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Synthesis, cytotoxicity, and DNA-topoisomerase inhibitory activity of new asymmetric ureas and thioureas

Andressa Esteves-Souza,[a] Kenia Pissinate,[a] Maria da Graça Nascimento,[b] Noema Faiga Grynberg[a] and Aurea Echevarria[a,*]

[a]*Universidade Federal Rural do Rio de Janeiro, Seropédica, 23851-970, RJ, Brazil*
[b]*Universidade Federal de Santa Catarina, Florianópolis, 88040-900, SC, Brazil*

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Abstract—A new series of *N*-3,3-diphenylpropyl-*N*-(*p*-X-benzyl)-*N*′-phenylureas (**5a**–**g**) and thioureas (**6a**–**g**) were synthesized by the reaction of secondary amines and phenyl isocyanate or isothiocyanate. The cytotoxic effects of the urea and thiourea derivatives were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against Ehrlich carcinoma and K562 human leukemia cells. Moreover, the activity of compounds in the inhibition of DNA topoisomerases I and II-α was tested. The results indicated that the compounds presented important and promising antiproliferative action.

1. Introduction

The DNA-topoisomerases are ubiquitous nuclear enzymes that play crucial roles in DNA metabolism events such as replication, transcription, recombination, repair, chromatin assembly, and chromosome segregation.[1–3] The type I enzymes (TOPO I) are monomeric and catalyze an ATP-independent relaxation of DNA supercoils by transiently breaking and religating single-stranded DNA, and the type II topoisomerases (TOPO II) are dimeric and relax supercoiled DNA through catalysis of a transient breakage of double-stranded DNA in an ATP-dependent manner.[1,4] Because of the crucial role of topoisomerases in the maintenance and replication of DNA during proliferation, cells become highly vulnerable when these functions are lost.[5] Consequently, topoisomerase-targeting agents that stabilize the clevable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer. The medical importance of these enzymes is highlighted by the fact that they are the specific targets for many promising anticancer drugs.[6,7]

Thus, there is now good evidence showing that topoisomerases are the principal intracellular targets for a number of clinically important antitumor drugs.[7]

Diarylsulfonylurea derivatives have been reported to possess a broad spectrum of antineoplasic activity in several tumor models. A series of compounds were initially discovered and assayed against CCRF-CEM, HT-29, K562, and HTB-54 tumor cell lines, showing good growth inhibition.[8]

In the literature, the pyrazolyl-ureas are described as P38 kinase inhibitors and the benzoylureas as new drugs with strong cancericidal activity.[9] Recently, hydroxyurea has been described as a clinically useful drug for the treatment of a wide range of solid tumors as well as acute and chronic leukemia.[10]

The urea and the thiourea derivatives have also been used for brain cancer treatment and as potent inhibitors of human DNA-topoisomerase II.[11] Moreover, other properties were attributed to urea derivatives such as HIV-1 protease and cholesterol acyltransferase (ACAT) inhibitory activities. They are also promising therapeutic agents for hypercholesteromia and atherosclerosis.[12,13]

Thus, due to the important properties presented by ureas and thiourea derivatives involving particularly antineoplasic activity, two new series were synthesized and evaluated for their cytotoxic activity.

In this work, we report the synthesis, cytotoxicity evaluation, and human DNA-topoisomerases I and II-α inhibitory activity of a series of *N*-[3,3-diphenyl

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* Corresponding author. Tel./fax: +55 21 26 82 28 07; e-mail: echevarr@ufrrj.br

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propyl]-*N*-[*p*-X-benzyl]-*N*′-phenylureas (**5a**–**g**) and thioureas (**6a**–**g**), in order to identify the essential structural elements required for the antiproliferative activity and to optimize the therapeutic index of these compounds.

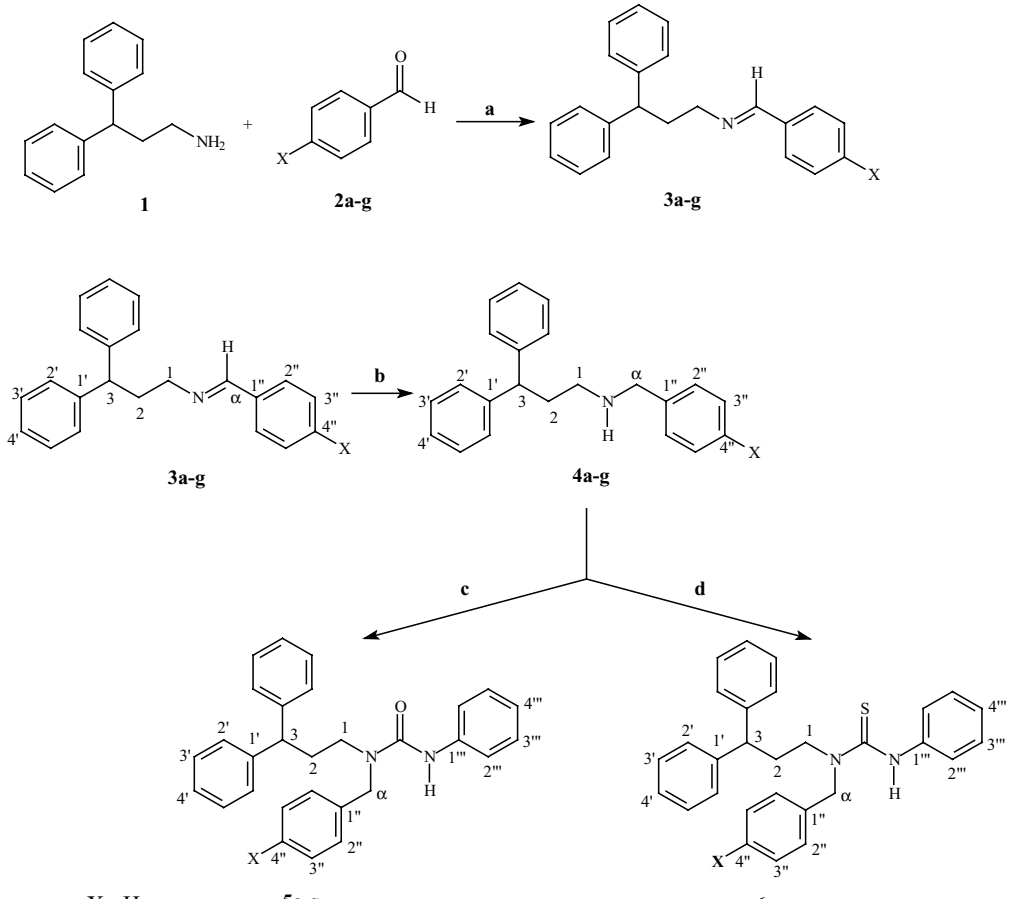
2. Results and discussion

2.1. Synthetic chemistry

The route adapted for the synthesis of asymmetric ureas and thioureas is outlined in Scheme 1. The secondary amines (**4a**–**g**) were obtained from the corresponding Schiff bases (**3a**–**g**) after the reduction reactions with sodium borohydride in 55 to 98% yield.[14] In the next step, the secondary amines were condensed with phenylisocyanate or phenylisothiocyanate in chloroform as the solvent, affording the target compounds after 2 h for unsubstituted derivatives (**5a** and **6a**) and derivatives with electron-donating substituent groups (**5b**–**d** and **6b**–**d**), and after 4h for the electron-withdrawing substituted derivatives (**5e**–**g** and **6e**–**g**). The reactions were monitored by thin layer chromatography (TLC) and the products were purified by recrystallization with ethanol. The yields were in the range of 52–98% for ureas and 45–98% for the thioureas. The best yields observed for ureas were obtained due to the higher reactivity of phenylisocyanate. The electrophilic character of the carbonylic carbon is stronger than that of the thiocarbonylic carbon, thus facilitating the nucleophilic attack of the secondary amines.

