128.5, 116.4, 114.6, 113.1, 39.5.

**4.4.3. 5-(3-Aminophenyl)pentanoic acid (13e).** Compound **13e** was synthesized from **19e** using Method B. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 30%; mp 70–72 °C; IR (KBr): $\nu$ 3347, 1694, 1353 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.11 (t, 1H, $J$ = 8.0, Ar), 6.61 (d, 1H, $J$ = 7.5, Ar), 6.54 (m, 2H, Ar), 6.17 (brs, 2H, $NH_2$), 2.57 (m, 2H, $CH_2$), 2.37 (m, 2H, $CH_2$), 1.68 (m, 4H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 179.6, 146.01, 143.4, 129.3, 119.2, 115.6, 113.1, 35.5, 34.0, 30.6, 24.4.

**4.4.4. 6-(3-Aminophenyl)hexanoic acid (13f).** Compound **13f** was synthesized from **19f** using Method B. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 64%; mp 131–133 °C; IR (KBr): $\nu$ 3402, 1705, 1420 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.08 (m, 1H, Ar), 6.64 (d, 1H, $J$ = 8.0, Ar), 6.59 (m, 2H, Ar), 6.14 (brs, 2H, $NH_2$), 2.55 (t, 2H, $J$ = 7.0, $CH_2$), 2.33 (t, 2H, $J$ = 7.0, $CH_2$), 1.64 (m, 4H, $CH_2$), 1.35 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 179.6, 145.9, 143.8, 129.2, 119.3, 115.6, 113.0, 35.6, 34.1, 30.9, 28.7, 24.6.

**4.4.5. 3-Aminobenzoic acid methyl ester (14a).** Compound **14a** was synthesized from **10a** using Method A. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (98/2)). Yield: 76%; IR (NaCl): $\nu$ 3372, 1712, 1238 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.28 (s, 1H, Ar), 7.15 (m, 2H, Ar), 6.77 (d, 1H, $J$ = 8.0, Ar), 5.49 (brs, 2H, $NH_2$), 2.52 (s, 3H, $CH_3$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 166.2, 148.9, 130.4, 129.0, 118.2, 116.3, 114.0, 51.8.

**4.4.6. 3-Aminophenylacetic acid methyl ester (14b).** Compound **14b** was synthesized from **10b** using Method A. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (98/2)). Yield: 59%; IR (NaCl): $\nu$ 3360, 1724, 1286 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 6.98 (m, 1H, Ar), 6.43 (m, 2H, Ar), 6.37 (d, 1H, $J$ = 8.0, Ar), 5.11 (brs, 2H, $NH_2$), 3.58 (s, 3H, $CH_3$), 3.44 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.7, 148.6, 134.7, 128.8, 116.6, 114.5, 112.5, 51.5, 40.5.

**4.4.7. 5-(3-Aminophenyl)pentanoic acid methyl ester (14e).** Compound **14e** was synthesized from **20e** using general Method B. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 50%; IR (NaCl): $\nu$ 3427, 1724, 1275 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.07 (m, 1H, Ar), 6.58 (d, 1H, $J$ = 7.5, Ar), 6.51 (m, 2H, Ar), 3.66 (s, 3H, $CH_3$), 3.55 (brs, 2H, $NH_2$), 2.54 (m, 2H, $CH_2$), 2.33 (m, 2H, $CH_2$), 1.65 (m, 4H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 174.1, 146.3, 143.4, 129.2, 118.8, 115.3, 112.8, 51.7, 33.5, 33.2, 30.7, 24.6.

**4.4.8. 6-(3-Aminophenyl)hexanoic acid methyl ester (14f).** Compound **14f** was synthesized from **20f** using general Method B. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 17%; IR (NaCl): $\nu$ 3402, 1742, 1170 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.08 (m, 1H, Ar), 6.68 (m, 3H, Ar), 3.65 (s, 3H, $CH_3$), 3.59 (brs, 2H, $NH_2$), 2.54 (m, 2H, $CH_2$), 2.38 (m, 2H, $CH_2$), 1.49 (m, 4H, $CH_2$), 1.28 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 174.2, 149.1, 143.8, 129.1, 120.1, 112.5, 109.9, 52.4, 36.1, 34.1, 31.0, 28.9, 24.8.

