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Design, Synthesis and Anticancer Activity of Unsymmetrically Substituted Urea Based Small Molecules against Human Breast Cancer (MCF7) Cell Lines

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Abstract: In the present investigation urea-based small molecules with unsymmetrical substitutions were synthesised and their *in vitro* cytocoxic activity was assessed against Human breast cancer (MCF7) cell lines. The title compounds (UD**a** - UD**l**) were synthesised in two steps and their *in vitro* cytotoxic activity was evaluated using MTT assay method. All the compounds showed effective cytotoxic activity against the tested cell line with the IC_{50} value ranges between 41.99 – 156.52 ug/ml. Among the compounds tested, UD**l** exhibited potent cytotoxic activity with a IC_{50} value of 41.99 ug/ml. Compound UD**f**, UD**i** and UD**k** showed IC_{50} values of 48.64, 51.08 and 52.27 ug/ml, respectively. Compounds UD**e** and UD**c** exhibited moderate cytotoxic activity and the remaining compounds showed mild activity against the tested cell lines. Camptothecin was used as a standard drug which showed the IC_{50} value as 19.95 ug/ml.

Keywords: Cytotoxicity, Breast cancer, Urea derivatives, MCF7, IC_{50}

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Introduction

Although there are significant advances in medical sciences, cancer is still an obstinate disease in humans and is a main cause of death around the world. Considering the existing cancer therapies, chemotherapy is one of the most principal treatments in the cancer management (Harrison *et al*., 2009). The disease originated due to the failure of cells to control growth, often leading to the

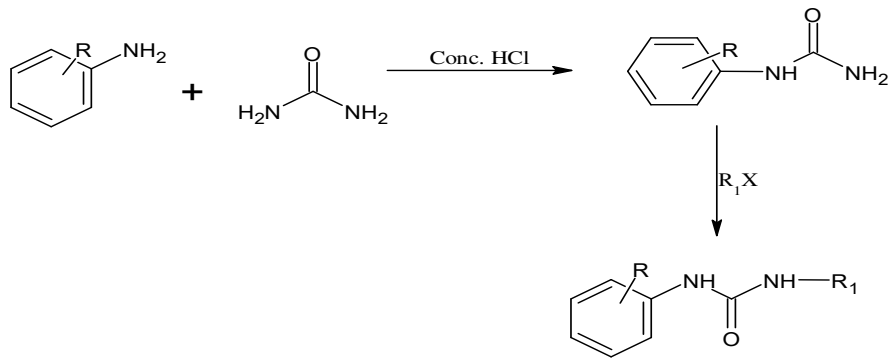


Fig. 1: Scheme for the synthesis of unsymmetrically substituted urea based small molecules.

formation of cancerous tumors. Cancer treatment consists of chemotherapy and radiotherapy where the former employs molecule-size drugs aiming at eradication and inhibition of cancer tumors. However, the existing anticancer agents often act on cells with swift metabolism and proliferation, and cannot differentiate between cancer and normal cells. Therefore, discovery of novel effective, selective and less toxic anticancer agents remains one of the most active area in the field of medicinal chemistry and drug design (Eckhardt, 2002). On the other hand, progress of resistance against the existing anticancer drugs keeps research window open in the search of newer small molecule chemotherapeutics (Sierra *et al*., 2010).

The urea functionality is intrinsic to numerous bioactive compounds, including a variety of clinically approved therapies. Urea containing compounds are increasingly used in medicinal chemistry and drug design in order to establish key drug-target interactions and fine-tune crucial drug-like properties (Ghosh and Margherita, 2020). Urea is a functional moiety that is commonly found in natural products and often displays a wide range of biological activities. In particular, substituted urea molecules have attracted attention due to their range of applications as inhibitory activity on lipopoly-saccharide-induced NO production (Yoon *et al*., 2007), MCHR1 antagonists for the treatment of obesity (Silvia *et al*., 2007), antibacterial activity (Salman *et al*., 2008), cytostatic and antioxidant activities (Simunovic *et al*., 2009), for blood glucose lowering effect (Rathish *et al*., 2009), antiproliferative activity (Huan *et al*., 2009) and antifungal activity (Qing *et al*., 2010). With the advances of protein structures and identification of new disease targets, there is a growing interest in urea-based derivatives for drug design and development.

