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UTILITY OF L-NOREPHEDRINE IN THE SEMISYNTHESIS OF NOVEL THIOUREA AND THIAZOLIDINE DERIVATIVES AS A NEW CLASS OF ANTICANCER AGENTS

MOSTAFA M. GHORAB[1,2]*, SALEH I. ALQASOUMI[1], MAGED S. ABDEL-KADER[3,4] and MANSOUR S. ALSAID[1]

[1]Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Kingdom of Saudi Arabia
[2]Department of Drug Radiation Research, National Center for Radiation Research and Technology, Atomic Energy Authority, P.O.Box 29 Nasr City, Cairo, Egypt
[3]Department of Pharmacognosy, College of Pharmacy, Salman Bin AbdulAziz University, P.O. Box 173, Al-Kharj 11942, Kingdom of Saudi Arabia
[4]Department of Pharmacognosy, College of Pharmacy, Alexandria University, Alexandria 21215, Egypt

Abstract: The natural alkaloid l-norephedrine **1** was utlized in the synthesis of some novel thiourea derivatives **2**, **5** and thiazolidinones **4a,b** and **6, 7**. Structures of the synthesized compounds were confirmed by analytical and spectral data. The synthesized compounds were evaluated *in vitro* for anticancer activity against the human breast (MCF-7), human liver (HEPG2) and human colon (HCT116) cancer cell lines. Thiazolidinone derivative **7** was the most active against all the cell lines with values IC_{50} = 2.60, 2.80 and 2.60 µg/mL compared with doxorubicin (IC_{50} = 5.40, 2.97 and 5.26 µg/mL). Thiazolidinone derivative **6** exhibited higher activity with IC_{50} value (3.20 µg/mL) against HCT116 when compared with doxorubicin with IC_{50} value (5.26 µg/ mL) as positive control.

Keywords: l-norephedrine, thiourea, oxazolidine, thiazolidinones, anticancer activity

Most cancer patients are subjected to chemotherapy for the treatment of advanced cancers. However, most metastatic solid tumors eventually remain incurable even by treatment with recent anticancer drugs. Also, cancer is a disease of striking significance in the world today. It is the second leading cause of death in the world after cardiovascular diseases and it is projected to beginning the primary cause of death there within the coming years (1, 2). The identification of novel structures that can be potentially useful in designing new, potent selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers (3). Despite of the important advances achieved over recent decades in the research and development of various cancerostatic drugs, current antitumor chemotherapy still suffers from two major limitations - the first is the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, bringing about unwanted side effects. The second is the acquisition by cancer cells of multiple-drug resistance. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action (4). The current scenario highlights the need for the discovery and development of new lead compounds of simple structure, exhibiting optimal *in vivo* antitumor potency and new mechanisms of action. Recent advances in clinical techniques, including large cooperative studies, are allowing more rapid and reliable evaluation of new drugs. The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds. In this regard, it should be emphasized that National Cancer Institute (NCI) *in vitro* primary anticancer drug screen represents a valuable research tool to facilitate the drug discovery of new

* Corresponding author: e-mail: mmsghorab@gmail.com

structural/mechanistic types of antitumor agents (5). The main sources of lead compounds for drug development are natural products, new synthetic compounds and analogs of new agents (6). The thiourea derivatives (**I**) (7–9) represent one of the generally most useful classes of anticancer agents (Fig. 1). In addition, thiazolidinone template is a privileged structure fragments in modern medicinal chemistry considering its broad pharmacological spectrum and affinity for various biotargets of these class heterocyclic compounds. It is among the usually occurred heterocyclic nuclei in many marine as well as natural plant products possessing wide range of biological applications (10–12). On the other hand, thiazolidinone derivatives are well known class of biological active substances (13–15) that became basic for the whole number of innovative medicinal agents, such as hypoglycemic thiazolidinediones (pioglitazone and its analogs) (16), aldose reductase inhibitors (epalrestat) (17), dual inhibitors of COX-2/5-LOX (darbufelon) (18), modern diuretics. (etozoline) (19), Mur family inhibitors (UDP-MurNAc/L-Ala ligases) (20). Recently, thiazolidinones research area unexpectedly became interesting and promising for oncology. In depth study of PPARs allowed to put forward and validate the concept of anticancer potential existence of PPAR agonists including thiazolidinediones (21, 22). In addition, inhibitors of antiapoptotic proteins Bcl-XL and BH3 (23), which contribute to modulation of programmed cell death (apoptosis), as well as inhibitors of tumor necrosis factor TNFα (24), necroptosis inhibitors (25), integrin antagonists (26), inhibitors of JSP-1 (27), Pim-2 and Pim-1 protein kinases (28), COX-2 (29) were identified among 4-thiazolidinones. Figure 1 presents «hit-compounds» (**I** – **IV**) from different groups (30, 31) that possess high antimitotic effect *in vitro* in submicromolar concentrations (10, 5, 10 and 7 M, respectively) and are characterized by the low *in vivo* toxicity level.