**4.4.9. 3-Aminobenzoic acid ethyl ester (15a).** Compound **15a** was synthesized from **11a** using Method A. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (98/2)). Yield: 56%; IR (NaCl): $\nu$ 3378, 112, 1237 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.24 (s, 1H, Ar), 7.17 (m, 2H, Ar), 6.81 (d, 1H, $J$ = 7.5, Ar), 5.54 (brs, 2H, $NH_2$), 4.31 (q, 2H, $J$ = 7.0, $CH_2$), 1.29 (t, 3H, $J$ = 7.0, $CH_3$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 173.8, 148.8, 131.0, 129.0, 118.3, 116.4, 114.1, 60.3, 14.2.

**4.4.10. 3-Aminophenylacetic acid ethyl ester (15b).** Compound **15b** was synthesized from **11b** using Method A. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 51%; IR (NaCl): $\nu$ 3372, 1724, 1292 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 6.99 (t, 1H, $J$ = 7.5, Ar), 7.47 (m, 2H, Ar), 6.34 (d, 1H, $J$ = 8.0, Ar), 5.17 (brs, 2H, $NH_2$), 4.08 (q, 2H, $J$ = 7.0, $CH_2$), 3.47 (s, 2H, $CH_2$), 1.15 (t, 3H, $J$ = 7.0, $CH_3$);$^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.2, 148.5, 134.8, 128.8, 116.7, 114.6, 112.5, 60.1, 40.7, 14.0.

**4.4.11. 5-(3-Aminophenyl)pentanoic acid ethyl ester (15e).** Compound **15e** was synthesized from **20e** using Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 50%; IR (NaCl): $\nu$ 3420, 1732, 1186 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 7.07 (m, 1H, Ar), 6.57 (d, 1H, $J$ = 7.5, Ar), 6.51 (m, 4H, Ar, $NH_2$), 4.13 (q, 2H, $J$ = 7.0, $CH_2$), 2.55 (m, 2H, $CH_2$), 2.31 (m, 2H, $CH_2$), 1.63 (m, 4H, $CH_2$), 1.22 (t, 3H, $J$ = 7.0, $CH_3$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 173.7, 146.4, 143.5, 129.2, 118.8, 115.2, 122.7, 60.2, 35.6, 34.2, 30.7, 24.6, 14.3.

**4.4.12. 6-(3-Aminophenyl)hexanoic acid ethyl ester (15f).** Compound **15f** was synthesized from **20f** using Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 63%; IR (NaCl): $\nu$ 3390, 1651, 1073 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 6.89 (t, 1H, $J$ = 7.5, Ar), 6.34 (m, 3H, Ar), 4.92 (brs, 2H,

$NH_2$), 4.04 (q, 2H, $J = 7.0$, $CH_2$), 2.39 (t, 2H, $J = 7$, $CH_2$), 2.27 (t, 2H, $J = 7$, $CH_2$), 1.51 (m, 6H, $CH_2$), 1.17 (t, 3H, $J = 7.0$, $CH_3$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 173.5, 148.5, 142.6, 128.7, 115.9, 113.9, 111.5, 59.7, 38.2, 33.5, 30.5, 28.2, 25.9, 14.2.

**4.4.13. 3-Aminobenzoic acid amide (16a).** Compound **16a** was synthesized from **12a** using general Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 31%; mp 72–74 °C; IR (KBr): $\nu$ 3384, 3183, 1687 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 7.74 (brs, 2H, $NH_2$), 7.07 (m, 3H, Ar), 6.72 (d, 1H, $J = 7.5$, Ar), 5.48 (brs, 2H, $NH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 168.8, 148.0, 135.3, 128.6, 116.8, 115.1, 113.5.

**4.4.14. 3-Aminophenylacetic acid amide (16b).** Compound **16b** was synthesized from **12b** using Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 28%; mp 111–113 °C; IR (KBr): $\nu$ 3402, 3177, 1645 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 7.76 (s, 1H, Ar), 7.03 (m, 2H, Ar), 6.68 (d, 1H, $J = 8.0$, Ar), 5.19 (brs, 2H, $NH_2$), 3.46 (brs, 2H, $NH_2$), 3.84 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.3, 147.5, 138.7, 136.1, 129.6, 123.7, 121.3, 42.0.

