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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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 \(\omega\)-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 \(\omega\)-carboxyl, \(\omega\)-ethyl esters or \(\omega\)-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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{5-7} However, recent results published by Ravelli\textsuperscript{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. 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.\textsuperscript{9} The three methoxyl groups on the ring A of colchicinoids act as a complex-stabilizing anchor\textsuperscript{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 a $\pi$-bond interactions.\textsuperscript{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, Nguyen\textsuperscript{12} 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,\textsuperscript{13} combretastatin,\textsuperscript{14} phenstatin,\textsuperscript{15} chalcones,\textsuperscript{16} A-105972 and A-289099,\textsuperscript{17} AVE8062\textsuperscript{18} and
Oxi4503\textsuperscript{19}. Second, a group of structurally unrelated molecules combining miscellaneous compounds such as nocodazole,\textsuperscript{20} 2-methoxyestradiol,\textsuperscript{21} curacin A,\textsuperscript{22} E7010,\textsuperscript{23} aryl 2-haloacetamides\textsuperscript{24} and \textit{N}-phenyl-\textit{N}'-(2-chloroethyl)urea (CEUs).\textsuperscript{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, 1\textit{a-f}) while the drugs were still covalently binding to the C-BS.\textsuperscript{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 2\textit{a-f} and 3\textit{a-f}). Moreover, we have demonstrated also that the pharmacophoric moiety \textit{N}-(2-chloroethyl)urea plays an “anchoring” action similar to the trimethoxy phenyl moiety of colchicinoids.\textsuperscript{29} To improve specific steric contacts occurring between the 2-chloroethylamino moiety of CEUs and a key amino acid (either Glu\textsuperscript{198} in β\textit{IV}-tubulin\textsuperscript{26} or Cys\textsuperscript{239} in β\textit{II}-tubulin\textsuperscript{31}) 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.\textsuperscript{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.\textsuperscript{27}

2. Results and discussion

2.1 Chemistry
Four new series of lower alkyl CEUs derivatives functionalized by a \(\omega\)-terminal group, namely, carboxyl (4\(a\), 4\(b\), 4\(e\), 4\(f\)), ester (5\(a\), 5\(b\), 5\(e\), 5\(f\) and 6\(a\), 6\(b\), 6\(e\), 6\(f\)), amidyl (7\(a\), 7\(b\), 7\(e\), 7\(f\)) and ketyl (8\(a\), 8\(e\), 8\(f\)), 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-SOCl\(_2\); 2-NH\(_4\)OH/toluene; (d) H\(_2\), Pd/C; (e) Fe/HCl; (f) 2-chloroethylisocyanate; (g) (j) K\(_2\)CO\(_3\), Cul, PPh\(_3\), alcyne/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.** GI\textsubscript{50} values, electrophoretic mobility assays, and competition assays of 1-(2-chloroethyl)-3-phenylurea derivatives

