

***N*-phenyl-*N'*-(2-chloroethyl)ureas (CEU) as Potential Antineoplastic Agents. Part 3: Role of Carbonyl Groups in the Covalent Binding to the Colchicine-Binding Site**

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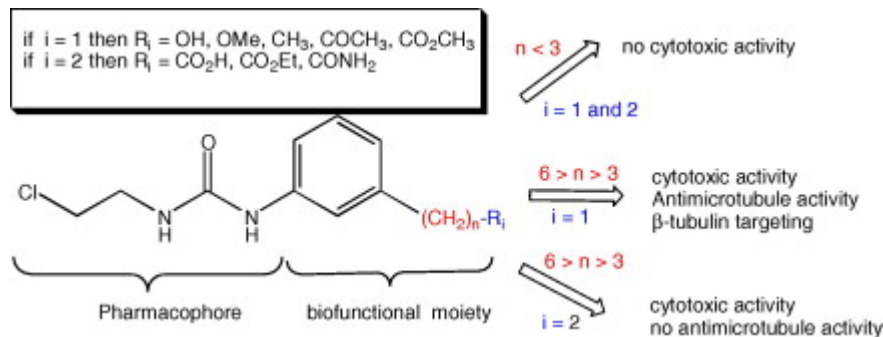
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Abstract

In the course of the development of *N*-phenyl-*N'*-(2-chloroethyl)ureas (CEUs) as potential antineoplastic agents, we have 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 G₂/M phase. Surprisingly, the presence of ω -carboxyl, ω -ethyl esters or ω -amides decreased significantly both the antiproliferative activity and the specificity towards tubulin.

Keywords: Phenyl chloroethylureas, antimicrotubule agents, antitubulin agents, antimitotics, soft alkylating agents, anticancer drugs, colchicine-binding site ligands

Graphical abstract



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 antimetotics 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 Ravelli⁸ and coworkers confirmed several assumptions concerning the molecular interactions occurring between colchicinoids and the C-BS that might help to the design of new antimetotics. In one hand, 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 sub sites on tubulin through thermodynamically independent reaction.⁹ The three methoxyl groups on the ring A of colchicinoids act as a complex-stabilizing anchor⁹, 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 a π -bond interactions.^{10, 11} 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, Nguyen¹² 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 moiety that gave rise to molecules such as ZD6126,¹³ combretastatin,¹⁴ phenstatin,¹⁵ chalcones,¹⁶ A-105972 and A-289099,¹⁷ AVE8062¹⁸ and

Oxi4503¹⁹. Second, a group of structurally unrelated molecules combining miscellaneous compounds such as nocodazole,²⁰ 2-methoxyestradiol,²¹ curacin A,²² E7010,²³ aryl 2-haloacetamides²⁴ and *N*-phenyl-*N'*-(2-chloroethyl)urea (CEUs).²⁵⁻³⁰ 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 (figure 1, **1a-f**) while the drugs were still covalently binding to the C-BS.^{27, 28}

We have recently studied the role of a ω -hydroxyl terminal group on the covalent binding of CEU to β -tubulin (figure 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.²⁹ To improve specific steric contacts occurring between the 2-chloroethylamino moiety of CEUs and a key amino acid (either Glu¹⁹⁸ in β_{IV} -tubulin²⁶ or Cys²³⁹ in β_{II} -tubulin³¹) 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**).

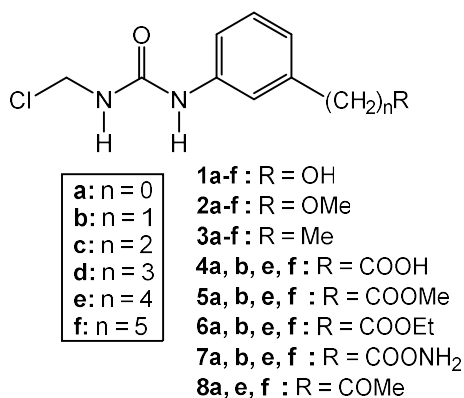


Figure 1. General formula of new 1-(2-chloroethyl)-3-phenylurea derivatives.