**4.4.15. 5-(3-Aminophenyl)pentanoic acid amide (16e).** Compound **16e** was synthesized from **22e** using Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 9%; IR (NaCl): $\nu$ 3384, 2928, 1650 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 7.12 (m, 1H, Ar), 6.63 (m, 3H, Ar), 5.27 (brs, 2H, $NH_2$), 3.46 (brs, 2H, $NH_2$), 2.55 (m, 2H, $CH_2$), 2.23 (m, 2H, $CH_2$), 1.67 (m, 2H, $CH_2$), 1.32 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.1, 146.5, 143.9, 130.9, 119.8, 115.1, 112.9, 36.4, 31.5, 28.2, 24.6.

**4.4.16. 6-(3-Aminophenyl)hexanoic acid amide (16f).** Compound **16f** was synthesized from **22f** using Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 17%; IR (NaCl): $\nu$ 3390, 3195, 1669 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 7.18 (m, 1H, Ar), 6.68 (m, 3H, Ar), 5.14 (brs, 2H, $NH_2$) $NH_2$, 2.59 (m, 2H, $CH_2$), 2.31 (m, 4H, $CH_2$), 1.71 (m, 2H, $CH_2$), 1.37 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.2, 146.2, 143.8, 130.0, 119.3, 115.5, 112.7, 35.8, 30.9, 28.8, 28.0, 24.9.

**4.4.17. 6-(3-Aminophenyl)hexan-2-one (17e).** Compound **17e** was synthesized from **23e** using general Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 18%; IR (NaCl): $\nu$ 3366, 1708 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 7.05 (m, 1H, Ar), 6.55 (m, 3H, Ar), 3.56 (brs, 2H, $NH_2$), 2.51 (m, 2H, $CH_2$), 2.42 (m, 2H, $CH_2$), 2.11 (s, 3H, $CH_3$), 1.59 (m, 4H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 209.1, 146.4, 143.5, 129.2, 118.7, 115.3, 112.7, 43.6, 35.7, 30.8, 29.9, 23.5.

**4.4.18. 6-(3-Aminophenyl)heptan-2-one (17f).** Compound **17f** was synthesized from **23f** using Method B. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 96%; IR (NaCl): $\nu$ 3368, 1710 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 7.03 (m, 1H, Ar), 6.50 (m, 3H, Ar), 3.56 (brs, 2H, $NH_2$), 2.48 (m, 2H, $CH_2$), 2.38 (m, 2H, $CH_2$), 2.08 (s, 3H, $CH_3$), 1.58 (m, 4H, $CH_2$), 1.30 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 209.4, 146.6, 143.7, 129.1, 118.6, 115.3, 112.6, 43.7, 35.7, 31.1, 29.8, 29.4, 23.6.

### 4.5. General preparation of compounds 10a, 10b, 11a, 11b, 20e, 20f, and 21e, 21f

In a bottom flask, the appropriate carboxylated compound (5.98 mmol) was dissolved in MeOH (20 mL) (Method C) or EtOH (20 mL) (Method D) and APTS (0.15 mmol) was added. The mixture was refluxed for 18 h. After cooling, the mixture was evaporated under reduced pressure. The residue was dissolved in a saturated solution of $Na_2CO_3$ (20 mL), extracted with dichloromethane ($3 \times 15$ mL), dried over $Na_2SO_4$, filtered, evaporated to dryness, and purified by chromatography on silica gel.

**4.5.1. 3-Nitrobenzoic acid methyl ester (10a).** Compound **10a** was synthesized from **9a** using Method C. The crude product was purified by flash chromatography ($CH_2Cl_2$). Yield: 84%; mp 68–74 °C; IR (KBr): $\nu$ 1724, 1523, 1268 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.89 (s, 1H, Ar), 8.42 (m, 2H, Ar), 7.71 (m, 1H, Ar), 4.01 (s, 3H, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 170.8, 148.2, 135.6, 129.4, 127.3, 124.3, 121.7, 52.7.