\[
\begin{align*}
\text{Cl} & \quad \text{N} & \quad \text{R} & \quad \text{O} & \quad \text{N} & \quad \text{H} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Compounds</th>
<th>GI\textsubscript{50} (µM)</th>
<th>HT-29</th>
<th>M21</th>
<th>MCF-7</th>
<th>Alkylated β-tubulin 24 h</th>
<th>48 h</th>
<th>Competition (48 h) with:</th>
<th>CTRL</th>
<th>Col (5 µM)</th>
<th>Vinb (5 µM)</th>
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<tr>
<td></td>
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<td>24 h</td>
<td>48 h</td>
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<tr>
<td>DMSO</td>
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<td>0.27</td>
<td>0.48</td>
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<td>0.84</td>
<td>0.94</td>
<td>1.9</td>
<td>2f R = -(CH\textsubscript{2})\textsubscript{5}-OMe</td>
<td>0.96</td>
<td>0.87</td>
<td>1.6</td>
<td>3f R = -(CH\textsubscript{2})\textsubscript{4}-CH\textsubscript{3}</td>
<td>68.1</td>
<td>67.9</td>
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<tr>
<td>4a R = -COOH</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>4b R = -CH\textsubscript{2}-COOH</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>4e R = -(CH\textsubscript{2})\textsubscript{4}-COOH</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>5a R = -COOMe</td>
<td>80.2</td>
<td>86.2</td>
<td>&gt;100</td>
<td>5b R = -CH\textsubscript{2}-COOMe</td>
<td>82.7</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>5e R = -(CH\textsubscript{2})\textsubscript{4}-COOMe</td>
<td>8.1</td>
<td>7.3</td>
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<tr>
<td>6a R = -COOEt</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>6b R = -CH\textsubscript{2}-COOEt</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>6e R = -(CH\textsubscript{2})\textsubscript{4}-COOEt</td>
<td>8.2</td>
<td>11.0</td>
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<tr>
<td>7a R = -CONH\textsubscript{2}</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>7b R = -CH\textsubscript{2}-CONH\textsubscript{2}</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>7e R = -(CH\textsubscript{2})\textsubscript{4}-CONH\textsubscript{2}</td>
<td>3.6</td>
<td>5.7</td>
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<td>8a R = -COCH\textsubscript{3}</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>8b R = -(CH\textsubscript{2})\textsubscript{5}-CONH\textsubscript{2}</td>
<td>5.7</td>
<td>8.9</td>
<td>21.8</td>
<td></td>
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<tr>
<td>8e R = -(CH\textsubscript{2})\textsubscript{5}-COCH\textsubscript{3}</td>
<td>0.6</td>
<td>1.1</td>
<td>2.0</td>
<td>8f R = -(CH\textsubscript{2})\textsubscript{5}-COCH\textsubscript{3}</td>
<td>0.9</td>
<td>2.1</td>
<td>3.9</td>
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</table>
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: OH > OCH$_3$ ≈ CH$_3$ ≈ COCH$_3$ >>> CO$_2$CH$_3$ > CONH$_2$ > CO$_2$C$_2$H$_5$ > CO$_2$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 \( \beta_{II} \)-tubulin indicates the presence of alkylated \( \beta_{II} \)-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 \( \beta_{II} \)-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 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 CEU using a competition assays with drugs having a high affinity to \( \beta_{II} \)-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 \( \beta_{II} \)-tubulin. The competition assay between colchicine and the CEUs showed the disappearance of the band corresponding to the CEU-tubulin complex, suggesting that 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

<table>
<thead>
<tr>
<th>CEU</th>
<th>Drug Conc. (µM)</th>
<th>Apoptotic cells (%)</th>
<th>G0/G1 (%)</th>
<th>S (%)</th>
<th>G2/M (%)</th>
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<tbody>
<tr>
<td>DMSO 0.50%</td>
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<tr>
<td>1f R = (CH$_2$)$_5$-OH</td>
<td>1.2</td>
<td>12</td>
<td>50</td>
<td>11</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>27</td>
<td>6</td>
<td>7</td>
<td>53</td>
</tr>
<tr>
<td>2f R = (CH$_2$)$_5$-OMe</td>
<td>3</td>
<td>14</td>
<td>29</td>
<td>14</td>
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</tr>
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<td>10</td>
<td>24</td>
<td>7</td>
<td>9</td>
<td>52</td>
</tr>
<tr>
<td>3e R = (CH$_2$)$_4$-CH$_3$</td>
<td>3</td>
<td>18</td>
<td>14</td>
<td>11</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>31</td>
<td>6</td>
<td>7</td>
<td>49</td>
</tr>
<tr>
<td>5e R = (CH$_2$)$_4$-COOCH$_3$</td>
<td>7</td>
<td>4</td>
<td>46</td>
<td>36</td>
<td>14</td>
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<td></td>
<td>21</td>
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<tr>
<td>8f R = (CH$_2$)$_5$-COCH$_3$</td>
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<td>21</td>
<td>10</td>
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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, 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 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 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 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 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% CO₂ 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, unbounded 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 x 10^5) were plated in 12-well plates and incubated overnight at 37 °C. The cells were treated with either 5 µM 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 µL of cold PBS, solubilized using Laemmli buffer. Samples (5 x 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 x 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 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 Brucker 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 (CH$_2$Cl$_2$/EtOH (95/5)).
Yield: 26%; mp 208-210 °C; IR (KBr): υ 3353, 1693, 1642, 1243 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): 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, CH$_2$), 3.45 (m, 2H, CH$_2$); $^{13}$C NMR (DMSO-d$_6$): 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 (CH$_2$Cl$_2$/EtOH (95/5)).
Yield: 25%; mp 214-215 °C; IR (KBr): υ 3311, 1658, 1632, 1245 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): 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, CH$_2$), 3.34 (s, 2H, CH$_2$); $^{13}$C NMR (DMSO-d$_6$): 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 37%; IR (NaCl): ν 3274, 1651, 1593, 1244 cm$^{-1}$; $^1$H NMR (acetone-d$_6$): 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, 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$_6$): 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 (CH$_2$Cl$_2$/EtOH (90/10)). Yield: 31%; mp 128-130 °C; IR (KBr): ν 3306, 1690, 1633, 1241 cm$^{-1}$; $^1$H NMR (acetone-d$_6$): 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, 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$_6$): 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 (CH$_2$Cl$_2$/EtOH (98/2)). Yield: 77%; mp 103-105 °C; IR (KBr): ν 3309, 1709,1638, 1235 cm$^{-1}$; $^1$H NMR (CDCl$_3$): 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, CH$_3$), 3.57 (m, 4H, CH$_2$); $^{13}$C NMR (CDCl$_3$): 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]$^+$.  