These various molecular modifications were selected, notably, on the basis that they had been shown beneficial for the biological affinity of colchicinoids such as ALLO, KAC, MAC, TCB, TKB and TMB.^{32, 33} (Fig. 2) and that they contain oxygen atoms susceptible to mimic the interactions of the oxygen atom present on the ring C of colchicinoids.

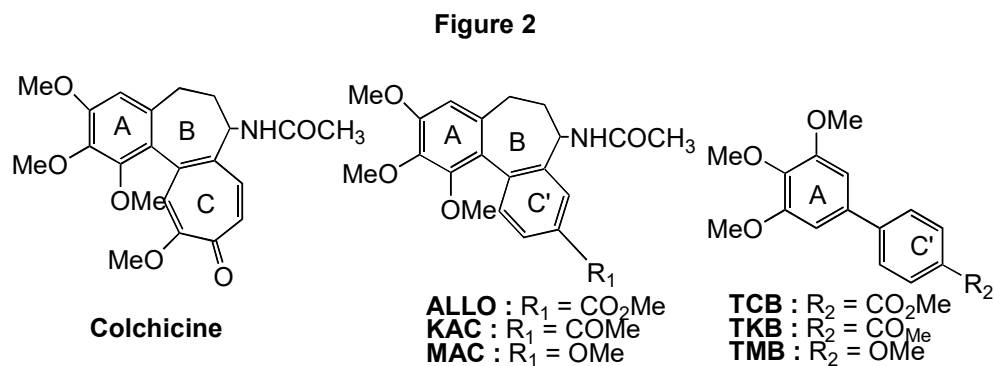
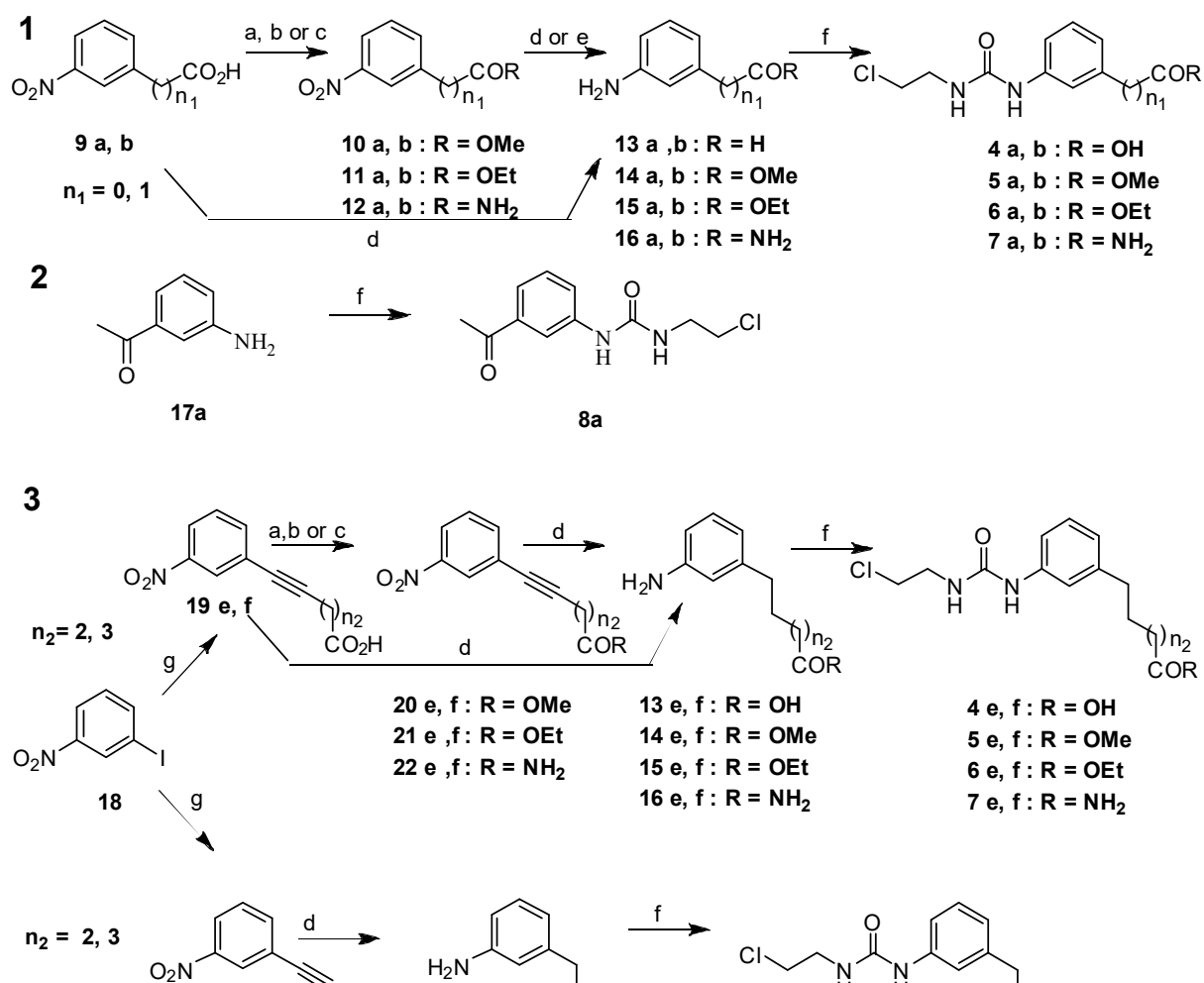


