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Original article

# Design, synthesis and structure–activity relationships of antiproliferative 1,3-disubstituted urea derivatives

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**Abstract**

Twenty-four new 1,3-disubstituted urea derivatives (compounds **1**–**24**) were synthesized and reported for the first time. The antiproliferative activities of these compounds were evaluated against a panel of one human liver cell line (L02) and two human tumor cell lines (KB and K562) by applying the MTT colorimetric assay. The series of 1,3-disubstituted urea derivatives show good antiproliferative activity against human cancer cell lines (KB and K562) and no antiproliferative activity against liver cell line (L02). The potent in vitro antiproliferative activity of these derivatives and their selectivity for L02 are quite important points for an anticancer drug candidate with fewer side effects. Structure–activity relationships were also discussed based on the obtained experimental data. The hydroxyl groups on the phenyl ring reduced the antiproliferative activities of 1,3-disubstituted urea derivatives. The OH groups could be responsible for a reduction in the permeability of the cell membrane. Generally, an aromatic ring on N-3 seems to be in favor of enhancing the inhibitory activity, compounds introduced a nitro group substituent at C-3 position on the aromatic ring approved to generally decrease activity.


*Keywords:* Urea derivatives; Antiproliferative; K562 cell line; KB cell line; Morpholine ring; SAR

## 1. Introduction

Despite progress made in discovering new compounds with antiangiogenic activity, this research field is still considered one of the most promising ways to treat diseases characterized by abnormal angiogenesis including tumors [1]. Protein tyrosine kinases (PTK) have been intensively investigated because of their role in the transduction of proliferative signals in mammalian cells. Many trans-membrane growth factor receptors possess intracellular PTK activity, with initiation of this activity following external binding of a growth factor being the first step in the cellular signal transduction pathway which controls mitogenesis and cell proliferation [2,3]. The over-expression or inappropriate expression of normal or mutant PTK activity in these receptors can thus result in loss of growth control and the unregulated cell proliferation associated with malignancy [4,5]. Therefore, selective interruption of signal transduction in tumor cells by specific inhibitors of PTK activity has recently emerged as a major new approach for the design of tumor-specific drugs [6,7]. Urea derivatives are synthesized largely in recent years and have become of particular interest to chemists and biologists because of their wide range of biological activities, such as anticonvulsant activity [8], colchicine-blinding antagonist [9] and CXCR3 antagonist [10]. Some of these compounds exhibit cell growth inhibition ($GI_{50}$) on numerous cancer cell lines and have developed various chemoresistance mechanisms, and to be potent inhibitors of the PTK activity of a number of trans-membrane growth factor receptors and cellular oncogene products, particularly epidermal growth factor receptor (EGFR) [11]. We previously investigated the effect of a series of urea-like compounds on the human-leukemia K562 cell line and found that incorporating a heterocycle, especially a morpholine ring can highly improve their activity [12].

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On the basis of the above work, we designed and prepared 24 new 1,3-disubstituted urea compounds (**1**–**24**), all of the compounds were assayed for their antiproliferative activities against a panel of one human liver cell line (L02) and two human tumor cell lines (KB and K562) by applying the MTT colorimetric assay. The neck carcinoma cell line KB are overexpressing EGFR, demonstrating EGF-independent cell growth, so the antiproliferative activity against KB was evaluated. Although in general leukemia cell lines K562 was not overexpressing EGFR, the antiproliferative activity against K562 was also evaluated for the possibility of another cellular target than EGFR for future research.

## 2. Results and discussion

Compounds **1**–**24** were prepared by the synthetic route illustrated in Scheme 1. Since introducing a morpholine ring can improve highly the cell growth inhibitory activity on the human-leukemia K562 cell line according to our previous report, to further optimize this activity, 12 compounds reported in this paper contain a morpholine ring. In the first step, condensation reaction of aldehydes with various amines containing functional groups (morpholine, furfurylamine, etc.), which are cheap and available easily in market afforded the schiff bases. Reduction of the latter with $NaBH_4$ afforded the corresponding saturated alkyl amines, to which dry DMF in $SOCl_2$ was added and refluxed for 4 h and excessive $SOCl_2$ was evaporated under reduced pressure. The residue was dissolved in dichloromethane, various amines obtained in the first step dissolved in dry pyridine and $CH_2Cl_2$ were added. As a result, 24 1,3-disubstituted urea compounds were synthesized, which were all first reported. All the compounds were purified by silica gel column and identified by elemental C, H, N analysis, NMR and MS and displayed in Table 1.

The in vitro antiproliferative activity of the synthesized 1,3-disubstituted urea derivatives was studied on a panel of one human liver cell line (L02) and two human tumor cell lines (KB, K562) by applying the MTT colorimetric assay. Compounds were tested over a range of concentrations from 0.1 to100 μg/mL, and the calculated $IC_{50}$ values, that is, the concentration (μM) of a compound able to cause 50% of cell death with respect to the control culture, are reported in Table 2. Etoposide was used as reference compounds. The results show that 1,3-disubstituted urea derivatives inhibited the growth of human cancer cell lines well, generally, the compounds **1**–**24** illustrated in Table 1 showed better activity compared with the 1-substituted urea derivatives reported before, indicating that an aromatic ring on N-3 seems to be in favor of enhancing the inhibitory activity. Against K562 and KB, compound **2** was found to show the most potent in vitro activity (with $IC_{50}$ of 0.27 and 0.9 μM, respectively) among the new compounds tested, and was comparable with the positive control etoposide ($IC_{50}$ of 1.27 and 0.42 μM, respectively).

