Med Chem Res (2014) 23:1099–1113
DOI 10.1007/s00044-013-0702-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antiproliferative effects of novel urea derivatives against human prostate and lung cancer cells; and their inhibition of β-glucuronidase activity

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Received: 8 March 2013 / Accepted: 8 August 2013 / Published online: 27 August 2013

Abstract Twenty-one novel urea derivatives were synthesized and their structures characterized by mass, NMR, IR, and UV spectroscopy. These compounds were evaluated for their antiproliferative profile against human PC-3 (prostate) and NCI-H460 (lung) cancer cell lines. Among them, compound **21** *N*-(3-nitrophenyl)-*N*′-(1-phenylethyl)urea was found to be active against both PC-3 ($IC_{50} \pm$ SEM: 20.13 ± 0.91 μM) and NCI-H460 (GI_{50}: 22 ± 2.6 μM) cell lines; hence has the potential to be further studied as anticancer agent. These compounds were also investigated for their ability to inhibit urease, β-glucuronidase, and phosphodiesterase enzymes. *N*-(2,6-Dimethylphenyl)-*N*′-(4′-nitrophenyl)urea (**1**) demonstrated 90 % inhibition of β-glucuronidase enzyme ($IC_{50} \pm$ SEM: 3.38 ± 0.043 μM).

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Keywords Urea derivatives · Prostate cancer (PC-3) cell line · Lung cancer (NCI-H460) cell line · β-Glucuronidase · Urease · Phosphodiesterase

Introduction

Cancer is the third leading cause of death in the developing world after cardiac and infectious diseases (Efferth *et al.*, 2008). Lung cancer is the most common malignancy worldwide (Globocan, 2008), whereas cancer of prostate is the second most frequently diagnosed malignancy in men (Youlden *et al.*, 2008). Since most of the known anticancer drugs have serious side effects, or the tumor cells acquire resistance to these agents, therefore the search for novel anticancer agents is an ongoing process. In the last decade, urea derivatives have attained importance as the focus of research for the rational design of new generation of urea anticancer drugs (Li *et al.*, 2009; Lam *et al.*, 1994; Castro *et al.*, 1996). Aromatic urea derivatives, such as *N*-phenyl-*N*′-(2-chloroethyl) ureas and benzoylureas, demonstrated excellent anticancer activity by acting as tubulin ligands and inhibiting the polymerization of tubulin (Fortin *et al.*, 2011). Moreover, a series of 3-haloacylamino benzoyl urea (HBU) exhibited anticancer activity with IC_{50} values between 0.01 and 0.30 μM against nine human tumor cell lines, including CEM (leukemia), Daudi (lymphoma), MCF-7 (breast cancer), Bel-7402 (hepatoma), DU-145 (prostate cancer), PC-3 (prostate cancer), DND-1A (melanoma), LOVO (colon cancer), and MIA Paca (pancreatic cancer) (Song *et al.*, 2008). Li *et al.* (2009) reported that heterocyclic urea derivatives demonstrated good inhibitory activity against various molecules involved in tumorigenesis, such as receptor tyrosine kinases (RTKs), raf kinases,

protein tyrosine kinases (PTKs), and NADH oxidase, and hence could be valuable as novel anticancer agents.

Previously, enzyme inhibition studies have led to the discovery of a wide variety of effective drugs. Specific inhibitors interact with enzymes like ureases, phosphodiesterases, and glucuronidases, and block their activity toward their corresponding natural substrates. Urease decomposes urea to ammonia and carbamates, which spontaneously decomposes to ammonia and carbonic acid. Bacterial ureases are directly involved in the formation of stones in the kidney and contribute to the pathogenesis of pyelonephritis, encephalopathy, hepatic coma, and peptic ulceration. Therefore, the strategies based on urease inhibition are now considered as the first line of treatment for infections caused by urease producing bacteria. In agriculture too, high urease activities cause significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. To reduce the problems encountered using urea fertilizers, several approaches have been suggested and the most promising one is to apply urease inhibitors (Perveen, 2008, 2010, 2011; Perveen *et al.*, 2008).

A phosphodiesterase (PDE) is an enzyme that breaks a phosphodiester bond and hydrolyzes cyclic nucleotides. Thus, phosphodiesterases play a key role in regulating intracellular levels of the second messengers cAMP (cyclic adenosine monophosphate) and cGMP (cyclic guanosine monophosphate), and hence cell function. Inhibitors of PDE can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE. Non-selective PDE inhibitors including theophylline and papaverine have been used therapeutically for a range of diseases for over 70 years. Sildenafil, a phosphodiesterase inhibitor, used for treating erectile dysfunction has made worldwide impact. Benzoyl salireposide and other salireposides are pure non-competitive inhibitors of phosphodiesterase from snake venom, and are potential candidates for the therapy of arthritis (Choudhary *et al.*, 2004).

Glucuronidases are enzymes that catalyze the hydrolysis of glucuronic acid residues from complex carbohydrates. Human β-glucuronidase is located in the lysosomes of cells, however, its high levels are present in necrotic areas of large tumors (De Graaf *et al.*, 2002). Glucuronide prodrugs hold promise for the treatment of cancer patients as enhancement of their therapeutic effects may possibly be achieved by techniques that target β-glucuronidase specifically at the site of the tumor. For example, a glucuronidated prodrug of the nitrogen mustard *bis*-(2-chloroethyl)amine was 27-fold more cytotoxic to colon cancer cells than the parent drug (Ray and Chaturvedi, 2004; Shing-Ming *et al.*, 1992). During the present study, 21 urea derivatives were synthesized including *N*-(2,6-dimethylphenyl)-*N*′-(4′-nitrophenyl)urea which showed 90.5 % inhibition of *E. coli* β-glucuronidase ($IC_{50} \pm$ SEM: 3.38 ± 0.043 μM) (Table 1). In addition, structure–activity relationships (SAR), and various other biological activities of novel urea derivatives were carried out.

Results and discussion

Chemistry

For the synthesis of compounds **1–3**, **5–13**, **17**–**19**, and **21**, *o*-, *m*-, and *p*-nitro phenyl isocyanates were treated with aliphatic/aromatic primary and secondary amines in the presence of 1,4-dioxane at room temperature (Scheme 1). On the other hand for the preparation of symmetrical 1,3-disubstituted urea "derivatives **14–16**", *o*-, *m*-, and *p*-nitrophenyl isocyanates treated with triethylamine in 1,4-dioxane (Scheme 2), whereas for the synthesis of compounds **4** and **20** (*o*- and *m*-nitrophenyl isocyanates, respectively) were treated with *N*-phenylpiperazine and 2-pyrrolidone separately in 1,4-dioxane at room temperature with continuous stirring. Progress of reaction was monitored by TLC. The reaction was completed in 3 h (Scheme 3). After completion of the reaction, mixture was poured into ice-cold water with continuous stirring. The solids obtained were filtered, and after purification afforded clear substituted urea "derivatives **1–13**" and **17–21**, and symmetrical 1,3-disubstituted urea "derivatives **14–16**". The structures of synthetic compounds are characterized by mass, NMR, IR, and UV spectroscopy, and melting points. Finally, different biological and enzyme inhibition activities were determined.

Antiproliferative and enzymes inhibition studies

*Antiproliferative effects of compounds **1–21** against PC-3 human prostate and NCI-H460 human non-small cell lung cancer cell lines*

The use of in vitro human cancer cell lines is commonly employed for the primary screening of compounds for their potential cytotoxic/cytostatic activity. Several in vitro assays have been developed for rapid assessment of cytotoxic or growth inhibitory activity, alone and in combinations. The SRB assay is an efficient method for screening a large number of samples within 7 days, the results are linear over a 20-fold range of cell concentration and the sensitivity is comparable to those of MTT colorimetric method (Keepers *et al.*, 1991). The amount of pink SRB dye (sulforhodamine B) bound to the cells after staining gives a measure of cell growth/growth inhibition and/or cell killing (Boyd and Paull, 1995). Whereas, the MTT

Table 1 Results of synthesized symmetrical/unsymmetrical 1, 3-disubstituted ureas **1–21**