Figure 2. Molecular structures of colchicine and colchicinoids. Colchicine is a semi-planar molecule where ring A and ring C are oriented at 54° with respect to each other.²⁷

2. Results and discussion

2.1 Chemistry

Four new series of lower alkyl CEUs 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.



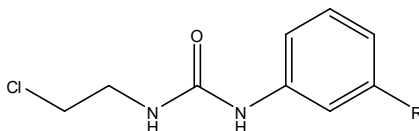
Scheme 1. General synthesis of 1-(2-chloroethyl)-3-phenylureas. Reagents and conditions: (a) MeOH, APTS; (b) EtOH, APTS; (c) 1-SOCl₂ ; 2-NH₄OH/toluene; (d) H₂, Pd/C; (e) Fe/HCl; (f) 2-chloroethylisocyanate; (g) (j) K₂CO₃, CuI, PPh₃, alkyne/1,2-DME/water.

CEUs bearing lower alkyl chains ($n < 3$) were prepared using compounds **9a**, **b** 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.^{27,28}

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 H₂/Pd afforded derivatives **13a**, **13b**, **13e**, **13f**, **14a**, **14b**, **14e**, **14f**, **15a**, **15b**, **15e**, **15f**, **16a**, **16b**, **16e**, **16f** and **17e**, **17f**.²⁷ 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.³⁴

Table 1. GI₅₀ values, electrophoretic mobility assays, and competition assays of 1-(2-chloroethyl)-3-phenylurea derivatives

Compounds	GI ₅₀ (μM)			Alkylated β-tubulin		Competition (48 h)		
	HT-29	M21	MCF-7	24 h	48 h	CTRL	Col (5 μM)	Vinb (5 μM)
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					

CEU 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.²⁸ 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.²⁸ Compounds **4f**, **5e**, **5f**, **6e**, **6f**, **7e**, **7f** and **8e**, **8f** showed GI_{50} values ranging from 0.6 to 47.5 μM . However, compounds **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 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 of the nature of the ω -terminal group. In summary, the antiproliferative activity of CEU increases according to the following order: $\text{OH} > \text{OCH}_3 \approx \text{CH}_3 \approx \text{COCH}_3 \gg \gg \text{CO}_2\text{CH}_3 > \text{CONH}_2 > \text{CO}_2\text{C}_2\text{H}_5 > \text{CO}_2\text{H}$.