In the present study a series of unsymmetrically substituted urea based small molecules (UD**a** – UD**l**) were synthesized and the *in vitro* cytotoxic activity was evaluated against human breast cancer cell lines (MCF7).

Materials and Methods

Instruments:

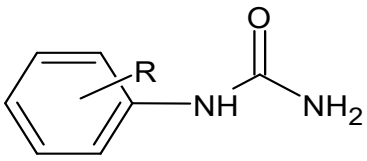
NMR spectra were recorded (DMSO) on Bruker (Bruker Bioscience, USA) 400 MHz spectrometer in DMSO-d6 using TMS as an internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument (Micromass, UK) at 70eV. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectro photometer using KBr optics (Perkin-Elmer, USA).

Synthesis:

The synthesis of the targeted compounds was

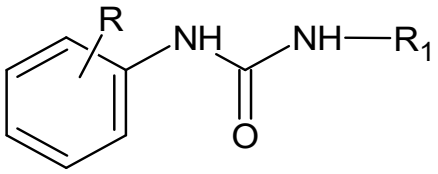
carried out using the procedure outlined in Figure 1.

Procedure for the preparation of substituted phenyl urea's (*UA-UL*):



Dissolved 0.5 moles of substituted aniline and 2 moles of urea in 200 ml of water taken in a round bottomed (RB) flask. Added 4 ml of conc. HCl and 4 ml of glacial acetic acid and fixed a reflux condenser to the RB flask, introduced few fragments of broken porcelain pieces and boiled the mixture for 1-2 h. Fine white crystals (diphenyl urea) appeared after about 15 min, and gradually increased in amount as the refluxing was continued. Cooled the flask in ice and filtered with suction. Separated the mixture of phenyl urea and diphenyl urea by boiling with 500 ml of water and filtered at the pump through a preheated Buchner funnel into a warm flask. The filtrate contained phenyl urea which was cooled to form the crystals, whereas the residue contains diphenylurea which was recrystallised from ethylacetate with addition of little decolorising carbon gives colourless crystals (Furnis *et al*., 1989).

Procedure for the preparation of unsymmetrically substituted urea based small molecules (*UDA-UDL*):



Taken 1 mole of substituted phenylurea in a beaker and added 0.5 moles of alkyl or aryl halide drop wise and stirred for about 1 h at 150°C, then cooled rapidly and dissolved it in toluene (Yasuo *et al*., 1989). Then filtered with suction and residue obtained was the product which was purified by column chromatography. All the compounds are soluble in DMSO.

Melting points of all the synthesized compounds were determined using digital melting point apparatus and are uncorrected values. The characterization of the compounds was done with the help of IR, 1H NMR and EI-MS spectral data. The IR spectra were recorded on Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics (Perkin-Elmer, USA). NMR spectra were recorded (DMSO) on Bruker (Bruker Bioscience, USA) 400 MHz spectrometer in DMSO-d6 using TMS as an internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument (Micromass, UK) at 70eV.

Anticancer activity:

The anticancer activity of each synthesized compounds UD**a** - UD**l** were evaluated against human breast cancer (MCF7) cells using MTT colorimetric assay according to the method of Mosmann (1983). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow coloured water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570 nm (Alley *et al*., 1986).

The MCF7 (Human breast adenocarcinoma cell lines) was purchased from NCCS (National Centre for Cell Science), Pune, India. The cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) with high glucose media supplemented with 10 % fetal bovine serum (FBS) (Gibco BRL) along with the 1% antibiotic-antimycotic solution (penicillin 100 U/ml and streptomycin 100 µg/ml) in the atmosphere of 5% CO_2, 18-20% O_2 at 37^{0}C temperature in the CO_2 incubator and sub-cultured for every 2 days. Passage number 70 was used for the present study.