The infra-red spectra for the ureas and thioureas showed the absorption stretching band values in the range of 1647–1623 cm^{-1} and 1350–1341 cm^{-1} for C=O and C=S groups, respectively. Higher values of stretching absorption bands were observed for the compounds with electron-withdrawing substituents, both for urea and thiourea derivatives.

The values obtained in the ^1H NMR chemical shifts permitted the characterization of the hydrogens showing similar values for both ureas and thioureas with the same substituents. However, deshield effects on H-1 and H-α NMR chemical shift values of thioureas were observed (c.a. δ 0.5) due to the heavy atom effect of



a: X= H
b: X= CH_3
c: X= $N(CH_3)_2$
d: X= OCH_2O
e: X= Br
f: X= CN
g: X= NO_2

Scheme 1. Reagents and conditions: (a) EtOH, reflux; (b) $NaBH_4$, EtOH, reflux; (c) PhNCO, $CHCl_3$, reflux; (d) PhNCS, $CHCl_3$, reflux.

sulfur. The signals of aromatic hydrogens occurred as multiplets in the characteristic region. The ^{1}H NMR chemical shift of the N–*H* in the ureas and thioureas presented a strong deshielding when compared with the N–*H* values of the amines, for example, δ 1.55 (**4a**) and δ 5.99 (**5a**), where X = H.

^{13}C NMR spectral analysis of the ureas and thioureas showed the typical absorptions for aliphatic carbons in the expected region, such as the signals for the carbonylic and thiocarbonylic carbons, in the range of δ 155.12–156.34 and δ 181.01–182.17, respectively.

2.2. Cytotoxic activity

The cytotoxic activity of the *N*-3,3-diphenylpropyl-*N*-(*p*-X-benzyl)-*N*′-phenylureas and thioureas on ascitic Ehrlich carcinoma and K562 human leukemia cells was measured by MTT assay[15] and the IC_{50} values were determined in μM and μg/mL, at least in three independent experiments.

The results of the cytotoxic activities for ureas and thioureas against Ehrlich carcinoma cells for 48 h cultures indicated that the ureas had a higher activity (Table 1). The ureas with electron-withdrawing groups were more cytotoxic with IC_{50} values of 12(±1) and 11(±1) μM for **5e** and **5f**, respectively. However, the thioureas with electron acceptors of groups did not present significant cytotoxicity until a dose of 50 μM.

The preliminary quantitative relationship analysis between –log (1/IC_{50}) of ureas and the electronic substituent parameter σ_p (Hammett substituent constant)[16] showed a significant correlation [correlation equation: $-\log(1/IC_{50}) = (1.21 \pm 0.05) - (0.34 \pm 0.11)\ \sigma_p$, sd = 0.12, $r = 0.88$, $n = 5$] indicating a linear relationship between the cytotoxic activity against Ehrlich cells and the substituent electronic effect. However, other parameters could have a significant importance, such as, the insolubility indicating an apparent inactivity, as, for example, the **5g** derivative.

Table 1. IC_{50} (μM) values of the ureas and thioureas against Ehrlich carcinoma and K562 human leukemia cells, and substituent constants

Urea/thiourea	IC_{50} (μM ± sd[a])		Substituent constant σ_p[16]
	Ehrlich carcinoma	K562	
5a	20 ± 3	42 ± 1	0
5b	13 ± 3	41 ± 6	−0.17
5c	36 ± 2	25 ± 2	−0.83
5d	na[b]	na	−0.16
5e	12 ± 1	22 ± 2	0.23
5f	11 ± 1	30 ± 2	0.66
5g	na	na	0.78
6a	33 ± 2	20 ± 4	0
6b	16 ± 2	26 ± 6	−0.17
6c	na	14 ± 1	−0.83
6d	na	16 ± 1	−0.16
6e	na	na	0.23
6f	na	na	0.66

[a] sd: standard deviation.
[b] na: no activity until 50 μM.

The cytotoxicity of the ureas and thioureas against K562 leukemia cells showed a dose-dependent effect and the thiourea derivatives presented the highest antiproliferative effect (Table 1). The thioureas with *N*,*N*-dimethyl (**6c**) and methylenedioxyl (**6d**) groups were the most active derivatives with IC_{50} of 14(±1) and 16(±1) μM, respectively. Moreover, no significant correlation was obtained for IC_{50} values of thioureas against K562 cells in relation to electronic parameters.

2.3. DNA-Topoisomerase inhibitory activity

The conversion of supercoiled plasmid DNA to relaxed DNA by human topoisomerases I and II-α was examined in the presence of urea (**5a**–**g**) and thiourea (**6a**–**f**) derivatives. The activity of the ureas and thioureas on the DNA-topoisomerase I enzyme was observed through relaxation assays of the supercoiled $\phi \times 174$ plasmid DNA. The campothecin, a well-known topoisomerase I enzyme inhibitor, was used as a positive control.[17,18]

The results were observed by the alteration of the electrophoretic mobility of $\phi \times 174$ plasmid DNA by the combined action of topoisomerase I and the drugs. The results were analyzed after development with ethidium bromide in UV light, and the record was photographed with a digital camera. As shown in Figure 1, the mobility of the supercoiled closed circular double-stranded plasmid DNA increased upon topoisomerase I mediated relaxation, when subjected to electrophoresis with ethidium bromide, line 3. In the presence of 100 μM campothecin (positive control), the relaxation effect was not observed, line 1. It can also be seen that in the presence of topoisomerase I with **6a** and **6d** (lines 5 and 9), as in the presence of campothecin a significant relaxation inhibitory effect was observed. The **5a** derivative (line 4) showed a lower inhibitory effect, and **5b**, **5e**, **5f**, and **6b** a very weak effect, but with nicked DNA formation.