2.3 β - tubulin alkylation

Concerning the alkylation of the β_{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.^{25,31} The

appearance of a second band exhibiting an apparent faster mobility β_{II} -tubulin indicates the presence of alkylated β_{II} -tubulin by CEU (see table 1).^{31, 35} Interestingly, compounds **6e** and **7e** were cytotoxic without any covalent binding to the C-BS. However, the methyl ester **5e**, **f** and the ketyl homologues **8e** and **8f** exhibited a significantly higher antiproliferative effect and a low affinity for C-BS. This suggests that the CEUs' inhibitory activity and affinity for β_{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 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 β_{II} -tubulin, we have assessed the irreversible binding of those CEU using a competition assays with drugs having a high affinity to β_{II} -tubulin such as colchicine and vinblastine. Compounds **1f**, **2f** and **3e** were also tested as references.^{27, 28} Afterwards, 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 β_{II} -tubulin. The competition assay between colchicine and the CEUs showed the disappearance of the band corresponding to the CEU-tubulin complex, suggesting that compound **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** and **8e**, **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 G₂/M phase through microtubule disruption. The analogy of action between CEUs and these microtubule-disrupting agents on the cytoskeleton prompted us to examine the effects of compound **5e**, **5f**, **8e** and **8f** on cell cycle progression. Exponentially growing M21 cells were treated with compounds **1f**, **2f**, **3e**, that were used as references, **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 G₂/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.

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

CEU	Drug Conc. (μ M)	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

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 -functionality 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 cytotoxic 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, CH₃, OCH₃, COOCH₃ and COCH₃, and *N*-(2-chloroethyl) pharmacophore seems essential for both antiproliferative and antitubulin activities. Most CEU tested were cytotoxic as previously demonstrated^{27, 36, 37} through the alkylation of the C-BS leading to microtubule depolymerization and arrest of the cell cycle progression in G₂/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 Section

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.²² 96-well tissue culture plates were seeded with 100 μ L 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% CO₂ for 24 h. Drugs, freshly solubilized in DMSO were diluted in fresh medium and aliquots of 100 μ L containing sequential dilution were added. Final drug concentrations ranged from 100 to 0.1 μ M. 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 μ L) 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 μ L of 20 mM Tris base, and the absorbance was read using a μ Quant 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 (2.2×10^5) were plated in 12-well plates and incubated overnight at 37 °C. The cells were treated with either 5 μ M of colchicine or vinblastine and a concentration of CEU equivalent to 10-times 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 3 min at 8000 rpm. The pellets were washed with 500 μ L of cold PBS, solubilized using Laemmli buffer. Samples (5×10^4 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 dry 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 drug 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, 5×10^5 cells from each sample were centrifuged for 3 min at 8000 rpm. Cell pellets were resuspended in PBS containing 50 μ g/mL of propidium iodide and 40 units/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 were 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 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. 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.^{27, 28} Purification of compounds were 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 F₂₅₄).

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 (CH₂Cl₂/EtOH (95/5)). Yield: 26%; mp 208-210 °C; IR (KBr): ν 3353, 1693, 1642, 1243 cm⁻¹; ¹H NMR (DMSO-d₆): δ 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, CH₂), 3.45 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 25%; mp 214-215 °C; IR (KBr): ν 3311, 1658, 1632, 1245 cm⁻¹; ¹H NMR (DMSO-d₆): δ 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, CH₂), 3.34 (s, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 37%; IR (NaCl): ν 3274, 1651, 1593, 1244 cm⁻¹; ¹H NMR (acetone-d₆): δ 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, CH₂), 3.46 (m, 4H, CH₂), 2.59 (m, 2H, CH₂), 1.63 (m, 2H, CH₂); ¹³C NMR (acetone-d₆): δ 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 (CH₂Cl₂/EtOH (90/10)). Yield: 31%; mp 128-130 °C; IR (KBr): ν 3306, 1690, 1633, 1241 cm⁻¹; ¹H NMR (acetone-d₆): δ 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, CH₂), 3.54 (m, 2H, CH₂), 2.55 (m, 2H, CH₂), 2.20 (m, 2H, CH₂), 1.71 (m, 4H, CH₂), 1.22 (m, 2H, CH₂); ¹³C NMR (acetone-d₆): δ 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 (CH₂Cl₂/EtOH (98/2)). Yield: 77%; mp 103-105 °C; IR (KBr): ν 3309, 1709, 1638, 1235 cm⁻¹; ¹H NMR (CDCl₃): δ 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, CH₃), 3.57 (m, 4H, CH₂); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (98/2)). Yield: 61%; mp 96-97 °C; IR (KBr): ν 3330, 1732, 1650, 1234 cm⁻¹; ¹H NMR (CDCl₃): δ 7.57 (brs, 1H, NH), 7.21 (m, 3H, Ar), 6.79 (m, 1H, Ar), 5.91 (brs, 1H, NH), 3.57 (m, 9H, CH₂, CH₃); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (97/3)). Yield: 19%; IR (NaCl): ν 3326, 1702, 1629, 1212 cm⁻¹; ¹H NMR (CDCl₃): δ 8.21 (brs, 1H, NH), 7.07 (m, 1H Ar), 6.57 (m, 3H, Ar), 6.17 (brs, 1H, NH), 3.66 (s, 3H, CH₃), 3.51 (m, 4H, CH₂), 2.58 (m, 2H, CH₂), 2.33 (t, 2H, J = 7.0, CH₂), 1.65 (m, 2H, CH₂), 1.25 (m, 2H, CH₂); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (97/3)). Yield: 32%; IR (NaCl): ν 3323, 1702, 1631, 1251 cm⁻¹; ¹H NMR (CDCl₃): δ 8.11 (brs, 1H, NH), 7.48 (m, 2H, Ar), 6.51 (m, 2H, Ar), 6.38 (brs, 1H, NH), 3.81 (s, 3H, CH₃), 3.57 (m, 6H, CH₂), 2.51 (m, 2H, CH₂), 2.17 (m, 4H, CH₂), 1.78 (m, 2H, CH₂); ¹³C NMR (CDCl₃): δ 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): ν 3335, 1730, 1642, 1238 cm^{-1} ; 1H NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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): ν 3341, 1727, 1595, 1230 cm^{-1} ; 1H NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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): ν 3311, 1728, 1636, 1184 cm^{-1} ; 1H NMR ($CDCl_3$): δ 7.23 (m, 4H, Ar, NH), 6.89 (m, 1H, Ar), 5.63 (brs, 1H, NH),