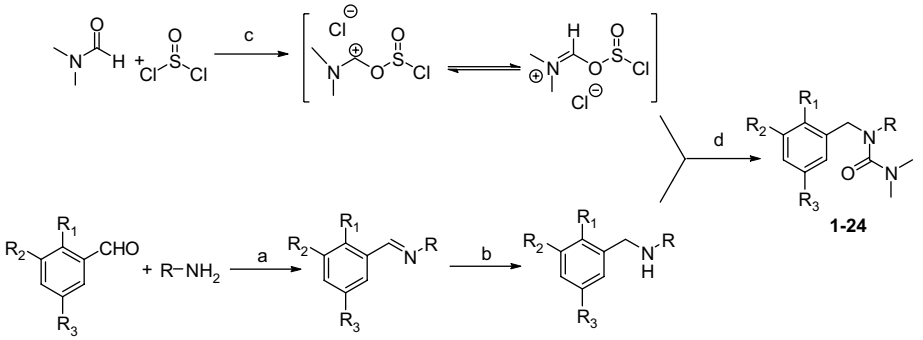
These compounds containing a morpholine ring (**1**, **2**, **6**, **7**, **9**, **10**, **13**, **14**, **18**, **19**, **22**, **23**) exhibited the most potent in vitro activities in vitro compared to the rest, implying that a morpholine ring can improve highly the antiproliferative activity on the K562 and KB cell line. The effects of substituents on the phenyl ring were investigated, when a nitro group placed on the phenyl ring, derivatives exhibited most potent in vitro activity, especially substituted on C-3 position. Against all two human cancer cell lines, compounds **18** and **19** were less active than **1** and **2**, respectively. This result indicates that the hydroxyl groups on the phenyl ring reduced the antiproliferative activities of 1,3-disubstituted urea derivatives. The OH groups on phenyl ring lead to the decrease of their permeability of the cell membrane, which may be the reason of decreased biological activity of the corresponding derivatives [13]. Other evidences are also found in Table 2 such as compounds **20** and **21**.

Moreover, compounds (**1**, **2**, **6**, **7**, **9**, **10**, **13**, **14**, **18**, **19**, **22**, **23**) with a morpholine ring and etoposide were also evaluated for a one human normal cell line (L02). The results (Table 3) showed that these 1,3-disubstituted urea derivatives with potent in vitro antiproliferative activity scarcely inhibited normal cell lines, while etoposide showed no selectivity to inhibition growth of the three cell lines (L02, K562 and KB).

## 3. Experimental part

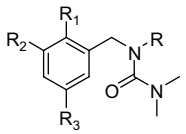
### 3.1. General

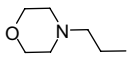
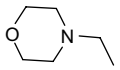
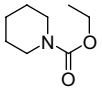
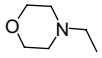
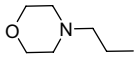
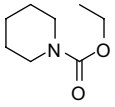
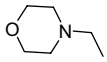
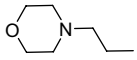
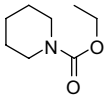
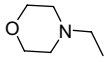
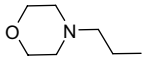
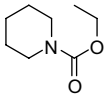
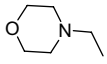
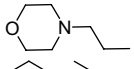
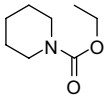
All chemicals and reagents used in current study were of analytical grade. TLC was run on the silica gel coated




Scheme 1. General synthesis of urea compounds **1**–**24**. Reagents and conditions: (a) ethanol, reflux, 1 h; (b) $NaBH_4$, ethanol, 50 °C, 2–3 h; (c) dry $CH_2Cl_2$, 70 °C, 4 h; (d) dry $CH_2Cl_2$, dry pyridine, 60 °C, 5–6 h.

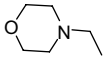
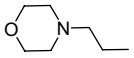
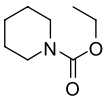
Table 1
Physical properties of 1,3-disubstituted urea compounds