The effects of the ureas (**5a**–**g**) and thioureas (**6a**–**f**) on the human DNA topoisomerase II-α enzyme were observed in the relaxation assays using supercoiled pBR322 plasmid DNA in the presence of ATP. The reaction products were analyzed by electrophoretic mobility and developed in ethidium bromide in the presence of UV light. As shown in Figure 2, **5g** and **5f** (100 μM) presented a significant inhibition of topo II-α

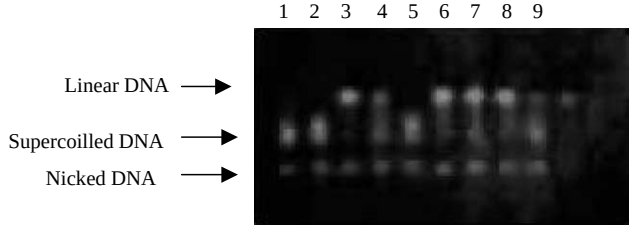


Figure 1. Effect of ureas and thioureas on DNA-topo I. Line 1: topo I + campothecin 100 μM; line 2: $\phi \times 174$ (0.125 μg) only; line 3: $\phi \times 174$ + topo I; line 4: topo I + **5a** 100 μM; line 5: topo I + **6a** 100 μM; line 6: topo I + **5b** 100 μM; line 7: topo I + **6b** 100 μM; line 8: topo I + **5e** 100 μM; line 9: topo I + **6d** 100 μM.

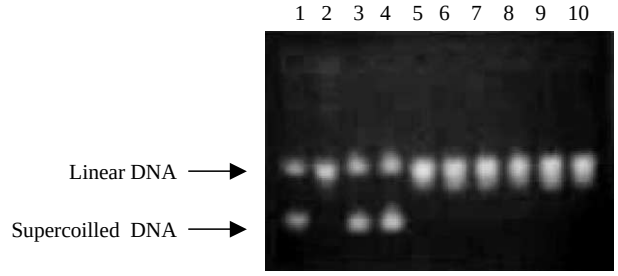


Figure 2. Effect of ureas and thioureas on DNA-topo II-α. Line 1: pBR322 (0.125 μg) only; line 2: pBR322 + topo II-α; line 3: topo II-α + **5g** 100 μM; line 4: topo II-α + **5f** 100 μM; line 5: topo II-α + **5e** 100 μM; line 6: topo II-α + **6e** 100 μM; line 7: topo II-α + **6d** 100 μM; line 8: topo II-α + **6b** 100 μM; line 9: topo II-α + **5b** 100 μM; line 10: topo II-α + **6d** 100 μM.

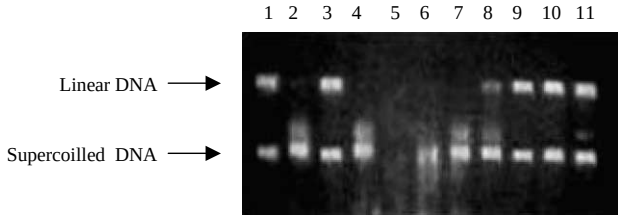


Figure 3. Effect of ureas and thioureas on DNA-topo II-α. Line 1: etoposide 100 μM + topo II-α; line 2: pBR322 (0.125 μg) only; line 3: pBR322 + topo II-α; line 4: topo II-α + 5 g 100 μM; line 5: topo II-α + **5g** 50 μM; line 6: topo II-α + **5g** 10 μM; line 7: topo II-α + **5f** 100 μM; line 8: topo II-α + **5f** 50 μM; line 9: topo II-α + **5f** 10 μM; line 10: topo II-α + **5a** 100 μM; line 11: topo II-α + **6a** 100 μM.

catalytic activity. In order to investigate the dose-dependent inhibitory effects of **5g** and **5f**, the reaction products of the relaxation assays were analyzed using 100, 50, and 10 μM drug concentrations. Figure 3 indicates the dose-dependent inhibition of topo II catalytic activity by **5g** and **5f**. The etoposide, a known topo II inhibitor, was used as a positive control at 100 μM.

3. Experimental

3.1. Chemistry

3.1.1. Reagents and apparatus. Melting points were determined with a MELTEMP II apparatus and are uncorrected. The microanalyses were carried out with elemental apparatus CHNS-O EA-1110, of Carlo Erba Instruments. Infra-red spectra (KBr pellets or as films on a NaCl disk) were recorded on a Perkin-Elmer 1605 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on Varian Unity-plus (299.95 MHz and 75.42 MHz) and Bruker AC200 (200 MHz and 50.3 MHz) spectrometers, with TMS as the internal reference, and $CDCl_3$ as the solvent. TLC analyses were performed on silica gel 60 F254 plates. All the reagents were purchased from Merck or Aldrich.

3.1.2. General procedure for Schiff base preparations (3a–g). To a solution of 3,3-diphenyl-propylamine **1** (2 mmol) the *p*-X-benzaldehyde (**2a**–**g**) (2 mmol) was added, in ethanol. The mixture was refluxed, with stirring and the water was collected in a Dean–Stark apparatus. The reaction was monitored by TLC. The products were filtered and recrystallized in ethanol.

3.1.3. *N*-Benzyliden-*N*-3,3-diphenylpropylamine (3a). 85% yield; mp 108–110 °C; IR (KBr): ν_{max} 3026, 2921–2853, 1641, 1538; ^{1}H NMR δ 2.45 (q, J = 7 Hz, 2H), 3.53 (t, J = 7 Hz, 2H), 4.03 (t, J = 7.9 Hz, 1H), 7.12–7.26 (m, 10H), 7.37–7.40 (m, 4H), 7.66–7.70 (m, 1H), 8.09 (s, 1H); ^{13}C NMR δ 36.32 (C-2), 48.61 (C-1), 59.60 (C-3), 126.15 (C-4′), 127.92 (C-3″, C-5″), 128.43 (C-3′,C-5′), 128.54 (C-2″, C-6″), 129.43 (C-2′, C-6′), 130.18 (C-4″), 136.18 (C-1″), 144.50 (C-1′), 161.36 (C-α).

3.1.4. *N-p*-Methyl-benzyliden-*N*-3,3-diphenylpropylamine (3b). 79% yield; mp 163–165 °C; IR (KBr): ν_{max} 3023, 2928–2832, 1643, 1493, 859, 740; ^{1}H NMR δ 2.36 (s, 3H), 2.45 (q, J = 7 Hz, 2H), 3.51 (t, J = 7 Hz, 2H), 4.02 (t, J = 7.2 Hz, 1H), 7.12–7.28 (m, 13H), 7.57 (d, J = 8.3 Hz, 1H), 8.05 (s, 1H); ^{13}C NMR δ 21.45 (CH$_3$), 36.36 (C-2), 48.60 (C-1), 59.59 (C-3), 126.12 (C-4′), 127.92 (C-3″, C-5″), 128.42 (C-3′-C-5′), 129.27 (C-2′, C-6′), 133.60 (C-1″), 140.74 (C-4″), 144.53 (C-1′), 161.27 (C-α).