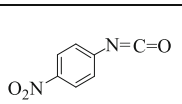
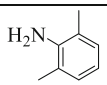
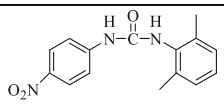
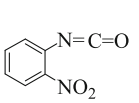
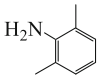
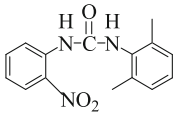
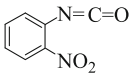
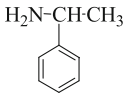
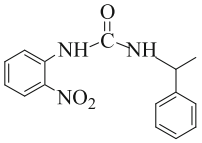
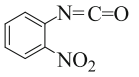
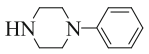
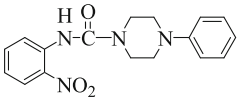
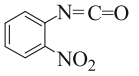
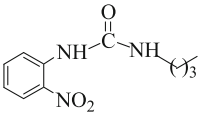
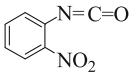
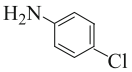
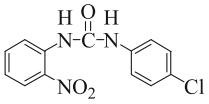
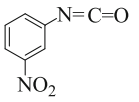
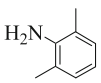
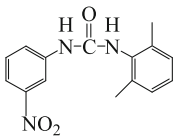
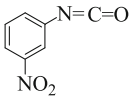
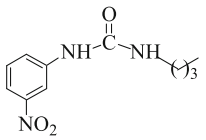
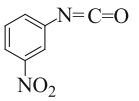
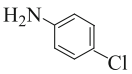
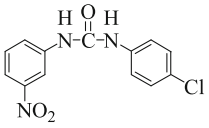
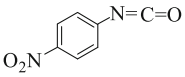
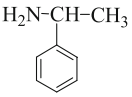
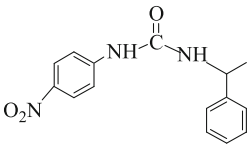
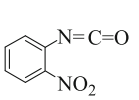
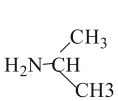
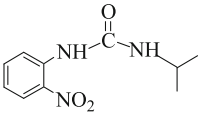
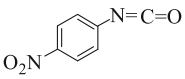
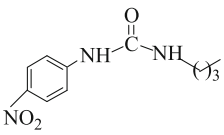
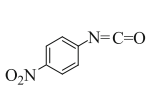
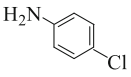
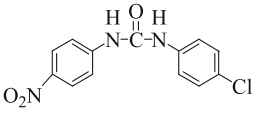
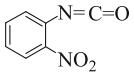
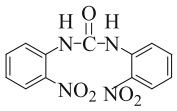
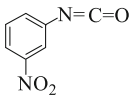
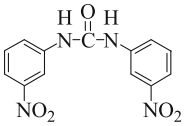
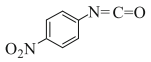
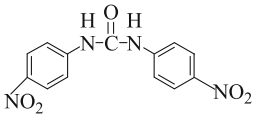
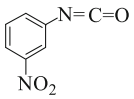
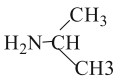
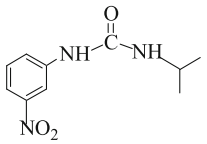
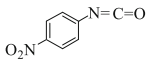
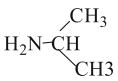
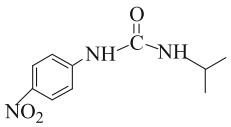
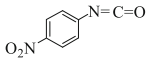
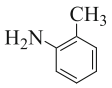
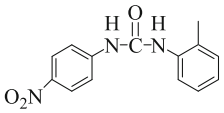
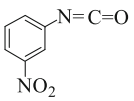
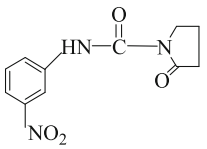
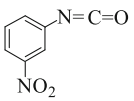
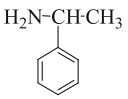
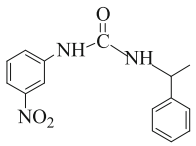
Compound no.	Substrate	Reagent	Product	%Yield	mp °C
1				93	186
2				98	209
3				68	209
4				71	269
5		$H_2N-(CH_2)_3CH_3$		100	127
6				77	241
7				92	243
8		$H_2N-(CH_2)_3CH_3$		91	138
9				79	241
10				64	186
11				89	198
12		$H_2N-(CH_2)_3CH_3$		100	137

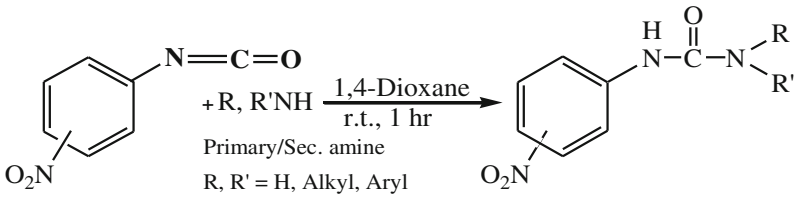
Table 1 continued

Compound no.	Substrate	Reagent	Product	%Yield	mp °C
13				72	250
14		$N-(C_2H_5)_3$		56	332
15		$N-(C_2H_5)_3$		100	258
16		$N-(C_2H_5)_3$		94	310
17				82	197
18				76	196
19				65	315
20				59	262
21				84	106

assay measures the activity of cellular enzymes that reduce the yellow tetrazolium dye to its insoluble purple colored formazan in living cells. This assay measures the cellular metabolic activity via NAD(P)H-dependent cellular oxidoreductase enzymes. It allows assessing the viability and the proliferation of cells since rapidly dividing cells exhibit high rates of MTT reduction. It can also be used to measure cytotoxicity or cytostatic activity of potential medicinal agents since those agents would result in cell toxicity, and therefore, metabolic dysfunction and decreased performance in the assay (Yung, 1989; Kim *et al.*, 2009).

All synthetic compounds **1–21** were screened for their cytotoxicity against human prostate and lung cancer cell lines using MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) and sulforhodamine B (SRB) assays according to literature protocols (Mosmann, 1983;

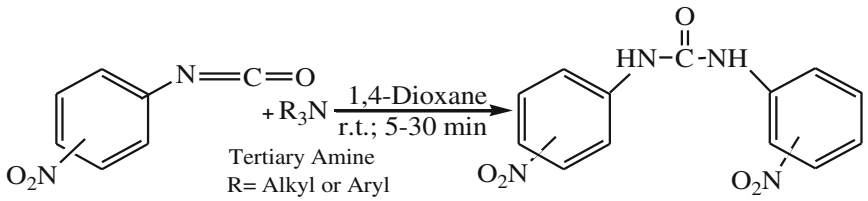
Scheme 1 Synthetic route for unsymmetrical ureas



o-,*m*-,*p*-Nitrophenyl isocynate

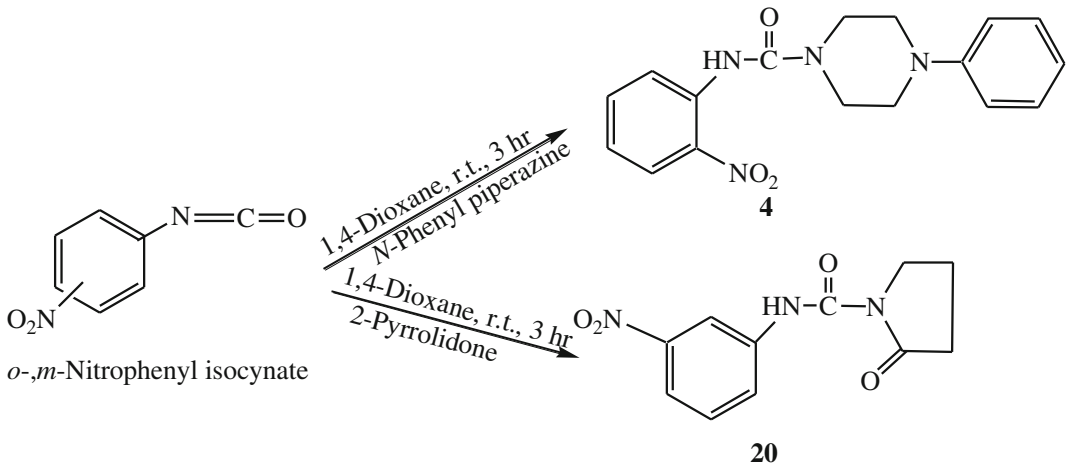
Unsymmetrical 1,3-disubstituted ureas **1-3, 5-13, 17-19** and **21**

Scheme 2 Synthetic route for symmetrical ureas



o-,*m*-,*p*-Nitrophenyl isocynate

o-,*m*-,*p*-Symmetrical 1, 3-disubstituted ureas **14-16**



o-,*m*-Nitrophenyl isocynate

Scheme 3 Synthetic routes for compounds **4** and **20**

Monks *et al.*, 1991). The standard drug doxorubicin was used as positive control against both the cell lines.

Five compounds **8**, **12**, **15**, "**20** and" **21** were found to be cytotoxic against PC-3 cell line (Table 2) having IC_{50} values of 66, 78.5, 20, "55 and" 20 μM, respectively, using the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay (Mosmann, 1983). The potency order regarding IC_{50} values was **21 = 15 > 20 > 8 > 12**.

All 21 synthesized urea derivatives were initially evaluated against NCI-H460 (human non-small cell lung cancer) cell lines at the dose of 100 μM (Table 3) using the sulforhodamine B (SRB) assay (Monks *et al.*, 1991). The compounds **3**, **8**, **12**, and **21** exhibited 14–58 % cell killing at this dose while compounds **6**, **9**, and **15** demonstrated substantial growth inhibition (62–95 %), and hence were further evaluated at five doses to determine their GI_{50}, TGI, and LC_{50} values (Table 4). This study indicates that the compound **21** (GI_{50}: 22, TGI: 36, and LC_{50}: 66 μM) possesses considerable cytotoxic activity (Table 4), whereas compounds **3** (GI_{50}: 12 μM), **12** (GI_{50}: 18 μM), **8** (GI_{50}: 34 μM), and **15** (GI_{50}: 44 μM) exhibit moderate growth inhibitory activity. The potency order regarding GI_{50} was **21–3–12 > 8–15 > 6–9**, and for TGI, it was **21 = 3 > 12 > 8**. The compound **21** was the only one for which LC_{50} could be calculated, while the compound **12** exhibited 48 % cell killing at 100 μM, hence its LC_{40} was found to be 91 μM.