Since 1990, we have been working on the synthesis of polycyclic systems containing quinoline, quinazoline and thienopyrimidine nucleus with a biologically active sulfonamide moiety in order to test their anticancer and radiosensitizing activities (32–38). In the light of these facts, and as a continuation of reported work (39, 40), we planned to synthesize novel thiourea and thiazolidinone derivatives by using l-norephedrine (phenylpropanolamine) **1** as starting material to evaluate their anticancer activities hoping to obtain compounds with significant anticancer potential.

EXPERIMENTAL

Chemistry

Reagents were obtained from commercial suppliers and were used without purification. Melting points were determined in open capillary tubes using Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus, and are uncorrected. Elemental analyses (C, H, N) were per-

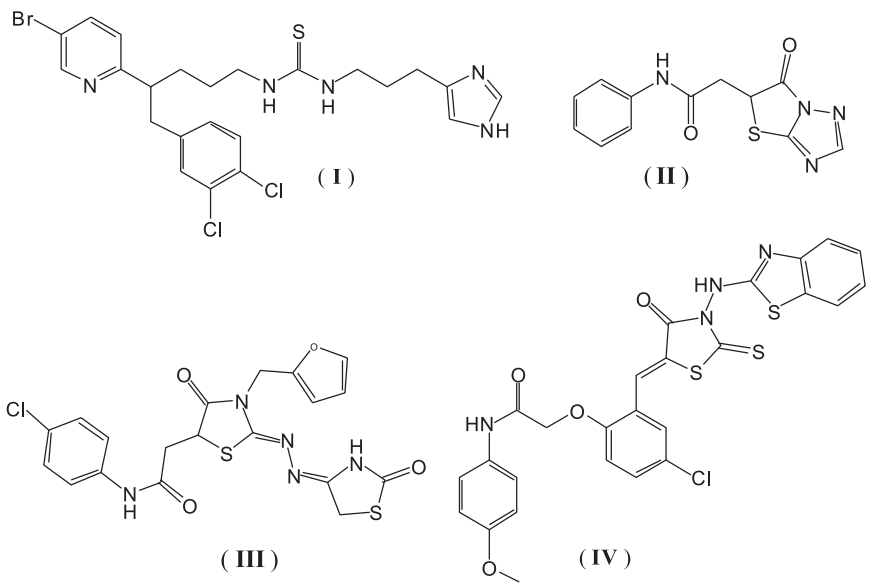


Figure 1. Structures of some anticancer lead compounds from the literature

formed on a Perkin- Elmer 2400 Instrument (USA). All results were within ±0.4% of the theoretical values. Infrared (IR) spectra (KBr disc) were recorded on FT-IR spectrophotometer (Perkin Elmer) at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. ^{1}H and ^{13}C NMR spectra were recorded on a UltraShield Plus 500 MHz (Bruker) (NMR Unite, College of Pharmacy, Salman Bin Abdulaziz University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the residual solvent peak, the coupling constants (*J*) are reported in Hertz (Hz). 2D-NMR experiments (COSY, NOESY, HSQC and HMBC) were obtained using standard Bruker programs. Mass spectra were run using a HP Model MS-5988 (Hewlett Packard).