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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$_2$Cl$_2$/EtOH (98/2)). Yield: 61%; mp 96-97 °C; IR (KBr): v 3330, 1732, 1650, 1234 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (97/3)). Yield: 19%; IR (NaCl): v 3326, 1702, 1629, 1212 cm$^{-1}$; $^1$H NMR (CDCl$_3$): $\delta$ 8.21 (brs, 1H, 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$_2$Cl$_2$/EtOH (97/3)). Yield: 32%; IR (NaCl): v 3323, 1702, 1631, 1251 cm$^{-1}$; $^1$H 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,
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$_2$Cl$_2$/EtOH (98/2)). Yield: 88%; mp 128-130 °C; IR (KBr): v 3335, 1730, 1642, 1238 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (98/2)). Yield: 52%; mp 95-97 °C; IR (KBr): v 3341, 1727, 1595, 1230 cm$^{-1}$; $^1$H 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$^{-1}$; $^1$H NMR (CDCl$_3$): $\delta$ 7.23 (m, 4H, Ar, NH), 6.89 (m, 1H, Ar), 5.63 (brs, 1H, NH),
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$_2$Cl$_2$/EtOH (98/2)). Yield: 81%; mp 72-74 °C; IR (KBr): $\tilde{\nu}$ 3323, 1725, 1634, 1247 cm$^{-1}$; $^1$H 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): $\tilde{\nu}$ 3320, 1660, 1635 cm$^{-1}$; $^1$H 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
(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 