4.31 (q, 2H, $J = 7.0$, CH₂), 3.63 (m, 4H, CH₂), 2.56 (m, 2H, CH₂), 2.39 (m, 2H, CH₂), 1.81 (m, 4H, CH₂), 1.23 (t, 3H, $J = 7.0$, CH₃); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (98/2)). Yield: 81%; mp 72-74 °C; IR (KBr): ν 3323, 1725, 1634, 1247 cm⁻¹; ¹H NMR (CDCl₃): δ 7.14 (m, 4H, Ar, NH), 6.86 (d, 1H, $J = 7.0$, Ar), 5.66 (brs, 1H, NH), 4.10 (q, 2H, $J = 7.0$, CH₂), 3.59 (m, 4H, CH₂), 2.54 (m, 2H, CH₂), 2.27 (m, 2H, CH₂), 1.62 (m, 4H, CH₂), 1.35 (m, 5H, CH₂, CH₃); ¹³C NMR (CDCl₃): δ 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): ν 3320, 1660, 1635 cm⁻¹; ¹H NMR (DMSO-d₆): δ 8.92 (brs, 1H, NH), 7.83 (m, 2H, Ar), 7.40 (d, 1H, $J = 8.0$, Ar), 7.32 (m, 3H, Ar, NH₂), 6.57 (brs, 1H, NH), 3.69 (m, 2H, CH₂), 3.44 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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