3.1.5. *N-p*-Dimethylamino-benzyliden-*N*-3,3-diphenylpropylamine (3c). 87% yield; mp 108–110 °C; IR (KBr): ν_{max} 3023, 2929–2856, 1603, 1531, 813, 749; ^{1}H NMR δ 2.50 (q, J = 6.6 Hz, 2H), 3.00 (s, 6H), 3.58 (t, J = 6.6 Hz, 2H), 4.10 (t, J = 7.6 Hz, 1H), 6.70 (dd, J_o = 9.4; J_p = 1.9 Hz, 2H), 7.30 (m, 10H), 7.62 (dd, J_o = 9.4; J_p = 1.9 Hz, 2H), 8.02 (s, 1H); ^{13}C NMR δ 36.52 (C-2), 40.13 (N(CH$_3$)$_2$), 48.56 (C-1), 59.42 (C-3), 111.51 (C-3″, C-5″), 124.24 (C-1″), 126.00 (C-4′), 127.64 (C-2″, C-6″), 128.33 (C-3′, C-5′), 129.37 (C-2′, C-6′), 144.62 (C-1′), 151.89 (C-4″), 161.33 (C-α).

3.1.6. *N*-3,4-Methylenedioxy-benzyliden-*N*-3,3-diphenylpropylamine (3d). 72% yield; mp 105–107 °C; IR (KBr): ν_{max} 3065, 2924–2820, 1649, 1495, 1195–1125, 819, 742; ^{1}H NMR δ 2.43 (q, J = 7 Hz, 2H), 3.48 (t, J = 7 Hz, 2H), 4.02 (t, J = 7.9 Hz, 1H), 5.98 (s, 2H), 6.80 (d, J = 7.9 Hz, 1H), 7.01 (d, J = 7.9 Hz, 1H), 7.24 (m, 10H), 7.32 (sl, 1H), 7.97 (s, 1H); ^{13}C NMR δ 36.78 (C-2), 48.59 (C-1), 59.32 (C-3), 101.38 (OCH$_2$O), 106.47 (C-2″), 108.02 (C-5″), 124.16 (C-6″), 126.15 (C-4′), 127.92 (C-3′, C-5′), 128.43 (C-2′, C-6′), 131.03 (C-1″), 144.52 (C-1′), 148.19 (C-3″), 153.28 (C-4″), 160.51 (C-α).

3.1.7. *N-p*-Bromo-benzyliden-*N*-3,3-diphenylpropylamine (3e). 65% yield; mp 99–102 °C; IR (KBr): ν_{max} 3021, 2929–2835, 1639, 1586, 819, 743; ^{1}H NMR δ 2.43 (q, J = 7 Hz, 2H), 3.48 (t, J = 7 Hz, 2H), 4.02 (t, J = 7.9 Hz, 1H), 7.14–7.31 (m, 12H), 7.53 (m, 2H), 8.04 (s, 1H); ^{13}C NMR δ 36.23 (C-2), 48.66 (C-1), 59.62 (C-3), 124.89 (C-4″), 126.21 (C-4′), 127.90 (C-3′, C-5′), 128.46 (C-2″, C-6″), 129.41 (C-2′, C-6′), 131.79 (C-3″, C-5″), 135.07 (C-1″), 144.43 (C-1′), 160.09 (C-α).

3.1.8. *N-p*-Cyano-benzyliden-*N*-3,3-diphenylpropylamine (3f). 80% yield; mp 78–80 °C; IR (KBr): ν_{max} 3056, 2934–2844, 2228, 1644, 1550, 836, 750; ^{1}H NMR δ 2.45 (q, J = 7 Hz, 2H), 3.57 (t, J = 7 Hz, 2H), 4.01 (t,

J = 7.8 Hz, 1H), 7.10–7.26 (m, 10H), 7.66 (d, J = 6.7 Hz, 2H), 7.77 (d, J = 6.7 Hz, 2H), 8.11 (s, 1H); ^{13}C NMR δ 36.13 (C-2), 48.68 (C-1), 59.76 (C-3), 118.53 (*C*N), 126.27 (C-4′), 127.47 (C-3″, C-5″), 128.40 (C-3′, C-5′), 128.50 (C-2′, C-6′), 127.86 (C-2″, C-6″), 129.85 (C-4″), 139.98 (C-1″), 144.29 (C-1′), 159.41 (C-α).

3.1.9. *N*-*p*-Nitro-benzyliden-*N*-3,3-diphenylpropylamine (3g). 92% yield; mp 96–98 °C; IR (KBr): ν_{max} 3058, 2933–2835, 1645, 1598, 832, 747; ^{1}H NMR 2.47 (q, J = 7.9 Hz, 2H), 3.59 (t, J = 7 Hz, 2H), 4.03 (t, J = 7.9 Hz, 1H), 7.14–7.27 (m, 10H), 7.83 (d, J = 10.4 Hz, 2H), 8.19 (d, J = 10.4 Hz, 2H), δ 8.26 (s, 1H); ^{13}C NMR δ 36.09 (C-2, C-3), 48.68 (C-1), 59.84 (C-4), 123.80 (C-4″), 126.25 (C-4′), 127.82 (C-2″, C-6″), 128.49 (C-3′, C-5′), 128.61 (C-2′, C-6′), 141.61 (C-1″), 144.25 (C-1′), 148.84 (C-3″, C-5″), 158.97 (C-α).

3.1.10. General procedure for amine preparations (4a–g). To an ethanolic solution of Schiff bases (**1a**–**g**), (2 mmol) $NaBH_4$ (2 mmol) was slowly added in an ice bath with stirring. The mixture was refluxed for 3–6 h. After this time, the solvent was evaporated and water (2 mL) was added. The product extraction was carried out with $CHCl_3$ (3× 2 mL). The organic layer was dried over Na_2SO_4, and after solvent evaporation the residue was purified by flash column chromatography (silica gel 60, 35–70 mesh) using $CHCl_3$ as the eluent.

3.1.11. *N*-[3,3-Diphenylpropyl]-*N*-benzylamine (4a). 94% yield; refraction index: 1.444; IR (KBr): ν_{max} 3408, 3025, 2923–2851, 1492, 1115, 744; ^{1}H NMR δ 1.55 (s, 1H), 2.26 (q, J = 7 Hz, 2H), 2.61 (t, J = 7 Hz, 2H), 3.71 (s, 2H), 4.02 (t, J = 7.8 Hz, 1H), 7.15–7.30 (m, 15H); ^{13}C NMR δ 35.82 (C-2), 47.71 (C-1), 49.01 (C-α), 53.89 (C-3), 126.15 (C-4′), 126.88 (C-4″), 127.82 (C-3′, C-5′), 128.06 (C-3″, C-5″), 128.36 (C-2′, C-6′), 128.43 (C-2″, C-6″), 140.31 (C-1″), 144.77 (C-1′).