| Compound | R | $R_1$ | $R_2$ | $R_3$ | Formula | M.p. (°C) | Yield (%) |
|---|---|---|---|---|---|---|---|
| **1** |  | $NO_2$ | H | H | $C_{17}H_{26}N_4O_4$ | 121–122 | 51 |
| **2** |  | $NO_2$ | H | H | $C_{16}H_{24}N_4O_4$ | 124–126 | 48 |
| **3** |  | $NO_2$ | H | H | $C_{15}H_{17}N_3O_4$ | 135–137 | 48 |
| **4** |  | $NO_2$ | H | H | $C_{18}H_{26}N_4O_5$ | Brown oil | 44 |
| **5** |  | Cl | H | OH | $C_{15}H_{17}ClN_2O_3$ | Yellow oil | 57 |
| **6** |  | Cl | H | OH | $C_{16}H_{24}ClN_3O_3$ | Yellow oil | 52 |
| **7** |  | Cl | H | OH | $C_{17}H_{26}ClN_3O_3$ | Yellow oil | 45 |
| **8** |  | Cl | H | OH | $C_{18}H_{26}ClN_3O_4$ | Yellow oil | 42 |
| **9** |  | H | OH | H | $C_{16}H_{25}N_3O_3$ | 144–146 | 55 |
| **10** |  | H | OH | H | $C_{17}H_{27}N_3O_3$ | Yellow oil | 41 |
| **11** |  | H | OH | H | $C_{15}H_{18}N_2O_3$ | Yellow oil | 51 |
| **12** |  | H | OH | H | $C_{18}H_{27}N_3O_4$ | Yellow oil | 40 |
| **13** |  | H | $NO_2$ | H | $C_{16}H_{24}N_4O_4$ | Yellow oil | 47 |
| **14** |  | H | $NO_2$ | H | $C_{17}H_{26}N_4O_4$ | Brown oil | 53 |
| **15** |  | H | $NO_2$ | H | $C_{15}H_{17}N_3O_4$ | 118–120 | 48 |
| **16** |  | H | $NO_2$ | H | $C_{18}H_{26}N_4O_5$ | Yellow oil | 42 |
| **17** |  | $NO_2$ | H | OH | $C_{15}H_{17}N_3O_5$ | Yellow oil | 51 |
| **18** |  | $NO_2$ | H | OH | $C_{16}H_{24}N_4O_5$ | Yellow oil | 53 |
| **19** |  | $NO_2$ | H | OH | $C_{17}H_{26}N_4O_5$ | Yellow oil | 45 |
| **20** |  | $NO_2$ | H | OH | $C_{18}H_{26}N_4O_6$ | Yellow oil | 41 |

(*continued on next page*)

Table 1 (*continued*)

| Compound | R | $R_1$ | $R_2$ | $R_3$ | Formula | M.p. (°C) | Yield (%) |
|---|---|---|---|---|---|---|---|
| **21** |  | Br | H | OH | $C_{15}H_{17}BrN_2O_3$ | Yellow oil | 55 |
| **22** |  | Br | H | OH | $C_{16}H_{24}BrN_3O_3$ | Yellow oil | 51 |
| **23** |  | Br | H | OH | $C_{17}H_{26}BrN_3O_3$ | Yellow oil | 41 |
| **24** |  | Br | H | OH | $C_{18}H_{26}BrN_3O_4$ | Yellow oil | 40 |

aluminum sheets (silica gel 60 GF254, E. Merk, Germany) and visualized in UV light (254 nm). All the NMR spectra were recorded on a Bruker DRX 500 model Spectrometer in either ($d_6$) DMSO or $CDCl_3$. Chemical shifts ($\delta$) for $^1$H-NMR spectra are reported in parts per million to residual solvent protons. Melting points were measured on a Boetius micro-melting point apparatus. The ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument.

### 3.2. Compounds

Aldehyde was dissolved in 25 ml of ethanol, and amine was added to the solution. The reaction mixture was refluxed for 1 h, and then 0.05 mmol of $NaBH_4$ was added to the reaction solution slowly, and stirred under 50 °C for 2–3 h. The mixture was evaporated under vacuum, and dissolved in EtOAc (30 ml). The solution was washed with 20 ml water twice, dried over anhydrous sodium sulfate, and evaporated. Purification by silica gel afforded pure products.

Table 2
Antiproliferative activity against K562 and KB cell line of compounds **1-24**

| Compound | IC50 (μM) | | Compound | $IC_{50}$ (μM) | |
|---|---|---|---|---|---|
| | KB | K562 | | KB | K562 |
| **1** | 0.17 ± 0.06 | 0.42 ± 0.15 | **13** | 0.75 ± 0.33 | 2.24 ± 0.95 |
| **2** | 0.09 ± 0.38 | 0.27 ± 0.13 | **14** | 0.52 ± 0.26 | 1.20 ± 0.46 |
| **3** | 23.8 ± 12.1 | 40.7 ± 25.2 | **15** | 17.5 ± 8.6 | 35.4 ± 11.6 |
| **4** | 15.6 ± 5.4 | 24.9 ± 10.1 | **16** | 16.4 ± 8.2 | 26.0 ± 9.8 |
| **5** | 49.5 ± 21.8 | 71.3 ± 36.7 | **17** | 20.8 ± 8.5 | 28.6 ± 10.1 |
| **6** | 2.24 ± 0.94 | 3.88 ± 1.35 | **18** | 4.8 ± 2.3 | 10.8 ± 3.7 |
| **7** | 1.75 ± 0.59 | 2.92 ± 1.14 | **19** | 3.3 ± 1.4 | 8.5 ± 3.8 |
| **8** | 25.7 ± 13.4 | 33.5 ± 12.8 | **20** | 24.2 ± 8.4 | 38.4 ± 17.0 |
| **9** | 4.25 ± 1.96 | 11.8 ± 4.9 | **21** | 32.8 ± 12.0 | 40.5 ± 14.5 |
| **10** | 2.88 ± 1.41 | 7.5 ± 1.9 | **22** | 7.3 ± 3.4 | 13.5 ± 5.5 |
| **11** | 52.1 ± 21.2 | 75.1 ± 34.1 | **23** | 9.0 ± 3.5 | 18.6 ± 6.5 |
| **12** | 48.6 ± 20.7 | 67.2 ± 33.6 | **24** | 24.6 ± 10.3 | 41.8 ± 14.8 |
| Etoposide[a] | $IC_{50}$/KB: 0.42 ± 0.21 | | | $IC_{50}$/K562: 1.27 ± 0.45 | |

[a] Used as a positive control. The data represent the mean of three experiments performed in triplicate and are expressed as means ± SD. The $IC_{50}$ value is the concentration at which 50% survival of cells was observed.