(CH₂Cl₂/EtOH (90/10)). Yield: 57%; mp 119-121 °C; IR (KBr): ν 3324, 1664, 1627 cm⁻¹; ¹H NMR (DMSO-d₆): δ 8.17 (brs, 1H, NH), 8.12 (d, 1H, J = 8.0, Ar), 7.65 (m, 5H, Ar, NH₂), 7.03 (brs, 1H, NH), 3.68 (s, 2H, CH₂), 3.45 (m, 4H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (90/10)). Yield: 23%; IR (NaCl): ν 3328, 1670, 1657 cm⁻¹; ¹H NMR (DMSO-d₆): δ 8.03 (brs, 1H, NH), 7.11 (m, 5H, Ar, NH₂), 6.69 (d, 1H, J = 7.0, Ar), 6.21 (brs, 1H, NH), 3.47 (m, 4H, CH₂), 3.34 (m, 2H, CH₂), 2.67 (t, 2H, J = 7.0, CH₂), 2.53 (m, 2H, CH₂), 2.39 (t, 2H, J = 7.0, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (90/10)). Yield: 27%; IR (NaCl): ν 3326, 1669, 1633 cm⁻¹; ¹H NMR (DMSO-d₆): δ 8.21 (brs, 1H, NH), 7.38 (m, 4H, Ar, NH₂), 6.55 (m, 2H, Ar), 6.38 (brs, 1H, NH), 3.45 (m, 6H, CH₂), 2.56 (m, 4H, CH₂), 2.23 (m, 4H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (90/10)). Yield: 94% (3.329 g); IR (NaCl): ν 3356, 1664, 1320; ¹H RMN (DMSO-d₆): δ 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, CH₂), 3.46 (t, 2H, J = 6.0, CH₂); ¹³C RMN (DMSO-d₆): δ 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): ν 3346 (NH), 1711, 1316; ¹H RMN (acetone-d₆) δ : 7.17 (m, 4H, Ar, NH), 6.87 (m, 1H, Ar), 5.70 (brs, 1H, NH), 3.59 (m, 4H, CH₂), 2.54 (m, 2H, CH₂), 2.42 (m, 2H, CH₂), 2.12 (s, 3H, CH₃), 1.57 (m, 4H, CH₂); ¹³C RMN (acetone-d₆): δ 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): ν 3324, 1710, 1306; ¹H NMR (CDCl₃): δ 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, CH₂), 2.43 (m, 4H, CH₂), 2.12 (s, 3H, CH₃), 1.54 (m, 4H, CH₂), 1.25 (m, 2H, CH₂); ¹³C NMR (CDCl₃): δ 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 x 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** as 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): ν 3427, 1638, 1390 cm^{-1} ; 1H NMR (DMSO- d_6): δ 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, NH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (95/5)). Yield: 74%; mp 166-169 °C; IR (KBr) ν 3390, 1639, 1402 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.19 (m, 2H, Ar), 6.69 (m, 2H, Ar), 5.17 (s, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (95/5)). Yield: 30%; mp 70-72 °C; IR (KBr): ν 3347, 1694, 1353 cm⁻¹; ¹H NMR (DMSO-d₆): δ 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, NH₂), 2.57 (m, 2H, CH₂), 2.37 (m, 2H, CH₂), 1.68 (m, 4H, CH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (95/5)). Yield: 64%; mp 131-133 °C; IR (KBr): ν 3402, 1705, 1420 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.08 (m, 1H, Ar), 6.64 (d, 1H, J = 8.0, Ar), 6.59 (m, 2H, Ar), 6.14 (brs, 2H, NH₂), 2.55 (t, 2H, J = 7.0, CH₂), 2.33 (t, 2H, J = 7.0, CH₂), 1.64 (m, 4H, CH₂), 1.35 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (98/2)). Yield: 76%; IR (NaCl): ν 3372, 1712, 1238 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.28 (s, 1H, Ar), 7.15 (m, 2H, Ar), 6.77 (d, 1H, J = 8.0, Ar), 5.49 (brs, 2H, NH₂), 2.52 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (98/2)). Yield: 59%; IR (NaCl): ν 3360, 1724, 1286 cm⁻¹; ¹H NMR (DMSO-d₆): δ 6.98 (m, 1H, Ar), 6.43 (m, 2H, Ar), 6.37 (d, 1H, J = 8.0, Ar), 5.11 (brs, 2H, NH₂), 3.58 (s, 3H, CH₃), 3.44 (s, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (95/5)). Yield: 50%; IR (NaCl): ν 3427, 1724, 1275 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.07 (m, 1H, Ar), 6.58 (d, 1H, J = 7.5, Ar), 6.51 (m, 2H, Ar), 3.66 (s, 3H, CH₃), 3.55 (brs, 2H, NH₂), 2.54 (m, 2H, CH₂), 2.33 (m, 2H, CH₂), 1.65 (m, 4H, CH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (95/5)). Yield: 17%; IR (NaCl): ν 3402, 1742, 1170 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.08 (m, 1H, Ar), 6.68 (m, 3H, Ar), 3.65 (s, 3H, CH₃), 3.59 (brs, 2H, NH₂), 2.54 (m, 2H, CH₂), 2.38 (m, 2H, CH₂), 1.49 (m, 4H, CH₂), 1.28 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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, CH₂Cl₂/EtOH (98/2)). Yield: 56%; IR (NaCl): ν 3378, 112, 1237 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.24 (s, 1H, Ar), 7.17 (m, 2H, Ar), 6.81 (d, 1H, J = 7.5, Ar), 5.54 (brs, 2H, NH₂), 4.31 (q, 2H, J = 7.0, CH₂), 1.29 (t, 3H, J = 7.0, CH₃); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (98/2)). Yield: 51%; IR (NaCl): ν 3372, 1724, 1292 cm⁻¹; ¹H NMR (DMSO-d₆): δ 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, NH₂), 4.08 (q, 2H, J = 7.0, CH₂), 3.47 (s, 2H, CH₂), 1.15 (t, 3H, J = 7.0, CH₃); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 50%; IR (NaCl): ν 3420, 1732, 1186 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.07 (m, 1H, Ar), 6.57 (d, 1H, J = 7.5, Ar), 6.51 (m, 4H, Ar, NH₂),