3.1.12. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-methyl-benzyl]-amine (4b). 98% yield; refraction index: volatile; IR (KBr): ν_{max} 3322, 2925–2822, 1493, 804, 749; ^{1}H NMR δ 1.58 (s, 1H), 2.26 (q, J = 7 Hz, 2H), 2.33 (s, 3H), 2.61 (t, J = 7 Hz, 2H), 3.68 (s, 2H), 4.03 (t, J = 7.8 Hz, 1H), 7.13–7.31 (m, 14H); ^{13}C NMR δ 21.01 (*C*H$_3$) 35.73 (C-2), 47.59 (C-1), 48.92 (C-α), 53.51 (C-3), 126.06 (C-4′), 127.73 (C-3″, C-5″), 127.94 (C-2″, C-6″), 128.36 (C-3′, C-5′), 128.95 (C-2′, C-6′), 136.32 (C-4″), 137.19 (C-1″), 144.72 (C-1′).

3.1.13. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-dimethylamino-benzyl]-amine (4c). 65% yield; refraction index: volatile; IR (KBr): ν_{max} 3306, 3058–3024, 2928–2854, 1491, 1125, 806, 750; ^{1}H NMR δ 2.33 (q, J = 6.5 Hz, 2H), 2.62 (t, J = 7 Hz, 2H), 2.91 (s, 3H), 3.65 (s, 2H), 4.01 (t, J = 6.5 Hz, 1H), 6.67 (d, J = 8.7 Hz, 2H), 7.13–7.26 (m, 12H); ^{13}C NMR δ 35.38 (C-2), 40.68 (N(*C*H$_3$)$_2$), 47.29 (C-1), 48.95 (C-α), 53.06 (C-3), 112.62 (C-3″, C-5″), 126.09 (C-4′), 127.42 (C-1″), 127.77 (C-3′, C-5′), 128.39 (C-2′, C-6′), 129.15 (C-2″, C-6″), 144.65 (C-1′), 149.82 (C-4″).

3.1.14. *N*-[3,3-Diphenylpropyl]-*N*-[3,4-methylenedioxy-benzyl]-amine (4d). 75% yield; refraction index: 1.450; IR (KBr): ν_{max} 3643, 3059–3026, 2926, 1493, 1189, 1107, 809, 750; ^{1}H NMR δ 1.72 (s, 1H), 2.26 (q, J = 7.5 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 3.62 (s, 2H), 4.02 (t, J = 7.5 Hz, 1H), 5.92 (s, 2H), 6.77 (1H), 6.71 (2H), 7.13–7.31 (m, 10H); ^{13}C NMR δ 35.52 (C–2), 47.29 (C-1), 48.86 (C-α), 53.42 (C-3), 107.92 (C-5″), 108.58 (C-2″), 110.74 (O*C*H$_2$O), 121.12 (C-6″), 126.07 (C-4′), 127.70 (C-3′, C-5′), 133.86 (C-1″), 144.62 (C-2′, C-6′), 146.38 (C-4″), 147.53 (C-3″).

3.1.15. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-bromo-benzyl]-amine (4e). 62% yield; refraction index: volatile; IR (KBr): ν_{max} 3322, 3059–3026, 2927–2846, 1489, 1116, 819, 749; ^{1}H NMR δ 1.55 (s, 1H), 2.23 (q, J = 7.7 Hz, 2H), 2.55 (t, J = 6.3 Hz, 2H), 3.62 (s, 2H), 4.00 (t, J = 7.7 Hz, 1H), 7.06–7.41 (m, 14H); ^{13}C NMR δ 35.60 (C-2), 47.37 (C-1), 48.74 (C-α), 52.90 (C-3), 120.40 (C-4″), 126.02 (C-4′), 127.61 (C-3′, C-5′), 128.29 (C-2′, C-6′), 129.57 (C-2″, C-6″), 131.16 (C-3″, C-5″), 139.19 (C-1″), 144.53 (C-1′).

3.1.16. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-cyano-benzyl]-amine (4f). 72% yield; refraction index: volatile; IR (KBr): ν_{max} 3314, 3058–3026, 2929–2854, 2226, 1494, 1113, 818, 747; ^{1}H NMR δ 2.28 (q, J = 7.5 Hz, 2H), 2.65 (t, J = 6.9 Hz, 2H), 3.78 (s, 2H), 4.03 (t, J = 7.5 Hz, 1H), 7.14–7.37 (m, 14H); ^{13}C NMR δ 35.65 (C-2), 47.57 (C-1), 48.89 (C-α), 53.22 (C-3), 126.24 (C-4′), 127.71 (C-3′, C-5′), 128.48 (C-2′, C-6′, C-3″, C-5″), 132.12 (C-2″, C-6″), 144.43 (C-1′), 145.80 (C-1″).

3.1.17. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-nitro-benzyl]-amine (4g). 55% yield; refraction: 1.456; IR (KBr): ν_{max} 3332, 3059–3026, 2929–2850, 1495, 845, 748; ^{1}H NMR δ 2.21 (s, 1H), 2.30 (q, J = 7 Hz, 2H), 2.59 (t, J = 7 Hz, 2H), 3.81 (s, 2H), 4.06 (t, J = 7.8 Hz, 1H), 7.16–7.29 (m, 10H), 7.76 (dd, J = 8.9 Hz, 2H), 8.05 (dd, J = 8.9 Hz, 2H); ^{13}C NMR δ 36.06 (C-2), 48.65 (C-1), 48.80 (C-α), 52.95 (C-3), 123.76 (C-3″, C-5″), 126.22 (C-4′), 127.79 (C-3′,C-5′), 128.46 (C-2′, C-6′), 128.58 (C-2″, C-6″), 141,56 (C-1′), 144.23 (C-1″,C-4″).

3.2. General procedure for urea (5a–g) and thiourea (6a–f) preparations

To chloroform solution of amines (**4a**–**g**) (1.5 mmol), phenylisocyanate or phenylisothiocyanate (1.5 mmol) was slowly added with stirring. The mixture was refluxed for 2–4 hours. After this time, the product was filtered and purified by recrystallization from ethanol.

3.2.1. *N*-[3,3-Diphenylpropyl]-*N*-benzyl-*N*′-phenylurea (5a). 98% yield; mp 123–125 °C; IR (KBr): ν_{max} 3284, 3057, 2930–2863, 1623, 1530, 750; ^{1}H NMR δ 2.38 (q, J = 8 Hz, 2H), 3.28 (t, J = 8 Hz, 2H), 3.89 (t, J = 8 Hz, 1H), 4.51 (s, 2H), 5.99 (s, 1H), 6.96–7.32 (m, 20H); ^{13}C NMR δ 33.81 (C-2), 46.43 (C-1), 48.62 (C-α), 50.53 (C-3), 119.69 (C-2‴, C-6‴), 122.90 (C-4‴), 126.58 (C-4′), 127.30 (C-4″), 127.61 (C-3′, C-5′, C-2″, C-3″, C-5″, C-6″), 128.46 (C-3‴, C-5‴) 128.78 (C-2′, C-6′), 137.56 (C-1‴), 138.89 (C-1″), 143.91 (C-1′), 155.26 (*C*═O); Found: C = 83.29, H = 6.77, N = 7.09. $C_{29}H_{28}N_2O$; Requires: C = 82.81, H = 6.72, N = 6.66.