Briefly, 200 µl cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Cells were allowed to grow for about 24 h. Appropriate concentrations (12.5, 25, 50, 100 and 200 µg/ml) of the test compounds (UD**a** – UD**l**) were added and the plates were incubated for 24 h at 37°C in a 5% CO_2 atmosphere. After the incubation period, the plates were removed from incubator, and the spent media was removed. MTT reagent to a final concentration of 0.5 mg/ml of total volume was added. The plates were wrapped with aluminium foil to avoid exposure to light and returned to the incubator and incubated for another 3 h. After incubation, the culture medium was then replaced with 100 µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. The absorbance of each well was measured on an ELISA reader at 570 nm. Camptothecin (20 µM/ml) was used as a positive control for cytotoxicity. For each compound, the concentration causing 50% cell growth inhibition (IC_{50}) was compared to the control and standard.

The % cell viability was calculated using the below formula:

% Cell viability = [OD of treated cells/OD of Untreated cells] X 100

The IC_{50} value was determined by using linear regression equation i.e. Y = mX+C.

Here, Y = 50, m and C values were derived from the viability graph.

Results

Synthesis:

Unsymmetrically substituted urea derivatives were synthesized successfully from reaction of substituted aromatic amines with different substituted aliphatic and aromatic alkyl halides. The compounds were purified either by crystallization or by column chromatography. The yields were obtained between 21 and 38 %.

1-ethyl-3-phenylurea (*UDa*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 204-208°C, yield 26%. IR (KBr, CM-1): 3286.01 (N-H stretch), 1643.28 (C=O stretch), -1228.91 (C-N stretch), 1542.42 (Ar C=C stretch), 3031.28 (Ar C-H stretch), 2919.75 (C-H stretch); 1H NMR (DMSO, δ ppm): 7.1 (s, 2H, -NH), 1.8 (t, 3H, -CH), 3.9 (q, 2H, CH), 6.8-7.5 (m, 10H, Ar-H); MS (EI-MS): 165 m/z; Elemental Analysis: Calc. for C9H12N2O: C, 65.83; H, 7.37; N, 17.06; O, 9.74. Found: C, 65.9; H, 7.3; N, 17.07; O, 9.7. Formula weight: 164.2045.

1-benzyl-3-phenylurea (*UDb*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 170-174°C, yield 22%. IR (KBr, CM-1): 3287.07 (N-H stretch), 1645.11 (C=O stretch), 1229.67 (C-N stretch), 1540.34 (Ar C=C stretch), 3032.83 (Ar C-H stretch), 2917.57 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.6 (s, 2H, -NH), 4.3 (s, 2H, -CH), 6.8-7.5 (m, 10H, Ar-H); MS (EI-MS): 227 m/z; Elemental Analysis (%): Calc. for C14H14N2O: C, 74.31; H, 6.24; N, 12.38; O, 7.07. Found: C, 74.3; H, 6.3; N, 12.4; O, 7.1. Formula weight: 226.2738.

1-(4-chlorobenzyl)-3-phenylurea (*UDc*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 198-200°C, yield 21%. IR (KBr, CM-1): 3291.12 (N-H stretch), 1642.0511 (C=O stretch), 1232.71 (C-N stretch), 1541.4234 (Ar C=C stretch), 3032.48 (Ar C-H stretch), 814.12 (C-Cl stretch), 2914.42 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.5 (s, 2H, -NH), 4.2 (s, 2H, -CH), 6.8-7.5 (m, 9H, Ar-H); MS (EI-MS): 262 m/z; Elemental Analysis (%): Calc. for C14H13ClN2O: C, 64.49; H, 5.03; Cl, 13.60; N, 10.74; O, 6.14. Found: C, 64.5; H, 5.0; Cl, 13.61; N, 10.8; O, 6.1. Formula weight: 260.7186.

1-ethyl-3-(2-methylphenyl) urea (*UDd*):

This compound was obtained as a white solid. It was purified by column chromatography using an

eluent hexane : ethylacetate (1:1 v/v). MP: 270-274°C, yield 30%. IR (KBr, CM-1): 3285.08 (N-H stretch), 1643.28 (C=O stretch), 1227.76 (C-N stretch), 1538.44 (Ar C=C stretch), 3030.38 (Ar C-H stretch), 2916.78 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.6 (s, 2H, -NH), 3.1 (q, 2H, -CH), 2.1 (s, CH), 1.1 (t, CH), 6.8-7.5 (m, 4H, Ar-H); MS (EI-MS): 178 m/z; Elemental Analysis: Calc. for C10H14N2O: C, 67.39; H, 7.92; N, 15.72; O, 8.98. Found: C, 67.4; H, 7.9; N, 15.71; O, 9.0. Formula weight: 178.231.