4.13 (q, 2H, J = 7.0, CH₂), 2.55 (m, 2H, CH₂), 2.31 (m, 2H, CH₂), 1.63 (m, 4H, CH₂), 1.22 (t, 3H, J = 7.0, CH₃); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 63%; IR (NaCl): ν 3390, 1651, 1073 cm⁻¹; ¹H NMR (DMSO-d₆): δ 6.89 (t, 1H, J = 7.5, Ar), 6.34 (m, 3H, Ar), 4.92 (brs, 2H, NH₂), 4.04 (q, 2H, J = 7.0, CH₂), 2.39 (t, 2H, J = 7, CH₂), 2.27 (t, 2H, J = 7, CH₂), 1.51 (m, 6H, CH₂), 1.17 (t, 3H, J = 7.0, CH₃); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 31%; mp 72-74 °C; IR (KBr): ν 3384, 3183, 1687 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.74 (brs, 2H, NH₂), 7.07 (m, 3H, Ar), 6.72 (d, 1H, J = 7.5, Ar), 5.48 (brs, 2H, NH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 28%; mp 111-113 °C; IR (KBr): ν 3402, 3177, 1645 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.76 (s, 1H, Ar), 7.03 (m, 2H, Ar), 6.68 (d, 1H, J = 8.0, Ar), 5.19 (brs, 2H, NH₂), 3.46 (brs, 2H, NH₂), 3.84 (s, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 9%; IR (NaCl): ν 3384, 2928, 1650 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.12 (m, 1H, Ar), 6.63 (m, 3H, Ar), 5.27 (brs, 2H, NH₂), 3.46 (brs, 2H, NH₂), 2.55 (m, 2H, CH₂), 2.23 (m, 2H, CH₂), 1.67 (m, 2H, CH₂), 1.32 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 17%; IR (NaCl): ν 3390, 3195, 1669 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.18 (m, 1H, Ar), 6.68 (m, 3H, Ar), 5.14 (brs, 2H, NH₂) NH₂, 2.59 (m, 2H, CH₂), 2.31 (m, 4H, CH₂), 1.71 (m, 2H, CH₂), 1.37 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 18%; IR (NaCl): ν 3366, 1708 cm⁻¹; ¹H NMR (CDCl₃): δ 7.05 (m, 1H, Ar), 6.55 (m, 3H, Ar), 3.56 (brs, 2H, NH₂), 2.51 (m, 2H, CH₂), 2.42 (m, 2H, CH₂), 2.11 (s, 3H, CH₃), 1.59 (m, 4H, CH₂); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 96%; IR (NaCl): ν 3368, 1710 cm⁻¹; ¹H NMR (CDCl₃): δ 7.03 (m, 1H, Ar),