The mixture of $CH_2Cl_2$ (15 ml) dry DMF (3 ml, 40 mmol) and $SOCl_2$ (7 ml, 0.10 mol) was stirred to reflux at 70 °C for 4 h and cooled. The solvents and excess $SOCl_2$ were then removed under reduced pressure. The residue dissolved in $CH_2Cl_2$ (15 ml) was added dry pyridine (4 ml) and various amines (40 mmol). The reaction mixture was stirred at 50–60 °C for 5–6 h, and then added to 20 ml ice-water, organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 10 ml). The organic layer was combined and washed with saturated $NaHCO_3$, dried with anhydrous $Na_2SO_4$ for 0.5 h and concentrated under vacuum; ultimately the residue was purified by silica gel column (eluent EtOAc / petroleum ether, 1:2 - 2:1).

#### 3.2.1. 1,1-Dimethyl-3-(3-morpholinopropyl)-3-(3-nitrobenzyl)urea (*1*)

Light brown solid. 3.57 g (51%). M.p. 121–122 °C. $^1$H-NMR (500 MHz, DMSO-$d_6$, $\delta$ppm): 1.77 (m, 2H); 2.83 (s, 6H); 3.20 (t, $J = 5.2$, 2H); 3.26 (t, $J = 4.5$, 4H); 3.44 (t, $J = 4.3$, 2H); 3.52 (t, $J = 4.1$, 4H); 4.23 (s, 2 H); 7.72 (t, $J = 7.8$, 1H); 8.14 (d, $J = 7.5$, 1H); 8.27 (d, $J = 8.0$, 1H); 8.48 (s, 1H). MS (ESI): 350.2 ($[M + H]^+$). Anal. Calcd. for $C_{17}H_{26}N_4O_4$: C, 58.27; H, 7.48; N, 15.99%. Found: C, 58.34; H, 7.35; N, 15.30%.

Table 3
Antiproliferative activity against human normal cell line L02 of compounds

| Compound | $IC_{50}$ (μM) | Compound | $IC_{50}$ (μM) |
|---|---|---|---|
| | L 02 | | L 02 |
| **1** | 134 ± 35 | **13** | 65 ± 17 |
| **2** | 155 ± 43 | **14** | 85 ± 19 |
| **6** | 82 ± 17 | **18** | 26 ± 7 |
| **7** | 81 ± 14 | **19** | 125 ± 34 |
| **9** | 81 ± 16 | **22** | 21 ± 4 |
| **10** | 109 ± 34 | **23** | 86 ± 23 |
| Etoposide[a] | $IC_{50}$/L02: 13 ± 3 | | |

[a] Used as a positive control. The data represent the mean of three experiments performed in triplicate and are expressed as means ± SD. The $IC_{50}$ value is the concentration at which 50% survival of cells was observed.

*3.2.2. 1,1-Dimethyl-3-(2-morpholinoethyl)-3-(3-nitrobenzyl)urea (**2**)*

Light brown solid. 3.22 g (48%). M.p. 124–126 °C. $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.61 (s, 6H); 2.82 (t, $J = 5.6$, 2H); 3.21 (t, $J = 4.2$, 4H); 3.24 (t, $J = 4.6$, 2H); 3.50 (t, $J = 4.7$, 4H); 4.28 (s, 2H); 7.73 (t, $J = 7.9$, 1H); 8.14 (d, $J = 7.5$, 1H); 8.28 (d, $J = 7.0$, 1H); 8.50 (s, 1H). MS (ESI): 336.18 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{24}N_4O_4$: C, 57.13; H, 7.19; N, 16.66%. Found: C, 57.24; H, 7.08; N, 16.62%.

*3.2.3. 1-(Furan-2-ylmethyl)-3,3-dimethyl-1-(3-nitrobenzyl)urea (**3**)*

Brown solid. 3.22 g (48%). M.p. 135–137 °C. $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.91 (s, 6H); 4.25 (s, 2H); 4.84 (s, 2H); 6.27 (d, $J = 2.4$, 1H); 6.42 (t, $J = 1.8$, 1H); 7.51 (d, $J = 2.6$, 1H); 7.59 (t, $J = 7.8$, 1H); 8.11 (d, $J = 7.6$, 1H); 8.28 (d, $J = 7.5$, 1H); 8.39 (s, 1H). MS (ESI): 303.1 ($[M + H]^+$). Anal. Calcd. for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85%. Found: C, 59.60; H, 5.82; N, 13.68%.