Although, these compounds exhibited <20-fold activity against both cell lines in comparison to doxorubicin which is a highly effective anthracycline antibiotic used to treat a

Table 2 Antiproliferative effects of compounds **1–21** against PC-3 human prostate cancer cell line

Compound no.	Compounds	IC_{50} (μM)
1	*N*-(2,6-Dimethylphenyl)-*N*′-(4′-nitrophenyl)urea	>100
2	*N*-(2,6-Dimethylphenyl)-*N*′-(2-nitrophenyl)urea	>100
3	*N*-(2-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	>100
4	*N*-(2-Nitrophenyl)-*N*′-(*N*-phenylpiprazinyl)urea	>100
5	*N*-Butyl-*N*′-(2′-nitrophenyl)urea	>100
6	*N*-(4-Chlorophenyl)-*N*′-(2′-nitrophenyl)urea	>100
7	*N*-(2,6-Dimethylphenyl)-*N*′-(3′-nitrophenyl)urea	>100
8	*N*-Butyl-*N*′-(3′-nitrophenyl)urea	66.31 ± 0.53[a]
9	*N*-(4-Chlorophenyl)-*N*′-(3′-nitrophenyl)urea	>100
10	*N*-(4-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	>100
11	*N*-Isopropyl-*N*′-(2′-nitrophenyl)urea	>100
12	*N*-Butyl-*N*′-(4′-nitrophenyl)urea	78.49 ± 1.2[b]
13	*N*-(4-Chlorophenyl)-*N*′-(4′-nitrophenyl)urea	>100
14	*N*-(2-Nitrophenyl)-*N*′-(2′-nitrophenyl)urea	>100
15	*N*-(3-Nitrophenyl)-*N*′-(3′-nitrophenyl)urea	20.0 ± 0.91[c]
16	*N*-(4-Nitrophenyl)-*N*′-(4′-nitrophenyl)urea	>100
17	*N*-Isopropyl-*N*′-(3′-nitrophenyl)urea	>100
18	*N*-Isopropyl-*N*′-(4′-nitrophenyl)urea	>100
19	*N*-(2-Methylphenyl)-*N*′-(4′-nitrophenyl)urea	>100
20	*N*-(3-Nitrophenyl)-2-one-1-pyrrolidine carboxamide	55.02 ± 1.74[d]
21	*N*-(3-Nitrophenyl)-*N*′-(1-phenylethyl)urea	20.13 ± 0.91[c]
	Doxorubicin	0.912 ± 0.12[e]

Data are mean ± SEM of three independent experiments analyzed by ANOVA

For comparison, least significant difference and Duncan's multiple range tests were used

In columns statistically different values are represented by dissimilar alphabetical superscripts

IC_{50} concentration of the compound causing 50 % growth inhibition of cells

wide array of cancers for over 30 years, but its use is limited owing to its toxicity to most major organs, especially life-threatening cardiotoxicity, which forces the treatment to become dose-limiting (Gibson *et al.*, 2013). Moreover, doxorubicin also induces apoptosis and necrosis in other healthy tissues causing toxicity in the brain, liver, and kidney (Tacar *et al.*, 2013). On the other hand, these compounds might lead to the development of effective and less toxic novel types of anticancer agents/therapies.

Structure–activity relationship

It was remarkable that compounds **8**, **12**, **15**, and **21** exhibited significant antiproliferative against both the cell lines even when two different anticancer assays were employed. It is suggested that the presence of a nitro group at *meta* position in compounds **8** and **21**, a nitro group at *para* position in compound "**12**, and" a nitro group at *meta* position at both the phenyl rings in the compound **15**, which is a symmetrical 1,3-disubstituted urea, could be responsible for their greater activity. The presences of nitro group at *meta* and *para* positions causes less steric hindrance as compared to its *ortho* analogs and another important thing in all these compounds except **21** have an aliphatic alkyl chain and alkyl benzylic group directly attached to nitrogen of the urea bridge enhancing the activity. These results demonstrate that in this series of compounds, the position of nitro group at the phenyl ring and the substituent group at the nitrogen of urea bridge induces a pronounced effect on the antiproliferative activities of these compounds. Furthermore, since the compound **21** demonstrated maximum cytotoxicity against both cell lines (Tables 2, 4), therefore has the potential to be developed as an anticancer agent.

Enzyme inhibition assays

Herein, all the 21 synthetic compounds were screened against three clinically important enzymes i.e., *β*-glucuronidase, urease, and phosphodiesterase.

β-Glucuronidase inhibition activity

Against *β*-glucuronidase (Table 5), *N*-(2,6-dimethylphenyl)-*N*′-(4′-nitrophenyl)urea (**1**) showed a 90 % inhibition with an IC_{50} = 3.38 μM, 14-fold more active than the standard, D-saccharic acid 1,4-lactone (IC_{50} = 48.7 ± 1.25 μM). The nitro group in *para* position of compound **1**, and an aryl group (2,6-dimethy phenyl) is directly attached to the nitrogen of the urea bridge, and we assume that both the structural properties are responsible for the *β*-glucuronidase activity. Two isomers, *N*-(2,6-dimethylphenyl)-*N*′-(2′-nitrophenyl)urea (**2**), and *N*-(2,6-dimethylphenyl)-*N*′-

Table 3 Antiproliferative effects of compounds **1–21** at the dose of 100 μM against NCI-H460 human non-small cell lung cancer cell line

Compound no.	Compounds	% Growth inhibition/cytotoxicity
1	*N*-(2,6-Dimethylphenyl)-*N*′-(4′-nitrophenyl)urea	+28
2	*N*-(2,6-Dimethylphenyl)-*N*′-(2-nitrophenyl)urea	+47
3	*N*-(2-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	−23
4	*N*-(2-Nitrophenyl)-*N*′-(*N*-phenylpiprazinyl)urea	+22
5	*N*-Butyl-*N*′-(2′-nitrophenyl)urea	+10
6	*N*-(4-Chlorophenyl)-*N*′-(2′-nitrophenyl)urea	+95
7	*N*-(2,6-Dimethylphenyl)-*N*′-(3′-nitrophenyl)urea	+08
8	*N*-Butyl-*N*′-(3′-nitrophenyl)urea	−14
9	*N*-(4-Chlorophenyl)-*N*′-(3′-nitrophenyl)urea	+62
10	*N*-(4-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	+02
11	*N*-Isopropyl-*N*′-(2′-nitrophenyl)urea	+04
12	*N*-Butyl-*N*′-(4′-nitrophenyl)urea	−48
13	*N*-(4-Chlorophenyl)-*N*′-(4′-nitrophenyl)urea	+06
14	*N*-(2-Nitrophenyl)-*N*′-(2′-nitrophenyl)urea	+02
15	*N*-(3-Nitrophenyl)-*N*′-(3′-nitrophenyl)urea	+68
16	*N*-(4-Nitrophenyl)-*N*′-(4′-nitrophenyl)urea	+01
17	*N*-Isopropyl-*N*′-(3′-nitrophenyl)urea	+03
18	*N*-Isopropyl-*N*′-(4′-nitrophenyl)urea	+01
19	*N*-(2-Methylphenyl)-*N*′-(4′-nitrophenyl)urea	+05
20	*N*-(3-Nitrophenyl)-2-one-1-pyrrolidine carboxamide	+02
21	*N*-(3-Nitrophenyl)-*N*′-(1-phenylethyl)urea	−58

+ Growth inhibition, − *c*ytotoxicity

Table 4 Antiproliferative effects of compounds **3**, **6**, **8**, **9**, **12**, **15**, and **21** against NCI-H460 human non-small cell lung cancer cell line

Compound no.	Compounds	GI_{50}	TGI (μM)	LC_{50}
3	*N*-(2-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	12 ± 1.7^{a}	35 ± 1^{a}	>100
6	*N*-(4-Chlorophenyl)-*N*′-(2′-nitrophenyl)urea	59 ± 8.5^{b}	>100	>100
8	*N*-Butyl-*N*′-(3′-nitrophenyl)urea	34 ± 7^{c}	87 ± 2.4^{b}	>100
9	*N*-(4-Chlorophenyl)-*N*′-(3′-nitrophenyl)urea	68 ± 10.2^{b}	>100	>100
12	*N*-Butyl-*N*′-(4′-nitrophenyl)urea	18 ± 8.8^{a}	65 ± 4.0^{c}	$91 \pm 2.6^{a,*}$
15	*N*-(3-Nitrophenyl)-*N*′-(3′-nitrophenyl)urea	44 ± 6.7^{c}	>100	>100
21	*N*-(3-Nitrophenyl)-*N*′-(1-phenylethyl)urea	22 ± 2.6^{a}	36 ± 2.6^{a}	66 ± 3.0^{b}
	Doxorubicin	0.05 ± 0.01^{d}	0.25 ± 0.01^{d}	0.8 ± 0.1^{c}

Data are mean ± SEM of three independent experiments analyzed by ANOVA

For comparison, least significant difference and Duncan's multiple range tests were used in columns statistically different values are represented by dissimilar alphabetical superscripts

GI_{50} concentration of the compound causing 50 % growth inhibition of cells

TGI concentration of the compound causing total growth inhibition of cells

LC_{50} lethal concentration of the compound that killed 50 % of cells

LC_{40} lethal concentration of the compound that killed 40 % of cells

* 91 ± 2.6

(3′-nitrophenyl)urea (**7**), in which a nitro group is attached at *ortho* and *meta* positions, respectively, were inactive. The difference in inhibitory potential of these compounds clearly indicates that the presences of a *para* nitro group is a prerequisite for inhibition of the *β*-glucuronidase enzyme, along with a 2,6-dimethyl group on the other phenyl residue. Conclusively, compound **1** seems to be a promising compound. The rest of the compounds showed less than 50 % inhibition, and therefore were not evaluated for their IC_{50} values.