6.50 (m, 3H, Ar), 3.56 (brs, 2H, NH₂), 2.48 (m, 2H, CH₂), 2.38 (m, 2H, CH₂), 2.08 (s, 3H, CH₃), 1.58 (m, 4H, CH₂), 1.30 (m, 2H, CH₂); ¹³C NMR (CDCl₃): δ 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 reduce pressure. The residue was dissolved in a saturated solution of Na₂CO₃ (20 mL), extracted with dichloromethane (3 x 15 mL), dried over Na₂SO₄, 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 (CH₂Cl₂). Yield: 84%; mp 68-74 °C; IR (KBr): ν 1724, 1523, 1268 cm⁻¹; ¹H NMR (CDCl₃): δ 8.89 (s, 1H, Ar), 8.42 (m, 2H, Ar), 7.71 (m, 1H, Ar), 4.01 (s, 3H, CH₃); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂). Yield: 70%; IR (NaCl): ν 1724, 1523, 1225 cm⁻¹; ¹H NMR (CDCl₃): δ 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, CH₂), 3.61 (s, 3H, CH₃); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂). Yield: 81%; mp 36-38 °C; IR (KBr): ν 1700, 1523, 1292 cm⁻¹; ¹H NMR (CDCl₃): δ 8.71 (s, 1H, Ar), 8.28 (m, 2H, Ar), 7.60 (m, 1H, Ar), 4.38 (q, 2H, J = 7.0, CH₂), 1.36 (t, 3H, J = 7.0, CH₃); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂). Yield: 81%; IR (NaCl): ν 1736, 1523, 1231 cm⁻¹; ¹H NMR (CDCl₃): δ 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, CH₂), 3.71 (s, 2H, CH₂), 1.24 (t, 3H, J = 7.0, CH₃); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (98/2)). Yield: 64%; IR (NaCl): ν 2229, 1718, 1536, 1268 cm⁻¹; ¹H NMR (CDCl₃): δ 8.17 (s, 1H, 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, CH₃), 2.73 (m, 2H, CH₂), 2.60 (m, 2H, CH₂); ¹³C NMR (CDCl₃): δ 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 (CH₂Cl₂/EtOH (95/5)). Yield: 57%; IR (NaCl): ν 2229, 1718, 1536, 1268

cm^{-1} ; ^1H NMR (CDCl_3): δ 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): δ 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 ($\text{CH}_2\text{Cl}_2/\text{EtOH}$ (98/2)). Yield: 69%; IR (NaCl): ν 2234, 1735, 1530, 1164 cm^{-1} ; ^1H NMR (CDCl_3): δ 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): δ 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 ($\text{CH}_2\text{Cl}_2/\text{EtOH}$ (95/5)). Yield: 91%; IR (NaCl): ν 2235, 1730, 1536, 1159 cm^{-1} ; ^1H NMR (CDCl_3): δ 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): δ 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 , 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 hydroxide were 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): ν 3360, 1663, 1523 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 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): δ 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): ν 3403, 1657, 1535 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 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): δ 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): ν 3397, 2222, 1663, 1511 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 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): δ 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): ν 3378, 2235, 1650, 1523 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 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, NH₂), 2.50 (t, 2H, J = 7.0, CH₂), 2.36 (t, 2H, J = 7.0, CH₂), 1.91 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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), K₂CO₃ (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), PPh₃ (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 x 15 mL). The organic extracts were combined, washed with brine, dried (Na₂SO₄) 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, CH₂Cl₂/EtOH (95/5)). Yield: 44%; mp 134-135 °C; IR (KBr): ν 3427, 1720, 1347 cm⁻¹; ¹H NMR (DMSO-d₆): δ 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, CH₂); ¹³C NMR (DMSO-d₆): δ 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,

CH₂Cl₂/EtOH (95/5)). Yield: 77%; IR (KBr): ν 3080, 2229, 1704, 1531, 1348 cm⁻¹; ¹H NMR (DMSO-d₆): δ 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, CH₂), 1.95 (m, 2H, CH₂); ¹³C NMR (DMSO-d₆): δ 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): ν 2228, 1713, 1525 cm⁻¹; ¹H NMR (CDCl₃): δ 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, CH₂), 2.66 (m, 2H, CH₂), 2.22 (s, 3H, CH₃); ¹³C NMR (CDCl₃): δ 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): ν 2223, 1714, 1528 cm⁻¹; ¹H NMR (CDCl₃): δ 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, CH₂), 2.46 (t, 2H, J = 7.0, CH₂), 2.17 (s, 3H, CH₃), 1.87 (m, 2H, CH₂); ¹³C NMR (CDCl₃): δ 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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