1-benzyl-3-(2-methylphenyl) urea (*UDe*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 260-264°C, yield 26%. IR (KBr, CM-1): 3287.27 (N-H stretch), 1644.91 (C=O stretch), 1227.48 (C-N stretch), 1541.34 (Ar C=C stretch), 3033.38 (Ar C-H stretch), 2919.75 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.5 (s, 2H, -NH), 4.6 (s, 2H, -CH), 2.1 (s, 3H, CH), 6.8-7.5 (m, 9H, Ar-H); MS (EI-MS): 242 m/z; Elemental Analysis: Calc. for C15H16N2O: C, 74.97; H, 6.71; N, 11.66; O, 6.66. Found: C, 75.0; H, 6.7; N, 11.7; O, 6.6. Formula weight: 240.3004.

1-(4-chlorobenzyl)-3-(2-methylphenyl) urea (*UDf*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 274-278°C, yield 29%. IR (KBr, CM-1): 3288.12 (N-H stretch), 1646.14 (C=O stretch), 1230.72 (C-N stretch), 1539.99 (Ar C=C stretch), 3033.33 (Ar C-H stretch), 815.14 (C-Cl stretch), 2917.91 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.6 (s, 2H, -NH), 4.3 (s, 2H, -CH), 2.1 (s, 3H, -CH3), 6.8-7.5 (m, 8H, Ar-H); MS (EI-MS): 275 m/z; Elemental Analysis: Calc. for C15H15ClN2O: C, 65.57; H, 5.50; Cl, 12.90; N, 10.20; O, 5.82. Found: C, 65.6; H, 5.5; Cl, 13.0; N, 10.21; O, 5.9. Formula weight: 274.7452.

1-ethyl-3-(4-methylphenyl) urea (*UDg*):

This compound was obtained as a white solid. It was purified by column chromatography using a eluent hexane : ethylacetate (1:1 v/v). MP: 240-244°C, yield 30%. IR (KBr, CM-1): 3294.08 (N-H stretch), 1658.24 (C=O stretch), 1238.76 (C-N stretch), 1548.3 (Ar C=C stretch), 3045.83 (Ar C-H stretch), 2928.57 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.6 (s, 2H, -NH), 1.1 (t, 3H, -CH), 2.3 (s, 3H, CH), 3.2 (q, 2H, CH), 6.8-7.5 (m, 4H, Ar-H); MS (EI-MS): 179 m/z; Elemental Analysis: Calc. for C10H14N2O: C, 67.39; H, 7.92; N, 15.72; O, 8.98. Found: C, 67.4; H, 7.9; N, 15.8; O, 9.0. Formula weight: 178.231.

1-benzyl-3-(4-methylphenyl) urea (*UDh*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 246-250°C, yield 30%. IR (KBr, CM-1): 3283.44 (N-H stretch), 1645.82 (C=O stretch), 1229.78 (C-N stretch), 1542.48 (Ar C=C stretch), 3033.09 (Ar C-H stretch), 2919.80 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.6 (s, 2H, -NH), 4.5 (s, 2H, -CH), 2.2 (-OCH), 6.8-7.5 (m, 9H, Ar-H); MS (EI-MS): 227 m/z; Elemental Analysis: Calc. for C15H16N2O: C, 74.97; H, 6.71; N, 11.66; O, 6.66. Found: C, 75.1; H, 6.7; N, 11.7; O, 6.7. Formula weight: 240.3004.