**4.5.2. 3-Nitrophenyl acetic acid methyl ester (10b).** Compound **10b** was synthesized from **9b** using Method C. The crude product was purified by flash chromatography ($CH_2Cl_2$). Yield: 70%; IR (NaCl): $\nu$ 1724, 1523, 1225 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.04 (s, 1H, Ar), 7.99 (d, 1H, $J = 7.0$, Ar), 7.53 (d, 1H, $J = 7.0$, Ar), 7.40 (m, 1H, Ar), 3.65 (s, 2H, $CH_2$), 3.61 (s, 3H, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 170.8, 148.2, 135.9, 135.3, 129.4, 124.3, 121.9, 52.4, 40.3.

**4.5.3. 3-Nitrobenzoic acid ethyl ester (11a).** Compound **11a** was synthesized from **9a** using Method D. The crude product was purified by flash chromatography ($CH_2Cl_2$). Yield: 81%; mp 36–38 °C; IR (KBr): $\nu$ 1700, 1523, 1292 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.71 (s, 1H, Ar), 8.28 (m, 2H, Ar), 7.60 (m, 1H, Ar), 4.38 (q, 2H, $J = 7.0$, $CH_2$), 1.36 (t, 3H, $J = 7.0$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 164.3, 148.1, 135.1, 132.1, 129.6, 127.1, 124.3, 61.9, 14.1.

**4.5.4. 3-Nitrophenyl acetic acid ethyl ester (11b).** Compound **11b** was synthesized from **9b** using Method D. The crude product was purified by flash chromatography ($CH_2Cl_2$). Yield: 81%; IR (NaCl): $\nu$ 1736, 1523, 1231 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.12 (s, 1H, Ar), 8.09 (d, 1H, $J = 8.0$, Ar), 7.62 (d, 1H, $J = 8.0$, Ar), 7.59 (m, 1H, Ar), 4.15 (q, 2H, $J = 7.0$, $CH_2$), 3.71 (s, 2H, $CH_2$), 1.24 (t, 3H, $J = 7.0$, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 170.4, 148.2, 136.1, 135.6, 129.8, 124.3, 122.1, 61.3, 40.6, 14.1.

**4.5.5. 5-(3-Nitrophenyl)pent-4-ynoic acid methyl ester (20e).** Compound **20e** was synthesized from **19e** using Method C. The crude product was purified by flash

chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 64%; IR (NaCl): $\nu$ 2229, 1718, 1536, 1268 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.17 (s, 1 H, Ar), 8.08 (d, 1H, $J$ = 7.5, Ar), 7.64 (d, 1H, $J$ = 7.5, Ar), 7.38 (t, 1H, $J$ = 7.5, Ar), 3.70 (s, 3H, $CH_3$), 2.73 (m, 2H, $CH_2$), 2.60 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 172.1, 148.1, 137.3, 129.2, 126.4, 125.3, 122.5, 91.1, 79.0, 51.9, 33.0, 15.2.

**4.5.6. 6-(3-Nitrophenyl)hex-5-ynoic acid methyl ester (20f).** Compound **20f** was synthesized from **19f** using Method C. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 57%; IR (NaCl): $\nu$ 2229, 1718, 1536, 1268 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.14 (s, 1H, Ar), 8.05 (d, 1H, $J$ = 8.0, Ar), 7.61 (d, 1H, $J$ = 8.0, Ar), 7.41 (t, 1H, $J$ = 8.0, Ar), 3.71 (s, 3H, $CH_3$), 2.57 (m, 4H, $CH_2$), 1.97 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 171.6, 148.0, 137.3, 129.2, 126.3, 125.4, 122.5, 91.2, 79.0, 60.7, 33.2, 15.2, 14.2.

**4.5.7. 5-(3-Nitrophenyl)pent-4-ynoic acid ethyl ester (21e).** Compound **21e** was synthesized from **19e** using Method C. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (98/2)). Yield: 69%; IR (NaCl): $\nu$ 2234, 1735, 1530, 1164 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.25 (s, 1H, Ar), 8.13 (d, 1H, $J$ = 8.0, Ar), 7.71 (d, 1H, $J$ = 7.5, Ar), 7.48 (m, 1H, Ar), 4.11 (q, 2H, $J$ = 7.0, $CH_2$), 2.81 (m, 2H, $CH_2$), 2.53 (m, 2H, $CH_2$), 1.19 (t, 3H, $J$ = 7.0, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 171.6, 148.0, 137.3, 129.2, 126.3, 125.4, 122.5, 91.2, 79.0, 60.7, 33.2, 15.3, 14.2.