3.2.2. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-methyl-benzyl]-*N*′-phenylurea (5b). 84% yield; mp 118–119 °C; IR (KBr): ν_{max} 3412, 3053–3027, 2925, 1632, 1530, 1244, 827, 752; ^{1}H NMR δ 2.31 (s, 3H), 2.38 (q, J = 7.4 Hz, 2H), 3.28 (t, J = 7.4 Hz, 2H), 3.89 (t, J = 8 Hz, 1H), 4.47 (s, 2H), 6.01 (s, 1H), 6.96–7.31 (m, 19H); ^{13}C NMR δ 20.99 (*C*H$_3$), 33.70 (C-2), 46.29 (C-1), 48.54 (C-α), 50.21 (C-3), 119.61 (C-2‴, C-6‴), 122.70 (C-4‴), 126.42 (C-4′), 127.22 (C-4″), 127.54 (C-2″, C-3″, C-5″,C-6″), 128.63 (C-3′, C-5′), 129.41 (C-2′, C-6′), 134.37 (C-3‴, C-5‴), 137.19 (C-1‴), 138.94 (C-1″), 143.89 (C-1′), 155.20 (*C*═O); Found: C = 83.07, H = 6.63, N = 6.74. $C_{30}H_{30}N_2O$; Requires: C = 82.90, H = 6.97, N = 6.45.

3.2.3. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-dimethylamino-benzyl]-*N*′-phenylurea (5c). 75% yield; mp 128–131 °C; IR (KBr): ν_{max} 3442, 3023, 2934-2859, 1635, 1535, 1243, 922, 750; ^{1}H NMR δ 2.06 (s, 6H), 2.25 (q, J = 6.4 Hz, 2H), 3.21 (t, J = 7.8 Hz, 2H), 3.94 (t, J = 6.4 Hz, 1H), 6.12 (s, 1H), 7.09–7.33 (m, 19H); ^{13}C NMR δ 35.66 (C-2), 38.94 (C-1), 39.17 (N(*C*H$_3$)$_2$), 48.53 (C-α), 48.78 (C-3), 120.62 (C-2‴, C-6‴), 123.37 (C-4‴), 126.43 (C-1″), 126.67 (C-4′), 127.67 (C-3′, C-5′), 128.49 (C-3‴, C-5‴), 128.57 (C-2′, C-6′), 138.74 (C-1‴), 143.78 (C-1′), 144.16 (C-4″), 156.34 (*C*═O); Found: C = 79.98, H = 7.04, N = 9.06. $C_{31}H_{33}N_3O$; Requires: C = 80.30, H = 7.19, N = 9.06.

3.2.4. *N*-[3,3-Diphenylpropyl]-*N*-[3,4-methylenodioxy-benzyl]-*N*′-phenylurea (5d). 83% yield; mp 83–85 °C; IR (KBr): ν_{max} 3419, 3058, 2932-2896, 1628, 1529, 1242, 809, 751; ^{1}H NMR δ 2.37 (q, J = 7.5 Hz, 2H), 3.25 (t, J = 7.9 Hz, 2H), 3.90 (t, J = 7.5 Hz, 1H), 4.42 (s, 2H), 5.92 (s, 2H), 6.00 (s, 1H), 6.64–7.33 (m, 19H); ^{13}C NMR δ 33.76 (C-2), 46.15 (C-1), 48.59 (C-α), 50.22 (C-3), 101.10 (O*C*H$_2$O), 107.96 (C-2″), 108.28 (C-6″), 119.58 (C-2‴, C-6‴), 120.64 (C-5″), 122.87 (C-4‴), 126.63 (C-4′), 127.61 (C-3′, C-5′), 128.82 (C-2′, C-6′, C-3‴, C-5‴), 131.50 (C-1″), 138.88 (C-1‴), 143.88 (C-1′), 147.00 (C-4″), 148.00 (C-3″), 155.15 (*C*═O); Found: C = 77.34, H = 6.06, N = 6.32. $C_{30}H_{29}N_2O_3$; Requires: C = 77.55, H = 6.07, N = 6.03.

3.2.5. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-bromo-benzyl]-*N*′-phenylurea (5e). 55% yield; mp 242–244 °C; IR (KBr): ν_{max} 3256, 3055, 2972-2874, 1640, 1536, 1238, 830, 753; ^{1}H NMR δ 2.37 (q, J = 7.5 Hz, 2H), 3.23 (t, J = 8.3 Hz, 2H), 3.87 (t, J = 7.5 Hz, 1H), 4.48 (s, 2H), 5.94 (s, 1H), 6.69–7.44 (m, 19H); ^{13}C NMR δ 33.74 (C-2), 46.02 (C-1), 48.44 (C-α), 49.69 (C-3), 119.66 (C-2‴, C-6‴), 120.95 (C-4″), 123.07 (C-6″, C-2″), 124.10 (C-4‴), 126.73 (C-4′), 127.57 (C-3′, C-5′), 128.79 (C-3‴, C-5‴),128.89 (C-2′, C-6′), 129.31 (C-3″, C-5″), 136.83 (C-1″), 138.68 (C-1‴), 143.71 (C-1′), 155.12 (*C*═O); Found: C = 69.57, H = 5.76, N = 5.42. $C_{29}H_{27}N_2OBr$; Requires: C = 69.73, H = 5.46, N = 5.61.

3.2.6. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-cyano-benzyl]-*N*′-phenylurea (5f). 74% yield; mp 243–246 °C; IR (KBr): ν_{max} 3324, 3063, 2924-2824, 1647, 1554, 1233, 895, 753; ^{1}H NMR δ 2.40 (q, J = 7 Hz, 2H), 3.21 (t, J = 7 Hz, 2H), 3.90 (t, J = 6.9 Hz,1H), 4.59 (s, 2H), 5.94 (s, 1H), 7.13–7.60 (m, 19H); ^{13}C NMR δ 33.81 (C-2), 46.14 (C-1), 48.36 (C-α), 49.99 (C-3), 119.72 (*C*N), 120.67 (C-2‴, C-6‴), 121.15 (C-2″, C-6″), 124.28 (C-4‴), 126.91 (C-4′), 127.55 (C-3′, C-5′), 128.86 (C-3‴, C-5‴), 129.04 (C-2′, C-6′), 132.53 (C-3″, C-5″), 143.53 (C-1′), 143.70 (C-1″); Found: C = 80.73, H = 6.07, N = 9.22. $C_{30}H_{27}N_3O$; Requires: C = 80.86, H = 6.12, N = 9.43.