1-(4-chlorobenzyl)-3-(4-methylphenyl) urea (*UDi*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:1 v/v). MP: 258-260°C, yield 27%. 1H NMR spectrum (DMSO, δ ppm) : 8.5(s, 2H, -NH), 3.9(s, 2H, -CH), 2.2(s, 3H, -CH), 6.8-7.5(m, 8H, Ar-H). IR (KBr, CM-1): 3296.69 (N-H stretch), 1638.07 (C=O stretch), 1235.85 (C-N stretch), 1555.99 (Ar C=C stretch), 2916.32 (Ar C-H stretch), 813.59 (C-Cl stretch); 2856.15 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.5 (s, 2H, -NH), 3.9 (s, 2H, -CH), 2.2 (s, 3H, -CH) 6.8-7.5 (m, 8H, Ar-H); MS (EI-MS): 275 m/z; Elemental Analysis: Calc. for C15H15ClN2O: C, 65.57; H, 5.50; Cl, 12.90; N, 10.20; O, 5.82. Found: C, 65.6; H, 5.5; Cl, 13.0; N, 10.2; O, 5.9. Formula weight: 274.7452.

1-ethyl-3-(4-methoxyphenyl) urea (*UDj*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:2 v/v). MP: 196-198°C, yield 38%. IR (KBr, CM-1): 3299.91 (N-H stretch), 1660.21 (C=O stretch), 1238.78 (C-N stretch), 1545.14 (Ar C=C stretch), 3085.10 (Ar C-

Table 1: Anticancer activity of synthesised compounds against Human Breast Cancer (MCF7) cell lines

Compound code	R	R1	MW	IC_{50} (µg/ml)	IC_{50} (µM/ml)
UD**a**	-H	$-C_2H_5$	164	156.52	0.95
UD**b**	-H	$-CH_2-C_6H_5-$	226	101.45	0.46
UD**c**	-H	$-CH_2$-p-Cl-C_6H_4-	260	61.53	0.24
UD**d**	-o-CH_3	$-C_2H_5$	178	94.81	0.53
UD**e**	-o-CH_3	$-CH_2-C_6H_5-$	240	58.2	0.24
UD**f**	-o-CH_3	$-CH_2$-p-Cl-C_6H_4-	274	48.64	0.18
UD**g**	-p-CH_3	$-C_2H_5$	178	101.25	0.57
UD**h**	-p-CH_3	$-CH_2-C_6H_5-$	240	128.11	0.53
UD**i**	-p-CH_3	$-CH_2$-p-Cl-C_6H_4-	274	51.08	0.18
UD**j**	-p-OCH_3	$-C_2H_5$	194	106.52	0.55
UD**k**	-p-OCH_3	$-CH_2-C_6H_5-$	256	52.27	0.2
UD**l**	-p-OCH_3	$-CH_2$-p-Cl-C_6H_4-	290	41.99	0.14
Standard		Camptothecin		19.95	0.06

H stretch), 2917.75 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.2 (s, 2H, -NH), 3.8 (s, 3H, -OCH); 1.3 (t, 3H, -CH), 3.2 (q, 2H, -CH), 6.8-7.5 (m, 4H, Ar-H); MS (EI-MS): 196 m/z; Elemental Analysis: Calc. for C10H14ClN2O2: C, 61.84; H, 7.27; N, 14.42; O, 16.47. Found: C, 61.8; H, 7.3; N, 14.4; O, 16.4. Formula weight: 194.2304.

1-benzyl-3-(*4-methoxyphenyl*) *urea* (*UDk*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:2 v/v). MP: 178-180°C, yield 33%. IR (KBr, CM-1): 3286.53 (N-H stretch), 1629.96 (C=O stretch), 1243.21 (C-N stretch), 1507.76 (Ar C=C stretch), 2891.05 (Ar C-H stretch), 2834.73 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.2 (s, 2H, -NH), 3.0 (s, 2H, -CH), 3.8 (s, 3H, -OCH); 6.8-7.5 (m, 9H, Ar-H); MS (EI-MS): 257 m/z; Elemental Analysis: Calc. for C15H16N2O2: C, 70.29; H, 6.29; N, 10.93; O, 12.48. Found: C, 70.1; H, 6.3; N, 11.0; O, 12.5. Formula weight: 256.2998.