*3.2.4. Ethyl 4-(3,3-dimethyl-1-(3-nitrobenzyl)ureido) piperidine-1-carboxylate (**4**)*

Brown oil. 3.33 g (44%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.26 (t, $J = 3.7$, 3H); 1.74 (m, 4H); 2.85 (s, 6H); 3.18 (m, 4H); 3.49 (m, 1 H); 4.15 (q, $J = 7.0$, 2H); 4.26 (s, 2H); 7.75 (t, $J = 7.8$, 1H); 8.12 (d, $J = 7.8$, 1H); 8.26 (d, $J = 7.5$, 1H); 8.52 (s, 1H). MS (ESI): 378.2 ($[M + H]^+$). Anal. Calcd. for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.93; N, 14.81%. Found: C, 57.42; H, 6.86; N, 14.73%.

*3.2.5. 1-(5-Chloro-2-hydroxybenzyl)-1-(furan-2-ylmethyl)-3,3-dimethylurea (**5**)*

Yellow oil. 3.51 g (57%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.82 (s, 6H); 4.27 (s, 2H); 4.54 (s, 2H); 6.27 (d, $J = 2.3$, 1H); 6.39 (t, $J = 1.7$, 1H); 6.73 (d, $J = 2.4$, 1H); 7.08 (m, 1H); 7.10 (d, $J = 7.8$, 1H); 7.17 (s, 1H). MS (ESI): 308.1 ($[M + H]^+$). Anal. Calcd. for $C_{15}H_{17}ClN_2O_3$: C, 58.35; H, 5.55; N, 9.07%. Found: C, 58.50; H, 5.62; N, 8.89%.

*3.2.6. 1-(5-chloro-2-hydroxybenzyl)-3,3-dimethyl-1-(2-morpholinoethyl)urea (**6**)*

Light yellow oil. 3.55 g (52%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.62 (t, $J = 5.2$, 2H); 2.94 (s, 6H); 3.16 (t, $J = 4.8$, 4H); 3.26 (t, $J = 4.2$, 2H); 3.52 (t, $J = 4.2$, 4H); 4.36 (s, 2H); 6.88 (d, $J = 8.8$, 1H); 7.32 (d, $J = 8.2$, 1H); 7.52 (s, 1H) 8.53 (s, 1H). MS (ESI): 341.1 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{24}ClN_3O_3$: C, 56.22; H, 7.08; N, 12.29%. Found: C, 56.40; H, 7.12; N, 12.09%.

*3.2.7. 1-(5-Chloro-2-hydroxybenzyl)-3,3-dimethyl-1-(3-morpholinopropyl)urea (**7**)*

Light yellow oil. 3.20 g (45%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.76 (m, 2H); 2.83 (s, 6 H); 2.96 (t, $J = 5.4$, 2H); 3.22 (t, $J = 4.6$, 4H); 3.36 (t, $J = 4.7$, 2H); 3.54 (t, $J = 4.0$, 4H); 4.34 (s, 2H); 6.87 (d, $J = 7.8$, 1H); 7.32 (d, $J = 7.6$, 1H); 7.53 (s, 1H) 8.53 (s, 1H). MS (ESI): 355.2 ($[M + H]^+$). Anal. Calcd. for $C_{17}H_{26}ClN_3O_3$: C, 57.38; H, 7.36; N, 11.81%. Found: C, 57.25; H, 7.42; N, 11.90%.

*3.2.8. Ethyl 4-(1-(5-chloro-2-hydroxybenzyl)-3,3-dimethylureido)piperidine-1-carboxylate (**8**)*

Yellow oil. 3.22 g (42%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.28 (t, $J = 3.8$, 3H); 1.72 (m, 4H); 2.80 (s, 6H); 3.13 (m, 4H); 3.46 (m, 1H); 4.17 (q, $J = 7.0$, 2H); 4.29 (s, 2H); 6.92 (d, $J = 8.3$, 1H); 7.28 (d, $J = 7.8$, 1H); 7.43 (s, 1H) 8.36 (s, 1H). MS (ESI): 383.2 ($[M + H]^+$). Anal. Calcd. for $C_{18}H_{26}ClN_3O_4$: C, 56.32; H, 6.83; N, 10.95%. Found: C, 56.46; H, 6.56; N, 11.23%.

*3.2.9. 1-(4-Hydroxybenzyl)-3,3-dimethyl-1-(2-morpholinoethyl)urea (**9**)*

Yellow solid. 3.38 g (55%). M.p. 144–146 °C. $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.55 (t, $J = 5.0$, 4H); 2.94 (s, 6H); 3.08 (t, $J = 5.2$, 2H); 3.26 (t, $J = 4.6$, 2H); 3.61 (t, $J = 4.0$, 4H); 4.49 (s, 2H); 6.75 (d, $J = 8.5$, 2H); 7.52 (d, $J = 8.5$, 2H) 8.16 (s, 1H). MS (ESI): 307.2 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{25}N_3O_3$: C, 62.52; H, 8.20; N, 13.67%. Found: C, 62.46; H, 8.36; N, 13.53%.