**4.5.8. 6-(3-Nitrophenyl)hex-5-ynoic acid ethyl ester (21f).** Compound **21f** was synthesized from **19f** using Method D. The crude product was purified by flash chromatography ($CH_2Cl_2$/EtOH (95/5)). Yield: 91%; IR (NaCl): $\nu$ 2235, 1730, 1536, 1159 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.19 (s, 1H, Ar), 8.03 (d, 1H, $J$ = 8.0, Ar), 7.64 (d, 1H, $J$ = 8.0, Ar), 7.39 (t, 1H, $J$ = 8.0, Ar), 4.09 (q, 2H, $J$ = 7.0, $CH_2$), 2.43 (m, 4H, $CH_2$), 1.87 (m, 2H, $CH_2$), 1.23 (t, 3H, $J$ = 7.0, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 173.0, 148.3, 137.3, 129.2, 126.4, 125.6, 122.4, 92.0, 79.3, 60.5, 33.1, 23.6, 18.8, 14.2.

### 4.6. General preparation of compounds 12a, 12b and 22e, 22f

A mixture of compound **9a** or **9b** (5.52 mmol), 1.66 mL of $SOCl_2$, and 10 mL dry $CHCl_3$ was refluxed for 14 h. $CHCl_3$ and the excess of thionyl chloride were removed *in vacuo*, and the residue was evaporated twice with 25 mL of toluene to remove traces of thionyl chloride. The residue was taken into 10 mL of toluene and 30 mL of cold concentrated ammonium hydroxyde was added. The white solid formed was collected and dried with ethanol *in vacuo* and used without further purification.

**4.6.1. 3-Nitrobenzoic acid amide (12a).** Compound **12a** was synthesized from **9a**. Yield: 71%; mp 135–137 °C; IR (KBr): $\nu$ 3360, 1663, 1523 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 8.71 (s, 1H, Ar), 8.41 (m, 2H, Ar), 7.82 (m, 1H, Ar), 7.37 (brs, 2H, $NH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 165.7, 147.7, 135.7, 133.8, 130.0, 125.8, 122.2.

**4.6.2. 3-Nitrophenyl acetic acid amide (12b).** Compound **12b** was synthesized from **9b**. Yield: 57%; mp 133–135 °C; IR (KBr): $\nu$ 3403, 1657, 1535 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 8.14 (s, 1H, Ar), 7.71 (m, 3H, Ar), 7.06 (brs, 2H, $NH_2$), 3.38 (s, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 171.4, 147.5, 138.7, 136.5, 129.5, 123.7, 121.3, 41.2.

**4.6.3. 5-(3-Nitrophenyl)pent-4-ynoic acid amide (22e).** Compound **22e** was synthesized from **19e**. Yield: 65%; mp 124–127 °C; IR (KBr): $\nu$ 3397, 2222, 1663, 1511 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 8.21 (s, 1H, Ar), 8.11 (d, 1H, $J$ = 8.5, Ar), 7.66 (d, 1H, $J$ = 8.0, Ar), 7.45 (t, 1H, $J$ = 8.0, Ar), 5.78 (brs, 2H, $NH_2$), 2.78 (t, 2H, $J$ = 7.0, $CH_2$), 2.53 (t, 2H, $J$ = 7.0, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 173.1, 148.2, 137.3, 129.2, 126.5, 125.3, 122.7, 91.3, 79.3, 34.4, 15.5.

**4.6.4. 6-(3-Nitrophenyl)hex-5-ynoic acid amide (22f).** Compound **22f** was synthesized from **19f**. Yield: 69%; IR (NaCl): $\nu$ 3378, 2235, 1650, 1523 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 8.17 (s, 1H, Ar), 8.04 (d, 1H, $J$ = 8.0, Ar), 7.65 (d, 1H, $J$ = 8.0, Ar), 7.45 (t, 1H, $J$ = 8.0, Ar), 6.17 (brs, 2H, $NH_2$), 2.50 (t, 2H, $J$ = 7.0, $CH_2$), 2.36 (t, 2H, $J$ = 7.0, $CH_2$), 1.91 (m, 2H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 175.1, 148.1, 137.4, 129.3, 127.8, 125.6, 122.4, 92.3, 79.2, 34.6, 24.2, 19.1.