1-(*4-chlorobenzyl*)*-3-*(*4-methoxyphenyl*)*urea* (*UDl*):

This compound was obtained as a white solid. It was purified by column chromatography using an eluent hexane : ethylacetate (1:2 v/v). MP: 208-212°C, yield 33%. IR (KBr, CM-1): 3295.09 (N-H stretch), 1649.28 (C=O stretch), 1235.76 (C-N stretch), 1551.43 (Ar C=C stretch), 3038.38 (Ar C-H stretch), 818.28 (C-Cl stretch), 2928.58 (C-H stretch); 1H NMR (DMSO, δ ppm): 8.1 (s, 2H, -NH), 3.2 (s, 2H, -CH), 3.7 (s, 3H, -OCH); 6.8-7.5 (m, 8H, Ar-H); MS (EI-MS): 291 m/z; Elemental Analysis: Calc. for C15H15ClN2O2: C, 61.97; H, 5.20; Cl, 12.19; N, 9.64; O, 11.01. Found: C, 61.9; H, 5.22; Cl, 12.2; N, 9.6; O, 11.0. Formula weight: 290.7446.

Anticancer activity:

In the present study we applied the MTT test to evaluate the potency of 12 unsymmetrically substituted urea derivatives (UD**a** – UD**l**) using human breast cancer cell lines (MCF7) in an *in vitro* cell-based assay. In order to determine the concentration required to achieve 50% inhibition of cells induced by each compound, the dose response curve was plotted. The results of cytotoxic assay were mentioned as IC_{50} (µg/ml and µM/ml) of compounds in comparison with standard anti-cancer drug camptothecin (Table 1).

Discussion

One of the major goals of cancer treatment is to predict the response of patients with cancer to chemotherapeutic agents by employing laboratory methods variously called "tumor chemosensitivity assays", "drug response assays", or "drug sensitivity assays", *in vitro*. *In vitro* cytotoxicity assays were applied to evaluate anti-cancer drugs on target cell lines (Brown and Mire-Sluis, 2002). The cytotoxicity of the synthesised compounds were evaluated against human breast carcinoma (MCF-7) which represent one of the most common types of cancer with high rate of occurrence. Screening of cell viability after exposure to the compounds was performed using the MTT assay. It is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. The basis of the MTT method is the ability of mitochondrial dehydrogenase present in the mitochondria of metabolically active cells to convert the yellow tetrazolium salt (MTT) into purple formazan crystals. MTT assay was carried out according to the protocol (Mosmann, 1983). Cells were treated with various concentrations of test compounds for 24–72 h. The activity of test compounds is expressed by IC_{50} values, defined as 50% inhibition of cell growth compared to the untreated control.

Results from the present screening showed that six out of twelve compounds are able to inhibit more than 50% of viability of breast carcinoma cells (MCF-7) and their IC_{50} values were in the range of 41.99 - 61.93 µg/ml. Among this compound UD**l** exhibited potent activity with an IC_{50} value of 41.99 µg/ ml. The concentration of the six compounds that inhibited breast carcinoma cells (MCF-7) proliferation by 50% (IC_{50}) was greater than 0.1 µM/ ml indicating low toxicity. The remaining compounds showed moderate to mild cytotoxic activity against the tested cancer cell line while the standard drug camptothecin showed very potent activity against the tested cell line with the IC_{50} value of 19.95 µg/ml.

It was observed that the substitution of amino hydrogen of urea residue with an aromatic ring is essential for activity against MCF-7 cells, since the alkyl substitution at R1 (UD**a**, UD**d**, UD**g** and UD**j**) showed very less cytotoxic activity at the same concentration. The presence of o- or p-substitutions at R increases the activity rather than the unsubstituted aromatic ring. The derivatives with the p-chlorine substituted aromatic ring at R1 showed greater activity among all the tested compounds. Accordingly with the results obtained, we were able to conclude that the presence of electron withdrawing group, p-Cl on aromatic ring at R1 and p-methoxy on aromatic ring at R showed increase in cytotoxic activity (UD**l**).

Conclusion

A series of unsymmetrically substituted urea based small molecules (UD**a** – UD**l**) were synthesized in this study. All compounds were evaluated *in vitro* against human breast carcinoma (MCF-7) cell lines. Among the tested compounds, the derivatives UD**l,** UD**i,** UD**f** and UD**k** exhibited potent cytotoxicity activity against the tested cell lines. Among the active compounds, UD**l** displayed the best results in this preliminary study and can be considered a promising lead compound in the design of more potent analogues.

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