*3.2.10. 1-(4-Hydroxybenzyl)-3,3-dimethyl-1-(3-morpholinopropyl)urea (**10**)*

Light yellow oil. 2.63 g (41%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.67 (m, 2H); 2.76 (t, $J = 5.4$, 2H); 2.81 (s, 6H); 3.02 (t, $J = 4.6$, 4H); 3.26 (t, $J = 4.7$, 2H); 3.44 (t, $J = 4.0$, 4H); 4.28 (s, 2H); 6.73 (d, $J = 8.4$, 2H); 7.52 (d, $J = 8.8$, 2H) 8.15 (s, 1H). MS (ESI): 321.2 ($[M + H]^+$). Anal. Calcd. for $C_{17}H_{27}N_3O_3$: C, 63.53; H, 8.47; N, 13.07%. Found: C, 63.67; H, 8.42; N, 12.98%.

*3.2.11. 1-(Furan-2-ylmethyl)-1-(4-hydroxybenzyl)-3,3-dimethylurea (**11**)*

Yellow oil. 2.80 g (51%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.84 (s, 6H); 4.26 (s, 2H); 4.57 (s, 2H); 6.26 (d, $J = 2.2$, 1H); 6.35 (t, $J = 1.9$, 1H); 6.72 (d, $J = 2.4$, 1H); 6.85 (d, $J = 8.4$, 2H); 7.55 (d, $J = 8.8$, 2H) 8.05 (s, 1H). MS (ESI): 274.1 ($[M + H]^+$). Anal. Calcd. for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21%. Found: C, 65.50; H, 6.72; N, 10.29%.

*3.2.12. Ethyl 4-(1-(4-hydroxybenzyl)-3,3-dimethylureido) piperidine-1-carboxylate (**12**)*

Yellow oil. 2.79 g (40%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.26 (t, $J = 3.7$, 3H); 1.74 (m, 4H); 2.85 (s, 6H); 3.15 (m, 4H); 3.43 (m, 1H); 4.18 (q, $J = 5.2$, 2H); 4.33 (s, 2H); 6.83 (d, $J = 8.5$, 2H); 7.56 (d, $J = 8.9$, 2H) 8.16 (s, 1H). MS (ESI): 349.2 ($[M + H]^+$). Anal. Calcd. for $C_{18}H_{27}N_3O_4$: C, 61.87; H, 7.79; N, 12.03%. Found: C, 61.95; H, 7.86; N, 11.68%.

*3.2.13. 1,1-Dimethyl-3-(2-morpholinoethyl)-3-(4-nitrobenzyl)urea (**13**)*

Light yellow oil. 3.16 g (47%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.43 (t, $J = 5.2$, 4H); 2.61 (t, $J = 6.5$,

2H); 2.84 (s, 6H); 3.55 (t, $J = 4.9$, 2H); 3.76 (t, $J = 6.5$, 4H); 4.42 (s, 2H); 7.98 (d, $J = 9.0$, 2H); 8.29 (d, $J = 9.0$, 2H). MS (ESI): 336.2 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{24}N_4O_4$: C, 57.13; H, 7.19; N, 16.66%. Found: C, 57.42; H, 7.06; N, 16.53%.

*3.2.14. 1,1-Dimethyl-3-(3-morpholinopropyl)-3-(4-nitrobenzyl)urea (**14**)*

Brown oil. 3.71 g (53%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.70 (m, 2H); 2.38 (t, $J = 7.5$, 2H); 2.67 (t, $J = 6.6$, 4H); 2.88 (s, 6H); 3.28 (t, $J = 5.6$, 2H); 3.64 (t, $J = 4.8$, 4H); 4.32 (s, 2H); 7.49 (d, $J = 8.1$, 2H); 8.16 (d, $J = 7.8$, 2H). MS (ESI): 350.2 ($[M + H]^+$). Anal. Calcd. for $C_{17}H_{26}N_4O_4$: C, 58.27; H, 7.48; N, 15.99%. Found: C, 58.33; H, 7.28; N, 15.87%.

*3.2.15. 1-(Furan-2-ylmethyl)-3,3-dimethyl-1-(4-nitrobenzyl)urea (**15**)*

Brown solid. 3.22 g (48%). M.p. 121–122 °C. $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.88 (s, 6H); 4.28 (s, 2H); 4.85 (s, 2H); 6.35 (q, $J = 2.5$, 1H); 6.46 (t, $J = 1.9$, 1H); 7.41 (q, $J = 2.5$, 1H); 7.94 (q, $J = 7.8$, 2H); 8.27 (d, $J = 8.2$, 2H). MS (ESI): 303.1 ($[M + H]^+$). Anal. Calcd. for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85%. Found: C, 59.23; H, 5.87; N, 13.89%.

*3.2.16. Ethyl 4-(3,3-dimethyl-1-(4-nitrobenzyl)ureido) piperidine-1-carboxylate (**16**)*

Yellow oil. 3.18 g (42%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.24 (t, $J = 3.6$, 3H); 1.72 (m, 4H); 2.89 (s, 6H); 3.13 (m, 4H); 3.44 (m, 1H); 4.16 (*q*, $J = 5.6$, 2H); 4.35 (s, 2H); 7.48 (d, $J = 8.0$, 2H); 8.15 (d, $J = 7.9$, 2H). MS (ESI): 378.2 ($[M + H]^+$). Anal. Calcd. for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.93; N, 14.81%. Found: C, 57.55; H, 7.06; N, 14.68%.