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, 
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$_2$Cl$_2$/EtOH (90/10)). Yield: 94% (3.329 g); IR (NaCl): $\tilde{\nu}$ 3356, 1664, 1320; $^1$H 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, 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): $\tilde{\nu}$ 3346 (NH), 1711, 1316; $^1$H 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): $\tilde{\nu}$ 3324, 1710, 1306; $^1$H 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,
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$_2$CO$_3$ (20 mL) was added, and the mixture was extracted with dichloromethane (3 x 15 mL). The organic portions were combined, dried over Na$_2$SO$_4$ 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$_2$Cl$_2$/EtOH (95/5)). Yield: 57%; mp 246-250 °C; IR (KBr): ν 3427, 1638, 1390 cm$^{-1}$; $^1$H 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, 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$_2$Cl$_2$/EtOH (95/5)). Yield: 74%; mp 166-169 °C; IR (KBr) v 3390, 1639, 1402 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (95/5)). Yield: 30%; mp 70-72 °C; IR (KBr): v 3347, 1694, 1353 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (95/5)). Yield: 64%; mp 131-133 °C; IR (KBr): v 3402, 1705, 1420 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (98/2)). Yield: 76%; IR (NaCl): v 3372, 1712, 1238 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (98/2)). Yield: 59%; IR (NaCl): v 3360, 1724, 1286 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (95/5)). Yield: 50%; IR (NaCl): v 3427, 1724, 1275 cm$^{-1}$; $^1$H 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$_2$Cl$_2$/EtOH (95/5)). Yield: 17%; IR (NaCl): ν 3402, 1742, 1170 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 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$_6$): 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, CH$_2$Cl$_2$/EtOH (98/2)). Yield: 56%; IR (NaCl): ν 3378, 112, 1237 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 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$_6$): 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 (CH$_2$Cl$_2$/EtOH (98/2)). Yield: 51%; IR (NaCl): ν 3372, 1724, 1292 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): 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, 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$_6$): 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 50%; IR (NaCl): ν 3420, 1732, 1186 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 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, 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 9%; IR (NaCl): ν 3384, 2928, 1650 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 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$_6$): 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 17%; IR (NaCl): ν 3390, 3195, 1669 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 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$_6$): 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 18%; IR (NaCl): ν 3366, 1708 cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 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$_3$): 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 96%; IR (NaCl): ν 3368, 1710 cm$^{-1}$; $^1$H NMR (CDCl$_3$): 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 (CH$_2$Cl$_2$). Yield: 81%; mp 36-38 °C; IR (KBr): ν 1700, 1523, 1292 cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 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$_3$): 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 (CH$_2$Cl$_2$). Yield: 81%; IR (NaCl): ν 1736, 1523, 1231 cm$^{-1}$; $^1$H NMR (CDCl$_3$): 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, CH$_2$), 3.71 (s, 2H, CH$_2$), 1.24 (t, 3H, J = 7.0, CH$_3$); $^{13}$C NMR (CDCl$_3$): 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 (CH$_2$Cl$_2$/EtOH (98/2)). Yield: 64%; IR (NaCl): ν 2229, 1718, 1536, 1268 cm$^{-1}$; $^1$H NMR (CDCl$_3$): 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, CH$_3$), 2.73 (m, 2H, CH$_2$), 2.60 (m, 2H, CH$_2$); $^{13}$C NMR (CDCl$_3$): 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 (CH$_2$Cl$_2$/EtOH (95/5)). Yield: 57%; IR (NaCl): ν 2229, 1718, 1536, 1268
cm⁻¹; ¹H NMR (CDCl₃): 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, CH₃), 2.57 (m, 4H, CH₂), 1.97 (m, 2H, CH₂); ¹³C NMR (CDCl₃): 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 (CH₂Cl₂/EtOH (98/2)). Yield: 69%; IR (NaCl): ν 2234, 1735, 1530, 1164 cm⁻¹; ¹H NMR (CDCl₃): 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, CH₂), 2.81 (m, 2H, CH₂), 2.53 (m, 2H, CH₂), 1.19 (t, 3H, J = 7.0, CH₃); ¹³C NMR (CDCl₃): 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 (CH₂Cl₂/EtOH (95/5)). Yield: 91%; IR (NaCl): ν 2235, 1730, 1536, 1159 cm⁻¹; ¹H NMR (CDCl₃): 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, CH₂), 2.43 (m, 4H, CH₂), 1.87 (m, 2H, CH₂), 1.23 (t, 3H, J = 7.0, CH₃); ¹³C NMR (CDCl₃): 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 SOCl₂, 10 mL dry CHCl₃ was refluxed for 14 h. CHCl₃ 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 were added. The white solid formed was collected and dried with ethanol \textit{in vacuo} and used without further purification.

\subsection*{4.6.1. 3-nitrobenzoic acid amide (12a):} Compound 12a was synthesized from 9a. Yield: 71%; mp 135-137 °C; IR (KBr): v 3360, 1663, 1523 cm\(^{-1}\); \(^1\)H 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.

\subsection*{4.6.2. 3-nitrophenyl acetic acid amide (12b):} Compound 12b was synthesized from 9b. Yield: 57%; mp 133-135 °C; IR (KBr): v 3403, 1657, 1535 cm\(^{-1}\); \(^1\)H 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.

\subsection*{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): v 3397, 2222, 1663, 1511 cm\(^{-1}\); \(^1\)H 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.

\subsection*{4.6.4. 6-(3-nitrophenyl)hex-5-ynoic acid amide (22f):} Compound 22f was synthesized from 19f. Yield: 69%; IR (NaCl): v 3378, 2235, 1650, 1523 cm\(^{-1}\); \(^1\)H 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), 7.39 (t, 1H, J = 8.0, Ar), 7.10 (t, 2H, J = 7.0, CH\(_2\)), 2.52 (s, 2H, 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.
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): v 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): v 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): v 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): v 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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