Table 5 β-Glucuronidase, urease, and phosphodiesterase activities of unsymmetrical/symmetrical 1,3-disubstituted urea derivatives **1–21**

Compound no.	Compounds	β-Glucuronidase (%)	Urease (%)	Phosphodiesterase (%)
1	*N*-(2,6-Dimethylphenyl)-*N*′-(4′-nitrophenyl) urea	90.5	16.3	NA
2	*N*-(2,6-Dimethylphenyl)-*N*′-(2′-nitrophenyl) urea	NA	45.9	NA
3	*N*-(2-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	NA	10.9	NA
4	*N*-(2-Nitrophenyl)-*N*′-(*N*-phenylpiprazinyl) urea	N.A	29.1	15.6
5	*N*-Butyl-*N*′-(2′-nitrophenyl)urea	24.25	38.2	18.0
6	*N*-(4-Chlorophenyl)-*N*′-(2′-nitrophenyl)urea	NA	7.0	23.8
7	*N*-(2,6-Dimethylphenyl)-*N*′-(3′-nitrophenyl) urea	NA	35.1	NA
8	*N*-Butyl-*N*′-(3′-nitrophenyl)urea	28.4	7.9	NA
9	*N*-(4-Chlorophenyl)-*N*′-(3′-nitrophenyl)urea	NA	NA	NA
10	*N*-(4-Nitrophenyl)-*N*′-(1′-phenylethyl)urea	NA	43.7	24.4
11	*N*-Isopropyl-*N*′-(2′-nitrophenyl)urea	NA	5.0	N.A
12	*N*-Butyl-*N*′-(4′-nitrophenyl)urea	NA	42.1	26.1
13	*N*-(4-Chlorophenyl)-*N*′-(4′-nitrophenyl)urea	NA	38.2	16.5
14	*N*-(2-Nitrophenyl)-*N*′-(2′-nitrophenyl)urea	NA	NA	NA
15	*N*-(3-Nitrophenyl)-*N*′-(3′-nitrophenyl)urea	NA	30.35	NA
16	*N*-(4-Nitrophenyl)-*N*′-(4′-nitrophenyl)urea	N.A	$IC_{50} = 1.25$ μM	48.9
17	*N*-Isopropyl-*N*′-(3′-nitrophenyl)urea	NA	NA	NA
18	*N*-Isopropyl-*N*′-(4′-nitrophenyl)urea	35.9	0.2	26.0
19	*N*-(2-Methylphenyl)-*N*′-(4′-nitrophenyl)urea	NA	NA	NA
20	*N*-(3-Nitrophenyl)-2-one-1-pyrrolidine carboxamide	NA	NA	NA
21	*N*-(3-Nitrophenyl)-*N*′-(1-phenylethyl)urea	NT	6.4	NT
	D-Sacchuric acid,1,4-lactone[a]	$IC_{50} \pm$ SEM:[d] 48.7 ± 1.25 μM		
	Thiourea[b]		$IC_{50} \pm$ SEM: 21.0 ± 0.01 μM	
	EDTA[c]			$IC_{50} \pm$ SEM: 274.0 ± 0.007 μM

NA not active, *NT* not tested

[a] Standard inhibitor for β-glucuronidase

[b] Standard inhibitor for urease

[c] Standard inhibitor for phosphodiesterase

[d] Standard error mean

Urease inhibition activity

In assays of urease, we found that compounds **2**, **5**, **7**, **10**, **12**, **13**, and **15** showed % inhibition of 46, 38, 35, 44, 42, 38, and 30, respectively, (Table 5). Compounds **3**, **10**, and **21** which are *o*, *p*, and *m* derivatives, respectively, having 1-phenylethyl group at the nitrogen of urea bridge, among them the most active compound is **10** with nitro group at *para* position (44 %). In compounds **1**, **2**, and **7**, the 2,6-dimethyl phenyl group is present at the nitrogen of urea bridge the most active compound is **2** (46 %) with nitro group at *ortho* position of phenyl ring. When an alkyl group (butyl) is present at the nitrogen of urea bridge in compounds **5**, **8**, and **12**, the most active compound is **12** (42 %) having a nitro group at *para* position. Compounds **6**, **9**, and **13** with 4-chlorophenyl group at nitrogen of urea bridge; among them, compound **13** with nitro group at *para* position is active (38 %). A number of urea derivatives were reported to test for urease and α-chymotrypsin inhibitory properties; it was found that the symmetrical *N*-

(4-nitrophenyl)-*N*′-(4′-nitrophenyl)urea **16** is a potent urease inhibitor with IC_{50} value of 1.25 μM (Perveen, 2008, 2010, 2011; Perveen *et al.*, 2008).

These results demonstrate that the position (*para*) and number of nitro group play vital role for urease inhibition.

Phosphodiesterase inhibition activity

In case of phosphodiesterase enzymes inhibition, the same compound *N*-(4-nitrophenyl)-*N*′-(4′-nitrophenyl)urea (**16**) which is symmetrical 1,3-disubstituted urea having nitro group at *para* position in the phenyl ring is most active showed 49 % inhibition while compounds **6**, **10**, **12**, and **18** showed percentage inhibitions 24, 24, 26, and 26 (Table 5) less than 30 %, and are therefore not evaluated for their IC_{50} values and considered to be inactive. Figure 1 demonstrates the activity of compounds **1–21** against *β*-glucuronidase, urease, and phosphodiesterase enzymes.

Cytotoxicity assays

MTT assay

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed microplates by using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, PC-3 prostate cancer cells were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 5 % of fetal bovine serum (FBS), 100 IU/mL of penicillin, and 100 μg/mL of streptomycin in 25 cm^3 flasks, and kept in 5 % CO_2 incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer, and diluted with a particular medium. Cell culture with the concentration of 1×10^5 cells/mL was prepared and introduced (100 μL/well) into 96-well plates. After overnight incubation, medium was removed and 200 μL of fresh medium was added with different concentrations of compounds (1–100 μM). After 72 h, 50 μL MTT (2 mg/mL) was added to each well and incubated further for 4 h subsequently, 100 μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate ELISA reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50 % growth inhibition for 3T3 cells (Mosmann 1983).

Sulforhodamine B (SRB) assay

The sulforhodamine B (SRB) protein staining assay was employed for the evaluation of in vitro growth inhibition and cytotoxicity (Monks *et al.*, 1991). About 1×10^4 NCI-H460 cells were added in each well (100 μL) of 96-well plates, and then incubated for 24 h leading to the formation of a monolayer. Subsequently, the test compounds were added in five tenfold dilutions and the plates were incubated for an additional 48 h. This was followed by fixation of cells with ice-cold trichloroacetic acid (50 μL). The plates were then stained using SRB dye which was solubilized in *Tris*-base solution, and then the optical density measurements were measured at 545 nm. Initially, all the compounds were tested at a single dose (100 μM). The compounds displaying more than 50 % growth inhibition were further tested at five doses (1–100 μM) to obtain their GI_{50}, TGI, and LC_{50} values. Drug concentrations causing a growth inhibition of 50 % (GI_{50}), total growth inhibition (TGI), and killing 50 % cells (LC_{50}) were calculated from dose response curves. Doxorubicin (anthracycline anticancer drug) was used as positive control ($GI_{50} = 0.05$, TGI = 0.25, $LC_{50} = 0.8$ μM).

Statistical analysis

The data are expressed as the mean ± SEM for three experiments. Statistical comparisons between the various treatments were performed using SPSS 17 statistical software, and the data were evaluated by Analysis of Variance (ANOVA). Comparisons of data were conducted by least significant difference (LSD) and Duncan's multiple range tests. *P* values of <0.05 were considered statistically significant.

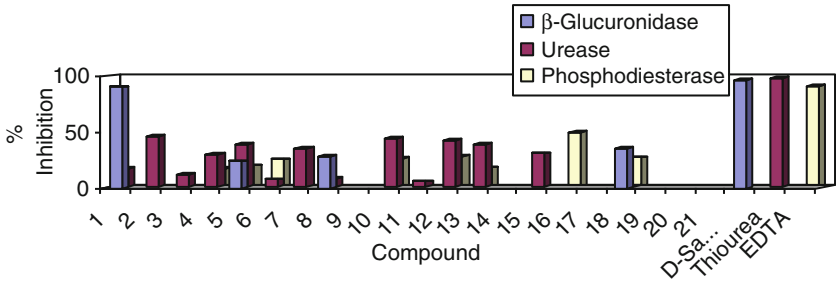


Fig. 1 Effect of synthetic compounds **1–21** against *β*-glucuronidase, urease, and phosphodiesterase enzymes

Enzyme inhibition assays

Urease inhibition assay

Reaction mixtures comprising one unit of urease solution in water (*Bacillus pasteurii* or Jack bean), and 55 μL of buffers (0.01 M $K_2HPO_4{\cdot}3H_2O$, 1 mM EDTA, 0.01 M LiCl; pH 8.2) containing 100 mM urea were incubated with 5 μL of test compounds (1 mM) at 30 °C for 15 min in 96-well plates.

Urease inhibitory activity was determined by measuring ammonia production using the indophenol method. Briefly, 45 μL of phenol reagent (1 % w/v phenol and 0.005 % w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5 % w/v NaOH and 0.1 % w/v NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using Soft-Max Pro software (Molecular Devices, CA, USA) (Khan *et al.*, 2004).

β-Glucuronidase inhibition assay

β-Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol formed from the substrate (50 μL of 0.4 mM *p*-nitrophenyl-*β*-D-glucuronide) by the spectrophotometric method. 185 μL of 0.1 M acetate buffer (13.608 g/L of sodium acetate, pH adjusted to 5.0 with 0.1 M acetic acid), 5 μL (0.5 mM) of test compound solution, and 10 μL of enzyme solution (30 U) were incubated at 37 °C for 30 min. The plates were read on a multiplate reader (Spectra Max plus 384) at 405 nm after the addition of 50 μL of 0.4 mM *p*-nitrophenyl-*β*-D-glucuronide counting for a total reaction volume of 250 μL. All the assays were performed in triplicate (Riaz *et al.*, 2003).

Phosphodiesterase inhibition assay

Activity against snake venom phosphodiesterase (PDE) was assayed by a modified method of (Mamillapalli *et al.*, 1998) by mixing 97 μL (33 mM) *Tris*–HCl buffer pH 8.8, 20 μL (30 mM) Mg-acetate with 0.000742 U/well of final enzyme concentration. A microtiter plate assay was applied and 60 μL (0.33 mM) of *bis*-(*p*-nitrophenyl) phosphate (Sigma N-3002) was used as substrate and 8 μL (0.5 mM) as test compound. 8 μL (0.5 mM) of EDTA (Merck, Darmstadt) were used as positive controls ($1C_{50}$ = 748 μM ± 0.015, 274 μM ± 0.007, respectively).

After 30 min pre-incubation of the enzyme with the test samples, the enzyme activity was monitored spectrophotometrically at 37 °C on a microtitre plate reader (Spectra Max, Molecular Devices, CA, USA) by following the rate (change in OD/min) of release of *p*-nitrophenol from *p*-nitrophenyl phosphate at 410 nm. All assays were conducted in triplicate (Mamillapalli *et al.*, 1998).