*3.2.17. 1-(Furan-2-ylmethyl)-1-(2-hydroxy-5-nitrobenzyl)-3,3-dimethylurea (**17**)*

Yellow oil. 3.25 g (51%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.86 (s, 6H); 4.26 (s, 2H); 4.56 (s, 2H); 6.28 (d, $J = 2.2$, 1H); 6.41 (t, $J = 1.9$, 1H); 6.75 (d, $J = 2.1$, 1H); 7.02 (m, 1H); 7.13 (d, $J = 7.8$, 1H); 7.27 (s, 1H), 8.24 (s, 1H). MS (ESI): 319.1 ($[M + H]^+$). Anal. Calc. for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16%. Found: C, 56.57; H, 5.42; N, 13.02%.

*3.2.18. 1-(2-Hydroxy-5-nitrobenzyl)-3,3-dimethyl-1-(2-morpholinoethyl)urea (**18**)*

Light yellow oil. 3.73 g (53%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.60 (t, $J = 4.2$, 2H); 2.96 (s, 6H); 3.06 (t, $J = 4.9$, 4H); 3.24 (t, $J = 4.8$, 2H); 3.54 (t, $J = 4.6$, 4H); 4.37 (s, 2H); 6.98 (d, $J = 8.9$, 1H); 7.42 (d, $J = 8.0$, 1H); 7.62 (s, 1H) 8.58 (s, 1H). MS (ESI): 352.2 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{24}N_4O_5$: C, 54.53; H, 6.86; N, 15.90%. Found: C, 54.40; H, 6.98; N, 15.92%.

*3.2.19. 1-(2-Hydroxy-5-nitrobenzyl)-3,3-dimethyl-1-(3-morpholinopropyl)urea (**19**)*

Light yellow oil. 3.30 g (45%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.74 (m, 2H); 2.82 (s, 6H); 2.98 (t, $J = 5.2$, 2H); 3.24 (t, $J = 4.8$, 4H); 3.38 (t, $J = 4.8$, 2H); 3.56 (t, $J = 4.4$, 4H); 4.36 (s, 2H); 6.92 (d, $J = 7.8$, 1H); 7.46 (d, $J = 7.6$, 1H); 7.67 (s, 1H) 8.50 (s, 1H). MS (ESI): 366.2 ($[M + H]^+$). Anal. Calcd. for $C_{17}H_{26}N_4O_5$: C, 55.72; H, 7.15; N, 15.29%. Found: C, 55.65; H, 7.23; N, 15.36%.

*3.2.20. Ethyl 4-(1-(2-hydroxy-5-nitrobenzyl)-3,3-dimethylureido)piperidine-1-carboxylate(**20**)*

Yellow oil. 3.23 g (41%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.26 (t, $J = 3.7$, 3H); 1.74 (m, 4H); 2.82 (s, 6H); 3.14 (m, 4H); 3.48 (m, 1H); 4.18 (q, $J = 4.0$, 2H); 4.32 (s, 2H); 6.94 (d, $J = 7.9$, 1H); 7.56 (d, $J = 7.8$, 1H); 7.89 (s, 1H) 8.52 (s, 1H). MS (ESI): 394.2 ($[M + H]^+$). Anal. Calcd. for $C_{18}H_{26}N_4O_6$: C, 54.81; H, 6.64; N, 14.20%. Found: C, 54.56; H, 6.78; N, 14.23%.

*3.2.21. 1-(5-Bromo-2-hydroxybenzyl)-1-(furan-2-ylmethyl)-3,3-dimethylurea (**21**)*

Yellow oil. 3.87 g (55%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.87 (s, 6H); 4.15 (s, 2H); 4.41 (s, 2H); 6.18 (d, $J = 2.5$, 1H); 6.38 (t, $J = 1.9$, 1H); 6.93 (d, $J = 2.2$, 1H); 7.18 (d, $J = 7.8$, 1H); 7.40 (d, $J = 7.8$, 1H); 7.62 (s, 1H); 8.82 (s, 1H). MS (ESI): 352.0 ($[M + H]^+$). Anal. Calcd. for $C_{15}H_{17}BrN_2O_3$: C, 51.01; H, 4.85; N, 7.93%. Found: C, 51.23; H, 4.72; N, 7.80%.

*3.2.22. 1-(5-Bromo-2-hydroxybenzyl)-3,3-dimethyl-1-(2-morpholinoethyl)urea (**22**)*

Light yellow oil. 3.93 g (51%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 2.57 (t, $J = 5.2$, 2H); 2.92 (s, 6H); 3.12 (t, $J = 4.6$, 4H); 3.28 (t, $J = 4.8$, 2H); 3.56 (t, $J = 4.3$, 4H); 4.42 (s, 2H); 7.06 (d, $J = 7.5$, 1H); 7.42 (d, $J = 7.6$, 1H); 7.70 (s, 1H); 8.67 (s, 1H). MS (ESI): 385.1 ($[M + H]^+$). Anal. Calcd. for $C_{16}H_{24}BrN_3O_3$: C, 49.75; H, 6.26; N, 10.88%. Found: C, 49.49; H, 6.38; N, 10.96%.