### 4.7. General preparation of compounds 19e, f and 23e, f

To a mixture of the compound **18** (4.56 mmol), $K_2CO_3$ (1.57 g, 11.4 mmol) in a mixture of 1,2-DME/water (1:1; 30 mL) were successively added CuI (34 mg, 0.18 mmol), $PPh_3$ (95.80 mg, 0.36 mmol), and Pd/C 10% (97.5 mg, 0.09 mmol). The mixture was stirred at room temperature for 1 h. Afterward, the appropriate alkyne (14.40 mmol) was added, and the mixture was refluxed overnight. After cooling, the mixture was filtered on Celite and the solvent was evaporated under reduced pressure. An aqueous solution of 1N HCl (20 mL) was then added to the residue. The aqueous solution was extracted with ethyl acetate (3 × 15 mL). The organic extracts were combined, washed with brine, dried ($Na_2SO_4$), and evaporated under reduced pressure.

**4.7.1. 5-(3-Nitrophenyl)-pent-4-ynoic acid (19e).** Compound **19e** was synthesized from 4-pentynoic acid. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 44%; mp 134–135 °C; IR (KBr): $\nu$ 3427, 1720, 1347 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 12.39 (brs, 1H, OH), 8.19 (d, 1H, $J$ = 8.0, Ar), 8.13 (s, 1H, Ar), 7.82 (d, 1H, $J$ = 8.0, Ar), 7.66 (t, 1H, $J$ = 8.0, Ar), 2.59 (m, 4H, $CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 172.8, 147.9, 137.5, 130.3, 125.7, 124.5, 122.9, 92.5, 78.5, 32.8, 14.8.

**4.7.2. 6-(3-Nitrophenyl)-hex-5-ynoic acid (19f).** Compound **19f** was synthesized from 5-hexynoic acid. The crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/EtOH (95/5)). Yield: 77%; IR (KBr): $\nu$ 3080, 2229, 1704, 1531, 1348 $cm^{-1}$; $^1C$ NMR (DMSO-$d_6$): $\delta$ 11.23 (brs, 1H, OH), 8.20 (s, 1H, Ar), 8.11 (d, 1H, $J$ = 8.0, Ar), 7.67 (d, 1H, $J$ = 8.0, Ar), 7.45 (m, 1H, Ar), 2.54 (m, 4H, $CH_2$), 1.95 (m, 2H,

$CH_2$); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 179.0, 148.0, 137.3, 130.7, 125.5, 123.7, 123.1, 91.9, 79.4, 32.9, 23.3, 18.7.

**4.7.3. 6-(3-Nitrophenyl)hex-5-yn-2-one (23e).** Compound **23e** was synthesized from hex-5-yn-2-one. The crude product was purified by flash chromatography (silica gel, Hexanes/ethyl acetate (90/10)). Yield: 61%; IR (KBr): $\nu$ 2228, 1713, 1525 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.21 (s, 1H, Ar), 8.12 (m, 1H, Ar), 7.66 (m, 1H, Ar), 7.44 (m, 1H, Ar), 2.79 (t, 2H, $J = 7.0$, $CH_2$), 2.66 (m, 2H, $CH_2$), 2.22 (s, 3H, $CH_3$);$^{13}C$ NMR ($CDCl_3$): $\delta$ 206.0, 148.9, 137.3, 129.2, 126.4, 125.5, 122.5, 91.7, 68.7, 42.1, 29.9, 13.8.

**4.7.4. 6-(3-Nitrophenyl)hept-6-yn-2-one (23f).** Compound **23f** was synthesized from **18** and hept-6-yn-2-one. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (60/40)). Yield: 58%; IR (NaCl): $\nu$ 2223, 1714, 1528 $cm^{-1}$; $^1C$ NMR ($CDCl_3$): $\delta$ 8.20 (s, 1H, Ar), 8.10 (m, 1H, Ar), 7.66 (m, 1H, Ar), 7.45 (t, 1H, $J = 8.0$, Ar), 2.61 (m, 2H, $CH_2$), 2.46 (t, 2H, $J = 7.0$, $CH_2$), 2.17 (s, 3H, $CH_3$), 1.87 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 208.0, 147.9, 137.2, 129.2, 126.1, 125.5, 122.3, 92.4, 79.0, 42.1, 29.9, 22.3, 18.6.

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