3.2.7. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-nitro-benzyl]-*N*′-phenylurea (5g). 52% yield; mp 110–112 °C; IR (KBr): ν_{max} 3418, 3058, 2940-2876, 1642, 1535, 1241, 841, 753; ^{1}H NMR δ 2.85 (q, J = 6.6 Hz, 2H), 3.62 (t, J = 6.6 Hz, 2H), 4.20 (t, J = 6.9 Hz, 1H), 4.85 (s, 2H), 6.13 (s, 1H), 7.03–7.34 (m, 19H); ^{13}C NMR δ 33.79 (C-2), 46.09 (C-1), 48.55 (C-α), 50.20 (C-3), 119.75 (C-2‴, C-6‴), 122.73 (C-2″, C-6″), 123.16 (C-3″, C-5″), 126.05 (C-4‴), 126.68 (C-4′), 127.57 (C-3′, C-5′), 128.85 (C-3‴, C-5‴), 128.90 (C-2′, C-6′), 139.49 (C-1‴), 142.01 (C-1′), 143.01 (C-4″), 143.65 (C-1″), 155.19 (*C*═O); Found: C = 74.78, H = 6.02, N = 9.08. $C_{29}H_{27}N_3O_3$; Requires: C = 74.81, H = 5.86, N = 9.03.

3.2.8. *N*-[3,3-Diphenylpropyl]-*N*-benzyl-*N*′-phenylthiourea (6a). 70% yield; mp 135–137 °C; IR (KBr): ν_{max} 3387, 3085, 2915, 1344, 1517, 1205, 741; ^{1}H NMR δ 2.50 (q, J = 7.6 Hz, 2H), 3.68 (t, J = 8 Hz, 2H), 3.90 (t, J = 7.6 Hz, 1H), 4.94 (s, 2H), 5.99 (s, 1H), 7.11–7.31 (m, 20H); ^{13}C NMR δ 32.70 (C-2), 48.68 (C-1), 50.22 (C-α), 54.70 (C-3), 125.49 (C-2″, C-6″), 125.69 (C-4″), 126.52 (C-4′), 127.52/127.05 (C-2″, C-3″, C-5″, C-6″), 127.91 (C-4″), 128.45 (C-3′, C-5′), 128.66 (C-3″, C-5″), 128.92 (C-2′, C-3′), 135.76 (C-1″), 139.46 (C-1″), 143.62 (C-1′), 181.94 (*C*═S), Found: C = 79.53, H = 6.51, N = 6.55, S = 1.29. $C_{29}H_{28}N_2S$; Requires: C = 79.64, H = 6.48, N = 6.42, S = 1.35.

3.2.9. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-methyl-benzyl]-*N*′-phenylthiourea (6b). 98% yield; mp 150–151 °C; IR (KBr): ν_{max} 3440, 3023, 2915, 1519, 1348, 1205, 836, 759; ^{1}H NMR δ 2.35 (s, 3H), 2.52 (q, J = 7.9 Hz, 2H), 3.71 (t, J = 8,1 Hz, 2H), 3.72 (t, J = 7.9 Hz, 1H), 4.89 (s, 2H), 6.80 (s, 1H), 7.15–7.29 (m, 19H); ^{13}C NMR δ 21.03 (*C*H$_3$), 32.66 (C-2), 48.68 (C-1), 50.43 (C-α), 54.86 (C-3), 125.39 (C-2″, C-6″), 125.58 (C-4″), 126.46 (C-4′), 126.91 (C-2″, C-6″), 127.50 (C-3″, C-5″), 128.40 (C-3″, C-5″), 128.60 (C-3′, C-5′), 129.58 (C-2′, C-3′), 132.50 (C-1″), 135.66 (C-1″), 139.47 (C-4″), 143.65 (C-1′), 181.82 (*C*═S); Found: C = 79.85, H = 6.69, N = 6.36, S = 7.14. $C_{30}H_{30}N_2S$; Requires: C = 79.95, H = 6.72, N = 6.22, S = 7.12.

3.2.10. *N*-[3,3-diphenylpropyl]-*N*-[3,4-methylenodioxy-benzyl]-*N*′-phenylthiourea (6c). 80% yield; mp 109–112 °C; IR (KBr): ν_{max} 3409, 3055, 2918-2889, 1345, 1519, 1203, 806; ^{1}H NMR: δ 2.46 (q, J = 7.8 Hz, 2H), 3.60 (t, J = 8.2 Hz, 2H), 3.88 (t, J = 7.8 Hz, 1H), 4.85 (s, 2H), 6.61–6.83 (m, 3H), 7.11–7.31 (m, 10H); ^{13}C NMR δ 32.66 (C-2), 48.67 (C-1), 49.64 (C-α), 54.58 (C-3), 101.17 (O*C*H$_2$O), 107.66 (C-5″), 108.39 (C-2″), 120.50 (C-6″), 125.52 (C-2″, C-6″), 125.78 (C-4″), 126.62 (C-4′), 127.52 (C-3′, C-5′), 128.52 (C-3″, C-5″), 128.73 (C-2′, C-3′), 129.64 (C-1″), 139.43 (C-1″), 143.59 (C-1′), 147.33 (C-3″), 148.20 (C-4″), 181.82 (*C*═S); Found: C = 74.78, H = 6.08, N = 5.82. $C_{30}H_{29}N_2O_2S$; Requires: C = 74.80, H = 6.08, N = 5.82.

3.2.11. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-bromo-benzyl]-*N*′-phenylthiourea (6d). 61% yield; mp 129–130 °C; IR (KBr): ν_{max} 3389, 3054, 2914, 1519, 1350, 1204, 820, 762; ^{1}H NMR δ 2.43 (q, J = 7.8 Hz, 2H), 3.50 (t, J = 8.3 Hz, 2H), 3.84 (t, J = 7.8 Hz, 1H), 4.95 (s, 2H), 6.80 (s, 1H), 7.10–7.27 (m, 18H), 7.41 (s, 1H); ^{13}C NMR δ 32.66 (C-2), 48.53 (C-1), 48.95 (C-α), 54.35 (C-3), 121.69 (C-4″), 125.61 (C-2″, C-6″), 125.93 (C-4″), 126.70 (C-4′), 128.53 (C-3′, C-5′), 128.79 (C-3″, C-5″), 129.10 (C-2′, C-3′, C-2″, C-6″), 131.86 (C-3″, C-5″), 135.18 (C-1″), 139.28 (C-1″), 143.38 (C-1′), 181.01 (*C*═S); Found: C = 67.79, H = 5.19, N = 5.58, S = 6.04. $C_{29}H_{27}N_2SBr$; Requires: C = 67.56, H = 5.29, N = 5.44, S = 6.23.