*3.2.23. 1-(5-Bromo-2-hydroxybenzyl)-3,3-dimethyl-1-(3-morpholinopropyl)urea (**23**)*

Light yellow oil. 3.27 g (41%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.68 (m, 2H); 2.86 (s, 6H); 2.89 (t, $J = 4.8$, 2H); 3.01 (t, $J = 4.4$, 4H); 3.23 (t, $J = 4.6$, 2H); 3.52 (t, $J = 4.2$, 4H); 4.38 (s, 2H); 7.08 (d, $J = 7.6$, 1H); 7.44 (d, $J = 7.6$, 1H); 7.72 (s, 1H); 8.66 (s, 1H). MS (ESI): 399.2 ($[M + H]^+$). Anal. Calcd. for $C_{17}H_{26}BrN_3O_3$: C, 51.01; H, 6.55; N, 10.50%. Found: C, 51.29; H, 6.41; N, 10.32%.

*3.2.24. Ethyl 4-(1-(5-bromo-2-hydroxybenzyl)-3,3-dimethylureido)piperidine-1-carboxylate (**24**)*

Yellow oil. 3.41 g (40%). $^1$H-NMR (500 MHz, DMSO-$d_6$, δppm): 1.32 (t, $J = 3.6$, 3H); 1.74 (m, 4H); 2.82 (s, 6H); 3.15 (m, 4H); 3.48 (m, 1H); 4.19 (q, $J = 4.0$, 2H); 4.32 (s, 2H); 7.06 (d, $J = 7.6$, 1H); 7.42 (d, $J = 7.6$, 1H); 7.67 (s, 1H); 8.56 (s, 1H). MS (ESI): 427.1 ($[M + H]^+$). Anal. Calcd.

for $C_{18}H_{26}BrN_3O_4$: C, 50.47; H, 6.12; N, 9.81%. Found: C, 50.78; H, 5.69; N, 9.77%.

### 3.3. Antiproliferative assay

The antiproliferative activity of **1–24** was determined using a standard (MTT)-based colorimetric assay (Sigma), using etoposide as reference drugs. Briefly, cell lines were seeded at a density of $7 \times 10^3$ cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 100 μg/mL. After 48 h, cell survival was determined by the addition of an MTT solution (10 μL of 5 mg/mL MTT in PBS). After 4 h, 100 μL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 18 h; optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. $IC_{50}$ values were determined from replicates of six wells from at least two independent experiments.

## 4. Conclusions

In approach of preparing new anticancer agents, we have synthesized a series of analogues of 1,3-disubstituted urea derivatives. For all the compounds synthesized, antiproliferative activity against one human normal cell line (L02) and two tumor cell lines (K562 and KB) was determined. The chemical modification of urease derivatives generated some potent compounds, with $IC_{50}$ values up to over 20-fold lower comparable to those of the reference compounds etoposide. The series of 1,3-disubstituted urea derivatives show good antiproliferative activity against human cancer cell lines and no antiproliferative activity against normal cell line (L02). Compounds with a morpholine ring exhibited remarkable antiproliferative toward human cancer cell lines; compounds introduced a nitro group substituent at C-3 position on the phenyl ring approved to generally decrease activity. It may be concluded that compounds **1** and **2**, possess promising anticancer activity combined with low antiproliferative to human normal cell line. These compounds are now undergoing further lead optimization in our laboratory for development as an anticancer agent.

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## References

[1] M. Gourley, J.S. Williamson, Curr. Pharm. Des. 6 (2000) 417–439.
[2] A. Ullrich, J. Schlessinger, Cell 61 (1990) 203–212.
[3] S.R. Hubbard, J.H. Till, Annu. Rev. Biochem. 69 (2000) 373–398.
[4] M.J. Hayman, P.J. Enrietto, Cancer Cells 3 (1991) 302–307.
[5] I. Dikic, S. Giordano, Curr. Opin. Cell Biol. 15 (2003) 128–135.
[6] F.M. Uckun, C. Mao, Curr. Pharm. Des. 10 (2004) 1083–1091.
[7] P. Traxler, J. Green, H. Mett, U. Sequin, P. Furet, J. Med. Chem. 42 (1999) 1018–1026.
[8] J.A. Shimshoni, M. Bialer, B. Wlodarczyk, R.H. Finnell, B. Yagen, J. Med. Chem. 50 (2007) 6419–6427.
[9] J.S. Fortin, J. Lacroix, M. Desjardins, P. Michel, P.E. Alexandre, C.G. Rene, Bioorg. Med. Chem. 15 (2007) 4456–4469.
[10] R.J. Watson, D.R. Allen, H.L. Birch, G.A. Chapman, A. Gayle, F.C. Frances, L.A. Knight, D. Meier, K. Oliver, J.W.G. Meissner, D.A. Owen, E.J. Thomas, N. Tremayne, S.C. Williams, Bioorg. Med. Chem. Lett. 18 (2008) 147–151.
[11] P. Be′chard, J. Lacroix, P. Poyet, Eur. J. Med. Chem. 29 (1994) 963–966.
[12] P. Cao, X.F. Huang, D. Hui, H.M. Ge, H.Q. Li, B.F. Ruan, H.L. Zhu, Chem. Biodiv. 4 (2007) 881–886.
[13] P. Krzysztof, K. P.JoannaPiotr, R. Jan, P. Wiesław, Z.S. Oliwia, B. Jerzy, S.S. Janusz, P.M. Aleksander, G. Grzegorz, Cancer Lett. 203 (2004) 59–65.