1-Ethyl-3-(1-hydroxy-1-phenylpropan-2-yl)thiourea (2) and 4-methyl-5-phenyloxazolidine-2-thione (3b)

l-Norephedrine (151 mg, 1 mmol) was added to a solution of ethyl isothiocyanate (87 mg, 1 mmol) dissolved in chloroform (20 mL) containing triethylamine (101 mg, 1 mmol). The reaction mixture was stirred for 5 min at room temperature. The solution was evaporated to dryness under reduced pressure and chromatographed on silica gel column (2 mm i.d., 30 g) eluted with dichloromethane. Polarity was increased with methanol in a gradient system. Fractions of 50 mL were collected, screened by TLC and similar fractions were pooled. Fractions 3–5 afforded 26 mg of **3b**. Fractions 8–10 were further purified on silica gel PTLC using CH_2Cl_2/MeOH 95 : 5 (v/v) as developing system to yield 135 mg of **2**.

2: Yield 68%; semisolid; IR (KBr, cm^{-1}): 3483 (OH), 3387, 3219 (2NH), 3087 (CH arom.), 2977, 2836 (CH aliph.), 1287 (C=S). ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 0.86 (d, *J* = 6.7 Hz, 3H, CH_3 of l-norephedrine), 1.08 (t, *J* = 7.2 Hz, 3H, NH-CH_2-C$\underline{H}_3$), 3.41 (NH-C$\underline{H}_2$-CH_3, signal partially obscured by H_2O signal), 4.47 (bs, 1H, NH-C$\underline{H}$- of l-norephedrine), 4.87 (bs, 1H, C$\underline{H}$-OH), 5.47 (bs, 1H, CH-O$\underline{H}$), 7.22–7.52 (m, 6H, aromatic and NH of l-norephedrine), 7.46 (t, *J* = 4.5 Hz, 1H, N$\underline{H}$-CH_2-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 12.68, 14.42, 38.77, 54.72, 73.43, 125.69–127.81 (5), 143.49, 181.31. MS m/z (%): 238 (12.8, M$^+$). Analysis: calcd. for $C_{12}H_{18}N_2OS$: C, 60.47; H, 7.61; N, 11.75%; found: C, 60.12; H, 7.39; N, 11.98%.

3b: Yield 29%, m.p. 90–92^{o}C, IR (KBr; cm^{-1}): 3280 (NH), 3099 (CH arom.), 2956, 2871 (CH aliph.), 1280 (C=S). ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 0.65 (d, *J* = 5.8 Hz, 3H, CH_3), 4.42 (bs, 1H, NH-C$\underline{H}$-CH_3), 6.00 (d, *J* = 8.5 Hz, 1H, -C$\underline{H}$-O), 7.25–7.42 (m, 5H, arom.), 10.20 (bs, 1H, NH) . ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 16.04, 54.89, 84.87, 126.10–128.41 (5), 134.82, 187.44. MS m/z (%): 193 (96.0, M$^+$). Analysis: calcd. for $C_{10}H_{11}NOS$: C, 62.15; H, 5.74; N, 7.25%; found: C, 62.44; H, 5.51; N, 7.61%.

2-(Ethylimino)-3-(1-hydroxy-1-phenylpropan-2-yl)thiazolidin-4-one (4a) and 3-ethyl-2-(1-hydroxy-1-phenylpropan-2-ylimino)thiazolidin-4-one (4b)

A solution of chloroacetyl chloride (70 mg, 0.6 mmol) in 15 mL of chloroform was added dropwise to a stirred solution of **2** (119 mg, 0.5 mmol) and triethylamine (60 mg, 0.6 mmol). The reaction was stirred overnight and then the mixture was evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column (2 mm i.d., 20 g) eluted with dichloromethane. Polarity was increased with methanol in a gradient system. Fractions 4–7 afforded 7 mg of **4a**, while fractions 9–15 afforded 54 mg of **4b**.

4a: Yield 19%; semisolid, IR (KBr, cm^{-1}): 3455 (OH), 3100 (CH arom.), 2960, 2848 (CH aliph.), 1699 (C=O), 1612 (C=N). ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 1.21 (m, 6H, 2×CH_3), 3.34 (bs, 2H, S-C$\underline{H}_2$-C=O), 3.88 (q, *J* = 8.4 Hz, 2H, N-C$\underline{H}_2$-CH_3), 4.80 (bs, 1H, N-CH- of l-norephedrine), 5.02 (bs, 1H, C$\underline{H}$-OH), 7.26–7.46 (m, 5H, arom.). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 12.26, 15.49, 32.66, 46.62, 58.65, 75.05, 126.05–128.32 (5), 142.06, 171.51. MS m/z (%): 278 (26.2, M$^+$). Analysis: calcd. for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06%; found: C, 60.18; H, 6.88; N, 10.27%.