Experimental

Melting points were taken on a Gallenkamp melting point apparatus. Thin layer chromatography was performed on pre-coated silica gel plates (Kieselgel 60 F_{254}, E. Merk, Germany) and spots were visualized under UV (Dual range, UK-Germany) at 254 and 365 nm and/or by spraying cerium sulfate (heat). EI MS were performed on MAT-312 and on JEOL JMS-HX 110 instruments. ^{1}H NMR spectra were recorded on a Bruker AVANCE instruments at 400 or 500 MHz, δ is given in ppm related to $SiMe_4$ (0 ppm) as internal standard. All the solvents used were of reagent grade. Bioscreenings for urease, *β*-glucuronidase, phosphodiesterase inhibition, and cytotoxicity were carried in various laboratories of the H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan. However, the compounds were synthesized in Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi.

Chemistry

*General procedure for the synthesis of symmetrical (**14–16**) and unsymmetrical 1,3-disubstituted ureas (**1–13** and **17–21**)*

Unsymmetrical 1,3-disubstituted ureas **1–3**, **5–13**, **17–19**, and **21** were synthesized (Table 1) by weighing 1.4 mol of primary/secondary amine in a round-bottom flask containing 20–25 mL of 1,4-dioxane. 0.2 Mol of *ortho*-, *meta*-, and *para*-nitrophenyl isocyanates, respectively, were added dropwise at room temperature separately with continuous stirring. The reaction was completed in 1 h (Scheme 1). For the preparation of the symmetrical 1,3-disubstituted ureas **14–16**, 0.3 mol of *ortho*-, *meta*-, and *para*-nitrophenyl isocyanates, respectively, in 20–25 mL of 1,4-dioxane, 2 mol of triethyl amine were added at room temperature (Scheme 2) (Hai *et al.*, 2003), and the progress of reaction was monitored via TLC. For the preparation of compounds **4** and **20**, 1.4 mol of *N*-phenylpiperazine and 2-pyrrolidone, respectively, were taken in a round-bottom flask containing 20-25 mL of 1,4-dioxane. Then, 0.2 mol of *ortho*- and *meta*-nitrophenyl isocyanates were added

dropwise at room temperature with continuous stirring. After completion of the reaction in 3 h (Scheme 3), the mixture was poured into ice-cold water with continuous stirring, the solid was filtered and crystallized from an appropriate solvent to obtain the desired product.

N-(2,6-Dimethylphenyl)-N′-(4′-nitrophenyl)urea (**1**) R_f = 0.65 (CH_2Cl_2/C_6H_{14}, 9:1). IR (KBr):ν_{max}: 3,326, 1,663, 1,540, 1,499, 1,332, 1,230, 1,103, 837, 751 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ; 8.13 (2H, d, $J_{3',2'=5',6'}$ = 9.0 Hz, $H^{3',5'}$), 7.49 (2H, d, $J_{2',3'=6',5'}$ = 9.0 Hz, $H^{2',6'}$), 7.22 (2H, d, $J_{3,4=5,4}$ = 9.0 Hz, $H^{3,5}$), 7.19 (1H, t, $J_{4,(3,5)}$ = 9.0 Hz, H^4), 6.41 (1H, br.s, NH), 5.94 (1H, br.s, NH), 2.32 (6H, s, $2CH_3$)—EI MS: *m/z* (rel. abund. %), 285 (M^+, 89), 270 (3), 255 (4), 164 (5), 147 (100), 138 (88), 132 (19), 121 (72), 108 (56), 92 (25)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 159.46 (C=O), 144.34 (C-1′), 140.80 (C-4′), 138.96 (C-1), 131.16 (C-2,6), 128.45 (C-3,5), 125.10 (C-3′,5′), 123.19 (C-4), 116.92 (C-2′,6′), 18.18 ($2CH_3$)—Anal. Calcd for $C_{15}H_{15}N_3O_3$: (285): C, 63.15; H, 5.30; N, 14.73 %. Found: C, 63.18; H, 5.28; N, 14.75 %.

N-(2,6-Dimethylphenyl)-N′-(2′-nitrophenyl)urea (**2**) R_f = 0.60 (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,322, 3,256, 1,646, 1,585, 1,548, 1,499, 1,336, 1,283, 1,225, 1,152, 1,090, 858, 764 cm^1. ^1H NMR (500 MHz, $CDCl_3$) δ; 9.63 (1H, br.s, NH), 8.75 (1H, d, $J_{3',4'}$ = 8.5 Hz, $H^{3'}$), 8.10 (1H, d, $J_{6',5'}$ = 8.2 Hz, $H^{6'}$), 7.60 (1H, dt, $J_{5',(4',6')}$ = 7.3, $J_{5',3'}$ = 0.8 Hz, $H^{5'}$), 7.26 (1H, m, H^4), 7.19 (2H, br.d, $J_{3,4=5,4}$ = 7.3 Hz, $H^{3,5}$), 7.03 (1H, dt, $J_{4',(3',5')}$ = 7.3, $J_{4',6'}$ = 0.9 Hz, $H^{4'}$), 6.03 (1H, br.s, NH), 2.33 (3H, s, CH_3), 1.50 (3H, s, CH_3)—EI MS: *m/z* (rel. abund. %), 285 (M^+, 45), 255 (2), 239 (5), 222 (2), 164 (5), 147 (89), 138 (100), 121 (70), 106 (26), 92 (38)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 156.16 (C=O), 136.33 (C-1), 134.68 (C-2′), 133.74 (C-5′), 133.25 (C-1′), 131.16 (C-2, 6), 128.45 (C-3,5), 123.19 (C-4), 122.23 (C-3′), 120.07 (C-4′), 119.75 (C-6′), 18.18 ($2CH_3$)—Anal. Calcd for $C_{15}H_{15}N_3O_3$: (285): C, 63.15; H, 5.30; N, 14.73 %. Found: C, 63.12; H, 5.32; N, 14.71 %.

N-(2-Nitrophenyl)-N′-(1′-phenylethyl)urea (**3**) R_f = 0.81 (CH_2Cl_2/C_6H_{14}, 9:1). IR (KBr): ν_{max}: 3,374, 3,305, 1,658, 1,552, 1,503, 1,315, 1,225, 1,111, 849, 747 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ; 9.72 (2H, br.s, NH), 8.62 (1H, d, $J_{3,4}$ = 8.4 Hz, H^3), 8.14 (1H, d, $J_{6,5}$ = 7.3 Hz, H^6), 7.53 (1H, t, $J_{5,(4,6)}$ = 7.3 Hz, H^5), 7.36 (4H, t, $J_{2',(3',1'')=3',(2',4')=4',(3',5'),5',(4',6')}$ = 6.1 Hz, $H^{2',3',4',5'}$), 7.29 (1H, dd, $J_{6',5'}$ = 6.1, $J_{6',4'}$ = 2.4 Hz, $H^{6'}$), 7.01 (1H, t, $J_{4(3,5)}$ = 7.3 Hz, H^4), 5.00 (1H, sex, $J_{1''(2'',2',6')}$ = 6.7 Hz, CH), 1.52 (3H, q, $J_{2'',(1'',2',6')}$ = 6.7 Hz, CH_3)—EI MS: *m/z* (rel. abund. %), 285 (M^+, 6), 268 (2), 164 (57), 147 (31), 138 (89), 132 (86), 120 (10), 106 (100), 92 (33), 90 (38)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 155.50 (C=O), 139.27 (C-1′), 134.29 (C-2), 133.33 (C-5), 131.95 (C-1), 127.62 (C-4′), 126.64 (C-3′, 5′), 126.63 (C-2′, 6′), 121.83 (C-3), 119.82 (C-4), 119.35 (C-6), 52.38 (CH), 20.95 (CH_3)—Anal. Calcd for $C_{15}H_{15}N_3O_3$: (285): C, 63.15; H, 5.30; N, 14.73 %. Found: C, 63.19; H, 5.28; N, 14.75 %.

N-(2-Nitrophenyl)-N′-(1-phenylpiprazinyl)urea (**4**) R_f = 0.76 (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,366, 2,835, 1,666, 1,599, 1,589, 1,499, 1,450, 1,344, 1,270, 1,229, 1,139 cm^{-1}. ^1H NMR (400 MHz, DMSO) δ; 9.3 (1H, br.s, NH), 7.92 (2H, d, $J_{3,4=6,5}$ = 8.0 Hz, $H^{3,6}$), 7.66 (2H, dd, $J_{3',2'=5'6'}$ = 8.1, $J_{3',4'=5'4'}$ = 7.0 Hz, $H^{3',5'}$), 7.23 (2H, t, $J_{4,(3,5)=5,(4,6)}$ = 8.0 Hz, $H^{4,5}$), 6.97 (2H, d, $J_{2',3'=6',5'}$ = 8.1 Hz, $H^{2',6'}$), 6.81 (1H, t, $J_{4',(3',5')}$ = 7.0 Hz, $H^{4'}$), 3.60 (4H, t, $J_{1'',2''}$ = 4.9 Hz, $2CH_2$), 3.17 (4H, t, $J_{2'',1''}$ = 4.9 Hz, $2CH_2$)—EI MS: *m/z* (rel. abund. %), 326 (M^+, 1), 162 (27), 147 (7), 132 (35), 119 (28), 105 (76), 104 (86), 91 (100)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 153.32 (C=O), 151.33 (C-1′ phenyl), 148.04 (C-3), 137.74 (C-1), 129.84 (C-5), 129.10 (C-3′, 5′ phenyl), 123.82 (C-6), 120.30 (C-4′ phenyl), 115.93 (C-2′, 6′ phenyl), 112.43 (C-2), 50.66 ($2CH_2$-3″, 5″ piprazine), 45.22 ($2CH_2$-2″, 6″ piprazine)—Anal. Calcd for $C_{17}H_{18}N_4O_3$: (326): C, 62.57; H, 5.56; N, 17.17 %. Found: C, 62.54; H, 5.58; N, 17.19 %.