3.2.12. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-cyano-benzyl]-*N*′-phenylthiourea (6e). 60% yield; mp 138–140 °C; IR (KBr): ν_{max} 3356, 3028, 2927, 2228, 1525, 1348, 1254, 815, 746; ^{1}H NMR δ 2.48 (q, J = 6.8 Hz 2H), 3.47 (t, J = 6.6 Hz, 2H), 3.85 (t, J = 6.8 Hz 1H), 4.17 (s, 2H), 6.85 (s, 1H), 7.20–7.36 (m, 19H); ^{13}C NMR δ 32.63 (C-2), 48.34 (C-1, C-α), 54.86 (C-3), 110.00 (C-4″), 125.82 (C-2″, C-6″), 126.10 (C-4″), 126.82 (C-4′), 127.37 (C-3′, C-5′), 128.10 (C-3″, C-5″), 128.55 (C-2′, C-3′), 128.86 (C-2″, C-6″), 132.37 (C-3″, C-5″), 139.00 (C-1″), 142.14 (C-1″), 143.17 (C-1′), 182.17 (*C*═S); Found: C = 77.96, H = 5.88, N = 9.27. $C_{30}H_{27}N_3S$; Requires: C = 78.04, H = 5.91, N = 9.10.

3.2.13. *N*-[3,3-Diphenylpropyl]-*N*-[*p*-nitro-benzyl]-*N*′-phenylthiourea (6f). 45% yield; mp 180–182 °C; IR (KBr): ν_{max} 3382, 3025, 2925-2858, 1517, 1341, 1196, 840, 748; ^{1}H NMR δ 2.49 (q, J = 6.6 Hz, 2H), 3.58 (t, J = 6.6 Hz, 2H), 3.89 (t, J = 6.8 Hz, 1H), 5.13 (s, 2H), 6.13 (s, 1H), 7.03–7.34 (m, 2H), 7.17–7.41 (m, 10H), 8.14 (d, J = 8.4 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H); ^{13}C NMR δ 32.67 (C-2), 48.56 (C-1), 49.41 (C-α), 54.60 (C-3), 122.71 (C-3″, C-5″), 125.53 (C-4″), 126.03 (C-2″, C-6″), 126.75 (C-4′), 127.77 (C-3′, C-5′), 128.54 (C-3″, C-5″), 128.73 (C-2′, C-3′), 128.79 (C-2″, C-6″), 137.70 (C-1″), 139.25 (C-1″), 143.38 (C-1′), 182.08 (*C*═S); $C_{29}H_{27}N_3O_2S$.

4. Biological assays

4.1. Cell culture

Ehrlich carcinoma cells were maintained for 12–14 days in Swiss mice. The tumor cell cultures were started from mouse Ehrlich ascites with at least one passage in vitro prior to use. The K562 cells were maintained in RPMI 1640 medium supplemented with fetal calf serum, 50 μM of 2-mercaptoethanol, 100 IU/mL penicillin, and 100 μg/mL streptomycin. The cultures were incubated at 37 °C in humidified atmosphere with 5% CO_2, and the culture medium was changed twice a week.

4.2. Drugs and MTT assay

The stock solutions of the ureas and thioureas were prepared in DMSO. The percentage of DMSO in assays was in maximum of 0.5% (v/v) in saline solution. The drug cytotoxicity assays were performed as previously described by Mosmann[15] using MTT for viable cell measurements. Aliquots of 5×10^5 cells/mL (Ehrlich) and 2×10^4 cells/mL (K562) were seeded onto 96-microtiter flat well plates in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin, containing the indicated concentration of the drug. All determinations were carried out in triplicate. After 48 h (Ehrlich) or 96 h (K562), at 37 °C under 5% of CO_2, the cultures were incubated with MTT (5 mg/mL) for 3 h. The formazan produced by live cells was solubilized with acidic isopropanol, and the absorbance was read at 570 nm. IC_{50} values were obtained by a linear regression analysis of the absorbance percent versus the log of the drug concentration.

4.3. Materials for DNA-relaxation

The DNA-topoisomerase I (topo I) and II-α (topo II-α) drug screening kits were from TopoGEN containing supercoiled (form I) plasmid substrate DNA (25 μg in 10 mL TE buffer). TE buffer 10 mM Tris–HCl at pH 7.5 and 1 mM EDTA), and the assay buffers (10 mM Tris–HCl pH 7.9, 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, 0.1 mM spermidine, and 5% glycerol (for topo I), and 50 mM Tris–HCl, pH 8, 120 mM KCl, 10 mM $MgCl_2$, 100 mM EDTA, 3 mg/mL bovine serum albumin, 0.5 mM dithiothreitol, and 0.5 mM ATP (for topo II-α). The DNAs utilized were supercoiled $\phi \times 174$ and *p*BR322 plasmid purchased from Sigma and Gibco, respectively. The loading buffer contained 25% bromophenol blue, 50% glycerol, and 10% SDS. The agarose and the substances utilized in these assays were purchased from Sigma.

4.4. Topo I assay

The topo I inhibition was determined by relaxation assay and was carried out as described in the TopoGEN screening kit. For topo I, one unit of the enzyme was utilized to relax 0.125 μg of the supercoiled $\phi \times 174$ plasmid DNA. The reaction mixture (10 μL) contained the drug, DNA, assay buffer, 1U of topo I, and water. The mixture was incubated at 37 °C for 30 min, and the reaction was finalized by the addition of 1 μL of dye solution containing 25% bromophenol blue, 50% glycerol, and 10% SDS.

Reaction products were loaded onto a 1% agarose gel containing ethidium bromide. Electrophoresis was carried out in Tris-acetate–EDTA, pH 8.5, at 15 V for 3.5 h and then photographed with a digital camera by illumination.

4.5. Topo II-α assay

The topo II-α inhibition was carried out as described in the TopoGEN screening kit. The reaction mixture (10 μL) contained the drug, DNA, assay buffer, 2U of topo II-α, and water. In the topo II-α assay, two units were utilized to relax 0.125 μg/mL *p*BR322 (Gibco). The mixture was incubated at 37 °C for 30 min, and the reaction was ended by the addition of 1 μL of dye

solution containing 25% bromophenol blue, 50% glycerol and 10% SDS.

The products were submitted to electrophoresis using 1% agarose gel in 1× TAE buffer (50x stock: 242 g Tris base, 57.1 mL glacial acetic acid, and 100 mL of 0.5 M EDTA) at 15 V for 3.5 h. Gels were stained with ethidium bromide (0.5 μg/mL) for 30–45 min, washed, and photographed under UV light.

4.6. Statistical analysis

The results of the MTT assays were presented as mean standard deviations of three independent experiments. Statistical significance was assessed by Student's *t*-test. A value of $P < 0.01$ was considered to show a significant difference.

5. Conclusion

In summary, the new *N*-3,3-diphenylpropyl-*N*-(*p*-X-benzyl)-*N*′-phenylureas and thioureas were synthesized in good yields. In preliminary in vitro studies of cytotoxicity the drugs were shown to be cytotoxic against Ehrlich carcinoma and K562 human leukemia cells. Moreover, the thiourea **6a** inhibited the DNA-topoisomerase I action and the ureas **5f** and **5g** showed a strong inhibition of the DNA-topoisomerase II-α activity.

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