N-Butyl-N′-(2′-nitrophenyl)urea (**5**) R_f = 0.71 (CH_2Cl_2/CH_3OH, 9:1). IR (KBr): ν_{max}: 3,342, 2,970, 1,665, 1,507, 1,465, 1,340, 1,262, 1,148, 743 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ; 9.76 (1H, br.s, NH), 8.64 (1H, d, $J_{3',4'}$ = 8.5 Hz, $H^{3'}$), 8.16 (1H, dd, $J_{6',5'}$ = 8.5, $J_{6',4'}$ = 1.1 Hz, $H^{6'}$), 7.57 (1H, dt, $J_{4',(3',5')}$ = 8.5, $J_{4',6'}$ = 1.1 Hz, $H^{4'}$), 7.02 (1H, dt, $J_{5',(6',4')}$ = 8.5, $J_{5',3'}$ = 0.6 Hz, $H^{5'}$), 4.76 (1H, s, NH), 3.29 (2H, q, $J_{1,(2,NH)}$ = 7.3 Hz, CH_2), 1.56 (2H, qin, $J_{2,(1,3)}$ = 7.3 Hz, CH_2), 1.39 (2H, sex, $J_{3,(2,4)}$ = 7.3 Hz, CH_2), 0.95 (3H, t, $J_{4,3}$ = 7.3 Hz, CH_3)—EI MS: *m/z* (rel. abund. %), 237 (M^+, 3), 148 (4), 139 (7), 138 (100), 122 (3), 108 (19), 92 (29)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 151.49 (C=O), 134.42 (C-2′), 133.33 (C-5′), 131.64 (C-1′), 121.83 (C-3′), 119.82 (C-4′), 119.49 (C-6′), 42.47 (CH_2-1), 32.00 (CH_2-2), 20.00 (CH_2-3), 13.70 (CH_3)—Anal. Calcd for $C_{11}H_{15}N_3O_3$: (237): C, 55.69; H, 6.37; N, 17.71 %. Found: C, 55.66; H, 6.40; N, 17.73 %.

N-(4-Chlorophenyl)-N′-(2′-nitrophenyl)urea (**6**) R_f = 0.68 (CH_2Cl_2/CH_3OH, 9:1). IR (KBr): ν_{max}: 3,334, 16,667, 1,618, 1,585, 1,540, 1,491, 1,334, 1,344, 1,274, 1,205, 1,095, 1,017 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ; 9.97 (1H, br.s, NH), 8.62 (1H, d, $J_{3',4,}$ = 8.5 Hz, $H^{3'}$), 8.18 (1H, d, $J_{6',5'}$ = 7.5 Hz, $H^{6'}$), 7.61 (1H, t, $J_{4',(3',5')}$ = 7.5 Hz, $H^{4'}$), 7.37 (2H, d, $J_{3,4=5,6}$ = 8.7 Hz, $H^{3,5}$), 7.32 (2H, d, $J_{2,3=6,5}$ = 8.7 Hz, $H^{2,6}$), 7.09 (1H, t, $J_{5',(4',6')}$ = 7.5 Hz,

$H^{5'}$), 6.76 (1H, br.s, NH)—EI MS: *m/z* (rel. abund. %), 293 (M^{2+}, 4), 291 (M^{+}, 11), 155 (6), 153 (21), 148 (4), 138 (100), 127 (90), 125 (15), 111 (9), 108 (14), 92 (39), 90 (52)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 153.26 (C=O), 135.41 (C-1), 134.68 (C-2′), 133.74 (C-5′), 133.24 (C-1′), 129.33 (C-3, 5), 128.52 (C-4), 122.23 (C-3′), 121.49 (C-2′, 6′), 120.07 (C-4′), 119.75 (C-6′)—Anal. Calcd for $C_{13}H_{10}N_3O_3Cl$: (293) C, 53.63; H, 3.46; N, 14.43 %. Found: C, 53.60; H, 3.44; N, 14.40 %.

N-(2,6-Dimethylphenyl)-N′-(3′-nitrophenyl)urea (**7**) R_f = 0.74 (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,326, 1,638, 1,524, 1,340, 1,217, 1,074, 764, 730 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.06 (1H, br.s, $H^{2'}$), 7.84 (2H, dt, $J_{5',(4',6')=6',5'} = 7.9$, $J_{6',(2',4')} = 1.6$ Hz, $H^{5',6'}$), 7.41 (1H, t, $J_{4,(3,5)} = 8.2$ Hz, H^{4}), 7.20 (1H, d, $J_{4',5'} = 7.9$ Hz, $H^{4'}$), 7.19 (2H, m, $H^{3,5}$), 6.27 (1H, br.s, NH), 5.87 (1H, br.s, NH), 2.34 (6H, s, $2CH_3$)—EI MS: *m/z* (rel. abund. %), 285 (M^{+}, 92), 270 (2), 255 (2), 238 (3), 164 (4), 147 (86), 138 (97), 121 (100), 106 (49), 92 (41)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 159.46 (C=O), 148.10 (C-3′), 138.96 (C-1), 138.75 (C-1′), 131.16 (C-2,6), 129.90 (C-5′), 128.45 (C-3, 5), 123.19 (C-4), 123.14 (C-6′), 115.95 (C-4′), 111.75 (C-2′), 18.18 (2 CH_3)—Anal. Calcd for $C_{15}H_{15}N_3O_3$: (285): C, 63.15; H, 5.30; N, 14.73 %. Found: C, 63.17; H, 5.35; N, 14.70 %.

N-Butyl-N′-(3′-nitrophenyl)urea (**8**) R_f = 0.72 (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,342, 2,942, 1,712, 1,638, 1,524, 1,422, 809, 735 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.14 (1H, t, $J_{2',(4',6')} = 2.0$ Hz, $H^{2'}$), 7.85 (1H, dd, $J_{6',5'} = 8.1$, $J_{6',4'} = 1.4$ Hz, $H^{6'}$), 7.78 (1H, dd, $J_{4',5'} = 8.1$, $J_{4',6'} = 1.4$ Hz, $H^{4'}$), 7.41 (1H, t, $J_{5',(4',6')} = 8.1$ Hz, $H^{5'}$), 6.50 (1H, br.s, NH), 4.66 (1H, br.s, NH), 3.27 (2H, q, $J_{1,(2,NH)} = 6.9$ Hz, CH_2), 1.53 (2H, qin, $J_{2,(1,3)} = 6.9$ Hz, CH_2), 1.37 (2H, sex, $J_{3,(2,4)} = 7.2$ Hz, CH_2), 0.93 (3H, t, $J_{4,3} = 7.2$ Hz, CH_3)—EI MS: *m/z* (rel. abund. %), 237 (M^{+}, 3), 270 (3), 165 (1), 138 (100), 108 (7), 92 (59), 65 (24)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 154.79 (C=O), 147.69 (C-3′), 137.14 (C-1′), 129.50 (C-5′), 122.88 (C-6′), 115.70 (C-4′), 111.49 (C-2′), 42.47 (CH_2-1), 32.00 (CH_2-2), 20.00 (CH_2-3), 13.70 (CH_3)—Anal. Calcd for $C_{11}H_{15}N_3O_3$: (237): C, 55.69; H, 6.37; N, 17.71 %. Found: C, 55.71; H, 6.34; N, 17.68 %.

N-(4-Chlorophenyl)-N′-(3′-nitrophenyl)urea (**9**) R_f = 0.88 (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,363, 1,687, 1,593, 1,540, 1,487, 1,344, 1,230, 1,095, 878 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ; 8.21 (1H, t, $J_{2',(4',6')} = 1.9$ Hz, $H^{2'}$), 7.91 (2H, dd, $J_{2,3=6,5} = 8.4$, $J_{2,6=6,2} = 1.4$ Hz, $H^{2,6}$), 7.82 (2H, dd, $J_{3,2=5,6} = 8.4$, $J_{3,5=5,3} = 1.3$ Hz, $H^{3,5}$), 7.4 (1H, d, $J_{4',5'} = 8.1$ Hz, $H^{4'}$), 7.44 (1H, dd, $J_{6',5'} = 8.5$, $J_{6',4'} = 1.7$ Hz, $H^{6'}$), 7.32 (1H, m, $H^{5'}$), 6.66 (1H, br.s, NH), 6.45 (1H, br.s, NH)—EI MS: *m/z* (rel. abund. %), 293 (M^{+2}, 4), 291 (M^{+}, 16), 165 (5), 155 (3), 153 (12), 138 (31), 127 (100), 111 (13), 99 (21), 92 (40)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 156.55 (C=O), 148.10 (C-3′), 138.74 (C-1′), 138.03 (C-1), 129.90 (C-5′), 129.33 (C-3, 5), 128.52 (C-4), 123.14 (C-6′), 121.49 (C-2, 6), 115.95 (C-4′), 111.75 (C-2′)—Anal. Calcd for $C_{13}H_{10}N_3O_3Cl$: (293): C, 53.63; H, 3.46; N, 14.43 %. Found: C, 53.60; H, 3.48; N, 14.41 %.

N-(4-Nitrophenyl)-N′-(1′-phenylethyl)urea (**10**) R_f = 0.74 (CH_2Cl_2/CH_3OH, 9:1). IR (KBr): ν_{max}: 3,350, 3,277, 2,978, 1,659, 1,565, 1,503, 1,450, 1,344, 1,283, 1,156 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.11(2H, d, $J_{3,2=5,6} = 9.1$ Hz, $H^{3,5}$), 7.41 (2H, d, $J_{2,3=6,5} = 9.1$ Hz, $H^{2,6}$), 7.35 (2H, d, $J_{2',3'=6',5'} = 5.9$ Hz, $H^{2',6'}$), 7.34 (1H, m, $H^{4'}$), 7.29 (2H, dd, $J_{3',2'=5',6'} = 5.9$, $J_{3',5'=5',3'} = 2.4$ Hz, $H^{3',5'}$), 6.57 (1H, br.s, NH), 4.93 (1H, quin, $J_{1'',(2'',NH)} = 6.6$ Hz, CH), 3.68 (1H, s, NH), 1.52 (3H, t, $J_{2'',(1'',2')} = 3.2$ Hz, CH_3)—EI MS: *m/z* (rel. abund. %), 285 (M^{+}, 2), 270 (4), 164 (5), 148 (53), 138 (98), 132 (17), 120 (11), 105 (100), 92 (17)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 158.79 (C=O), 143.04 (C-1), 140.55 (C-4), 139.27 (C-1′), 127.62 (C-4′), 126.64 (C-3′, 5′), 126.36 (C-2′, 6′), 124.70 (C-3, 5), 116.52 (C-2, 6), 52.38 (CH), 20.95 (CH_3)—Anal. Calcd for $C_{15}H_{15}N_3O_3$: (285): C, 63.15; H, 5.30; N, 14.73 %. Found: C, 63.21; H, 5.33; N, 14.69 %.

N-Isopropyl-N′-(2′-nitrophenyl)urea (**11**) R_f = 0.65 (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,396, 2,960, 1,641, 1,557, 1,503, 1,348, 1,285, 734 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 9.71 (1H, br.s, NH), 8.63 (1H, d, $J_{3',4'} = 8.4$ Hz, $H^{3'}$), 8.16 (1H, dd, $J_{6',5'} = 8.4$, $J_{6',4'} = 0.9$ Hz, $H^{6'}$), 7.56 (1H, t, $J_{5',(4',6')} = 8.4$ Hz, $H^{5'}$), 7.01 (1H, t, $J_{4',(3',5')} = 8.4$ Hz, $H^{4'}$), 4.70 (1H, d, $J_{NH,1} = 5.4$ Hz, NH), 3.98 (1H, sept, $J_{1,(2a,2b)} = 6.5$ Hz, CH), 1.22 (6H, d, $J_{2a,1=2b,1} = 6.5$ Hz, $2CH_3$)—EI MS: *m/z* (rel. abund. %), 223 (M^{+}, 23), 167 (4), 164 (6), 149 (19), 138 (100), 92 (68), 85 (15), 83 (16), 69 (22), 57 (37)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 155.86 (C=O), 134.53 (C-2′), 133.33 (C-5′), 131.48 (C-1′), 121.83 (C-3′), 119.82 (C-4′), 119.59 (C-6′), 42.26 (CH-1), 23.73 ($2CH_3$)—Anal. Calcd for $C_{10}H_{13}N_3O_3$: (223): C, 53.78; H, 5.87; N, 18.83 %. Found: C, 53.71; H, 5.82; N, 18.88 %.

N-Butyl-N′-(4′-nitrophenyl)urea (**12**) R_f = 0.67 (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,379, 3,305, 2,845, 1,650, 1,556, 1,503, 1,319, 1,225, 1,111, 849, 747, 678 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 9.35 (1H, br.s, NH), 8.15 (2H, d, $J_{3',2'=5',6'} = 8.7$ Hz, $H^{3',5'}$), 7.50 (2H, d, $J_{2',3'=6',5'} = 8.7$ Hz, $H^{2',6'}$), 6.68 (1H, br.s, NH), 3.27 (2H, t, $J_{1,(2,NH)} = 7.2$ Hz, CH_2), 1.52 (2H, qin, $J_{2,(1,3)} = 7.2$ Hz, CH_2), 1.36 (2H, sex, $J_{3,(2,4)} = 7.2$ Hz, CH_2), 0.92 (3H, t,

$J_{4,3}$ = 7.2 Hz, CH_3)—EI MS: *m/z* (rel. abund. %), 237 (M^+, 17), 219 (2), 219 (2), 207 (2), 164 (6), 138 (100), 122 (7), 108 (30), 92 (17), 78 (21)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 154.79 (C=O), 142.73 (C-1′), 140.55 (C-4′), 124.70 (C-3′, 5′), 116.66 (C-2′, 6′), 42.47 (CH_2-1), 32.00 (CH_2-2), 20.00 (CH_2-3), 13.70 (CH_3)—Anal. Calcd for $C_{11}H_{15}N_3O_3$: (237): C, 55.69; H, 6.37; N, 17.71 %. Found: C, 55.71; H, 6.39; N, 17.68 %.

N-(4-Chlorophenyl)-N′-(4′-nitrophenyl)urea ***(13)*** R_f = 0.70 (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,366, 1,723, 1,595, 1,539, 1,492, 1,345, 1270, 1173, 1113, 835 cm^{-1}. ^{1}H NMR (400 MHz, DMSO) δ; 9.45 (1H, br.s, NH), 9.04 (1H, br.s, NH), 8.19 (2H, d, $J_{3,2=5,6}$ = 9.3 Hz, $H^{3,5}$), 7.67 (2H, dd, $J_{2,3=6,5}$ = 9.3, $J_{2,6=6,2}$ = 1.4 Hz, $H^{2,6}$), 7.49 (2H, d, $J_{2',3'=6',5'}$ = 8.8 Hz, $H^{2',6'}$), 7.35 (2H, d, $J_{3',2'=5',6'}$ = 8.8 Hz, $H^{3',5'}$)—EI MS: *m/z* (rel. abund. %), 293 (M^{+2}, 4), 291 (M^+, 11), 164 (21), 155 (48), 153 (100), 138 (81), 129 (18), 127 (72), 125 (69), 108 (54), 92 (28), 90 (52), 65 (33)—^{13}C NMR (125 MHz, $CDCl_3$) δ;156.55 (C=O), 144.33 (C-1′), 140.80 (C-4′), 138.03 (C-1), 129.33 (C-3, 5), 128.52 (C-4), 125.10 (C-3′,5′), 121.49 (C-1, 6), 116.92 C-1′, 6′)—Anal. Calcd for $C_{13}H_{10}N_3O_3Cl$: (293): C, 53.63; H, 3.46; N, 14.43 %. Found: C, 53.68; H, 3.39; N, 14.46 %.

N-(2-Nitrophenyl)-N′-(2′-nitrophenyl)urea ***(14)*** R_f = 0.70 (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,366, 1,659, 1,589, 1,556, 1,499, 1,344, 1,344, 1,288, 1,209, 1,160, 849, 739, 727, 682 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 10.13 (1H, br.s, NH), 8.55 (1H, d, $J_{3,4}$ = 7.5 Hz, H^3), 8.23 (1H, d, $J_{3',4'}$ = 7.6 Hz, $H^{3'}$), 8.10 (1H, d, $J_{6,5}$ = 8.5 Hz, H^6), 7.65 (1H, t, $J_{4,(3,5)}$ = 7.5 Hz, H^4), 7.34 (1H, t, $J_{5,(4,6)}$ = 7.5 Hz, H^5), 7.16 (1H, t, $J_{4',(3',5')}$ = 7.6 Hz, $H^{4'}$), 6.78 (1H, d, $J_{6',5'}$ = 8.3 Hz, $H^{6'}$), 6.69 (1H, t, $J_{5',(4',6')}$ = 7.6 Hz, $H^{5'}$), 6.01 (1H, br.s, NH)—EI MS: *m/z* (rel. abund. %), 302 (M^+, 2), 256 (1), 164 (5), 138 (80), 121 (7), 92 (63), 90 (59), 80 (34), 78 (12), 77 (10), 76 (6), 66 (19), 65 (100), 52 (13)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 149.96 (C=O), 134.68 (C-2, 2′), 133.74 (C-5, 5′), 130.62 (C-1, 1′), 122.23 (C-3, 3′), 120.07 (C-4, 4′), 119.75 (C-6, 6′)—Anal. Calcd for $C_{13}H_{10}N_4O_5$: (302): C, 51.66; H, 3.33; N, 18.54 %. Found: C, 51.68; H, 3.31; N, 18.52 %.

N-(3-Nitrophenyl)-N′-(3′-nitrophenyl)urea ***(15)*** R_f = 0.77 (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,346, 1,720, 1,691, 1,524, 1,417, 1,352, 809, 756, 682 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.02 (2H, t, $J_{2,(4,6)=2',(4',6')}$ = 1.9 Hz, $H^{2,2'}$), 7.78 (2H, dd, $J_{4,5=4',5'}$ = 8.2, $J_{4,6=4',6'}$ = 1.6 Hz, $H^{4,4'}$), 7.80 (2H, dd, $J_{6,5=6',5'}$ = 8.2, $J_{6,4=6',4'}$ = 1.6 Hz, $H^{6,6'}$), 7.38 (2H, t, $J_{5,(4,6)=5',(4',6')}$ = 8.2 Hz, $H^{5,5'}$), 7.23 (1H, s, NH), 3.08 (1H, s, NH)—EI MS: *m/z* (rel. abund. %), 302 (M^+, 3), 164 (23), 138 (87), 118 (15), 108 (6), 106 (12), 92 (73), 90 (97), 65 (100)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 156.55 (C=O), 148.10 (C-3, 3′), 138.74 (C-1, 1′), 129.90 (C-5, 5′), 123.14 (C-6, 6′), 115.95 (C-4, 4′), 111.75 (C-2, 2′)—Anal. Calcd for $C_{13}H_{10}N_4O_5$: (302): C, 51.66; H, 3.33; N, 18.54 %. Found: C, 51.64; H, 3.30; N, 18.51 %.

N-(4-Nitrophenyl)-N′-(4′-nitrophenyl)urea ***(16)*** IR (KBr): $\nu_{max:}$ 3,286, 3,190, 1,649, 1,600, 1,556, 1,312 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ; 9.62 (2H, br.s, NH), 8.22 (4H, dd, $J_{3,2=5,6=3',2'=5',6'}$ = 8.1, $J_{3,6=5,2=3',6'=5',2'}$ = 0.2 Hz, $H^{3,5}$, $H^{3',5'}$), 8.13 (4H, dd, $J_{2,3=6,5=2',3'=6',5'}$ = 8.1, $J_{2,5=6,3=2',5'=6',3'}$ = 0.2 Hz, $H^{2,6}$, $H^{2',6'}$)—EI MS: *m/z* (rel. abund. %), 302 (M^+, 6), 257 (15), 181 (20), 162 (100), 123 (55)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 156.55 (C=O), 144.33 (C-1, 1′), 140.80 (C-4, 4′), 125.10 (C-3, 5, 3′, 5′), 116.92 (C-2, 6, 2′, 6′)—Anal. Calcd for $C_{13}H_{10}N_4O_5$: (302): C, 51.66; H, 3.34; N, 18.54 %. Found: C, 51.59; H, 3.28; N, 18.47 %.

N-Isopropyl-N′-(3′-nitrophenyl)urea ***(17)*** R_f = 0.72 (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,326, 1,638, 1,565, 1,527, 1,345, 728 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.16 (1H, t, $J_{2',(4',6')}$ = 1.7 Hz, $H^{2'}$), 7.84 (1H, dd, $J_{4',5'}$ = 8.1, $J_{4',6'}$ = 1.2 Hz, $H^{4'}$), 7.76 (1H, dd, $J_{6',5'}$ = 8.1, $J_{6',4'}$ = 1.2 Hz, $H^{6'}$), 7.41 (1H, t, $J_{5',(4',6')}$ = 8.1 Hz, $H^{5'}$), 6.30 (1H, br.s, NH), 4.39 (1H, br.s, NH), 3.98 (1H, sept, $J_{1,(2a,2b)}$ = 6.6 Hz, CH), 1.21 (6H, d, $J_{2a,1=2b,1}$ = 6.6 Hz, $2CH_3$)—EI MS: *m/z* (rel. abund. %), 223 (M^+, 51), 164 (8), 139 (84), 138 (100), 92 (100), 80 (35), 58 (22)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 159.15 (C=O), 147.69 (C-3′), 136.98 (C-1′), 129.50 (C-5′), 122.98 (C-6′), 115.70 (C-4′), 111.59 (C-2′), 42.26 (CH-1), 23.73 ($2CH_3$)—Anal. Calcd for $C_{10}H_{13}N_3O_3$: (223): C, 53.78; H, 5.87; N, 18.83 %. Found: C, 53.81; H, 5.91; N, 18.86 %.

N-Isopropyl-N′-(4′-nitrophenyl)urea ***(18)*** R_f = 0.75 (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,395, 3,302, 2,926, 1,662, 1,551, 1,507, 1,324, 1,236, 855 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.14 (1H, d, $J_{3',2'=5',6'}$ = 9.1 Hz, $H^{3',5'}$), 7.49 (2H, d, $J_{2',3'=6',5'}$ = 9.0 Hz, $H^{2',6'}$), 6.56 (1H, br.s, NH), 4.54 (1H, d, $J_{NH,1}$ = 6.5 Hz, NH), 3.99 (1H, sept, $J_{1,(2a,2b)}$ = 6.5 Hz, CH), 1.20 (6H, d, $J_{2a,1=2b,1}$ = 6.5 Hz, $2CH_3$)—EI MS: *m/z* (rel. abund. %), 223 (M^+, 52), 165 (184), 138 (100), 108 (36), 92 (98)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 159.15 (C=O), 142.57 (C-1′), 140.55 (C-4′), 124.70 (C-3′, 5′), 116.76 (C-2′, 6′), 42.26 (CH-1), 23.73 ($2CH_3$)—Anal. Calcd for $C_{10}H_{13}N_3O_3$: (223): C, 53.78; H, 5.87; N, 18.83 %. Found: C, 53.83; H, 5.82; N, 18.89 %.

N-(2-Methylphenyl)-N′-(4′-nitrophenyl)urea (**19**) $R_f = 0.73$ (CH_2Cl_2/C_6H_{14}, 7:3). IR (KBr): ν_{max}: 3,300, 1,692, 1,496, 1,298, 1,178, 1,114, 846 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ; 8.06 (2H, d, $J_{3',2'=5',6'} = 9.2$ Hz, $H^{3',5'}$), 7.63 (1H, d, $J_{3,4} = 7.5$ Hz, H^3), 7.51 (2H, d, $J_{2',3=6',5'} = 9.2$ Hz, $H^{2',6'}$), 7.10 (1H, d, $J_{6,5} = 7.5$ Hz, H^6), 7.08 (1H, t, $J_{5,(4,6)} = 7.5$ Hz, H^5), 6.93 (1H, t, $J_{4,(3,5)} = 7.5$ Hz, H^4)—EI MS: *m/z* (rel. abund. %), 271 (M^+, 41), 164 (1), 138 (40), 107 (100), 91 (42), 65 (47)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 158.00 (C=O), 144.33 (C-1′), 140.80 (C-4′), 137.93 (C-1), 132.58 (C-3), 129.88 (C-2), 125.94 (C-5), 125.10 (C-3′, 5′), 122.67 (C-6), 122.88 (C-4), 116.92 (C-2′, 6′), 17.49 (CH_3)—Anal. Calcd for $C_{14}H_{13}N_3O_3$: (271): C, 61.99; H, 4.83; N, 15.50 %. Found: C, 61.86; H, 4.80; N, 15.47 %.

N-(3-Nitrophenyl)-2′-one-1-pyrrolidine carboxamide (**20**) $R_f = 0.56$ (CH_2Cl_2/C_6H_{14}, 6:4). IR (KBr): ν_{max}: 3,300, 1,527, 1,343, 1,229, 1,172, 878, 735, 698 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ; 8.25 (1H, br.s, H^2), 7.95 (1H, d, $J_{4,5} = 7.9$ Hz, H^4), 7.84 (1H, d, $J_{6,5} = 7.9$ Hz, H^6), 7.50 (1H, t, $J_{5,(4,6)} = 7.9$ Hz, H^5), 6.77 (1H, br.s, NH), 3.47 (2H, d, $J_{5',4'} = 5.6$ Hz, $H^{5'}$), 1.24 (2H, br.s, $H^{4'}$), 0.85 (2H, m, $H^{3'}$)—EI MS: *m/z* (rel. abund. %), 249 (M^+, 30), 165 (10), 164 (83), 138 (100), 136 (40), 112 (82), 106 (18), 92 (82)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 171.93 (C=O), 154.03 (C=O), 147.61 (C-3), 137.25 (C-1), 129.41 (C-5), 122.10 (C-6), 116.40 (C-4), 110.72 (C-2), 47.99 (CH_2-5, pyrrolidine), 30.68 (CH_2-4, pyrrolidine), 16.59 (CH_2-3, pyrrolidine)—Anal. Calcd for $C_{11}H_{11}N_3O_4$: (249): C, 53.01; H, 4.45; N, 42.02 %. Found: C, 52.99; H, 4.42; N, 42.00 %.

N-(3-Nitrophenyl)-N′-(1-phenylethyl)urea (**21**) $R_f = 0.62$ (CH_2Cl_2/CH_3OH, 9:1). IR (Solid): ν_{max}: 3,366, 3,313, 2,361, 1,654, 1,556, 1,352, 1,233, 1,021 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (1H, t, $J_{2,(4,6)} = 1.4$ Hz, H^2), 7.82 (1H, dd, $J_{4,5} = 8.3$, $J_{4,2} = 1.4$ Hz, H^4), 7.68 (1H, dd, $J_{6,5} = 8.3$, $J_{6,2} = 1.2$ Hz, H^6), 7.39 (1H, t, $J_{5,(4,6)} = 8.3$ Hz, H^5), 7.36 (4H, m, $H^{2',3',5',6'}$), 7.28 (1H, m, $H^{4'}$), 6.38 (2H, br.s, NH), 4.92 (1H, m, CH), 1.52 (3H, d, $J_{2'',1''} = 7.4$ Hz, CH_3))—EI MS: *m/z* (rel. abund. %), 285 (M^+, 25), 270 (3), 227 (1), 181 (1), 164 (5), 138 (100), 120 (5), 105 (92), 92 (17)—^{13}C NMR (125 MHz, $CDCl_3$) δ; 158.79 (C=O), 147.69 (C-3), 139.27 (C-1′), 137.45 (C-1), 129.50 (C-5), 127.62 (C-4′), 126.64 (C-3′,5′), 126.63 (C-2′, 6′), 122.74 (C-6), 115.70 (C-4), 111.35 (C-2), 52.38 (CH), 20.95 (CH_3)—Anal. Calcd for $C_{15}H_{15}N_3O_3$: (285): C, 63.15; H, 5.30; N, 14.73 %. Found: C, 63.20; H, 5.31; N, 14.68 %.

Conclusion

Compounds **8**, **12**, **15**, and **21** demonstrated potent cytotoxic effects against prostate cancer cell line. Compound **21** with IC_{50} value of 20 μM against PC-3 and GI_{50} of 22 μM against NCI-H460 was most active against both the cell lines. These results revealed that the position, number of nitro groups, and presence of an aryl group play a vital role for the antiproliferative activities of these compounds.

While for the β-glucuronidase, the position of nitro group at *para* is a precondition for inhibition of β-glucuronidase enzyme along with aryl group on the second phenyl ring of the urea bridge.

Acknowledgments Authors gratefully acknowledge the Higher Education Commission (HEC) for granting fellowship to Ph. D. student Ms. Sana Mustafa. Dr. Shahnaz Perveen, and Prof. Dr. Khalid M. Khan are thankful to Deutscher Akademischer Austauschdienst (DAAD, Bonn, Germany) for a short term scholarship.

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