4b: Yield 61%; semisolid, IR (KBr, cm^{-1}): 3412 (OH), 3068 (CH arom.), 2969, 2855 (CH aliph.), 1702 (C=O), 1608 (C=N). ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 0.98 (m, 6H, 2×CH_3), 3.45 (bs, 1H, NH-C$\underline{H}$-CH_3), 3.60 (m, 2H, 2×CH_2), 4.47 (bs, 1H, N-CH- of l-norephedrine), 4.61 (bs, 1H, C$\underline{H}$-OH), 4.87 (bs, 1H, C$\underline{H}$-OH), 7.17–7.27 (m, 5H, arom.). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 12.28, 15.15, 32.64, 37.98, 63.16, 77.19, 126.61–128.14 (5), 141.21, 151.65, 171.44. MS m/z (%): 278 (18.3, M$^+$). Analysis: calcd. for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06%; found: C, 60.67; H, 6.28; N, 9.71%.

1-Cyclohexyl-3-(1-hydroxy-1-phenylpropan -2-yl) thiourea (5)

l-Norephedrine **1** (151 mg, 1 mmol) was added to a solution of cyclohexyl isothiocyanate (141 mg,

1 mmol) dissolved in chloroform (20 mL) containing triethylamine (101 mg, 1 mmol). The reaction mixture was stirred for 10 min at room temperature. The solution was evaporated to dryness under reduced pressure and loaded on the top of silica gel column (2 mm i.d., 30 g) eluted with dichloromethane. Fractions of 50 mL were collected, screened by TLC and similar fractions were pooled. Fractions 3–6 afforded 170 mg of **5**.

5: Yield 93%; m.p. 124–125°C, IR (KBr, cm^{-1}): 3446 (OH), 3327, 3276 (2NH), 3088 (CH arom.), 2949, 2868 (CH aliph.), 1288 (C=S). ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 0.84 (d, *J* = 6.7 Hz, 3H), 1.16–1.86 (m, 10H), 4.01 (bs, 1H, C<u>H</u>- cyclohexyl), 7.21–7.42 (m, 7H, arom., 2NH), 4.47 (bs, 1H, C<u>H</u>-NH l-norephedrine); 4.87 (bs, 1H, C<u>H</u>-OH), 5.50 (bs, 1H, CH-O<u>H</u>). ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 12.51, 24.35 (2), 25.19, 32.34 (2), 51.48, 54.57, 73.36, 125.63–127.83 (5), 143,54,181.57. MS m/z (%): 292 (6.4, M^+). Analysis: calcd. for $C_{16}H_{24}N_2OS$: C, 65.71; H, 8.27; N, 9.58%; found: C, 65.48; H, 8.52; N, 9.26%.

2-(Cyclohexylimino)-3-(1-hydroxy-1-phenylpropan-2-yl)-thiazolidin-4-one (6) and 2-(3-cyclohexyl-4-oxothiazolidin-2-ylideneamino)-1-phenylpropyl-2-chloroacetate (7)

A solution of chloroacetyl chloride (70 mg, 0.6 mmol) in chloroform (15 mL) was added dropwise to a stirred solution of **5** (146 mg, 0.5 mmol) and triethylamine (60 mg, 0.6 mmol). The reaction was stirred overnight and then the reaction mixture was evaporated to dryness under reduced pressure, The residue was chromatographed on a silica gel column (2 mm i.d., 30 g) eluted with dichloromethane. Polarity was increased with methanol in a gradient system. Fractions 3–5 afforded 94 mg of **7**, while fractions 7–11 afforded 35 mg of **6**.

6: Yield 23%; semisolid**,** IR (KBr, cm^{-1}): 3493 (OH), 3094 (CH arom.), 2944, 2863 (CH aliph.), 1689 (C=O), 1598 (C=N). ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 1.16 (d, *J* = 6.5 Hz, 3H), 1.17–1.72 (m, 10H), 3.08 (bs, 1H, C<u>H</u>- cyclohexyl), 3.79 (q, *J* = 10.5 Hz, 2H, O=C-C<u>H$_2$</u>-S), 4.71 (d, *J* = 5.5 Hz, 1H, C<u>H</u>-NH l-norephedrine); 4.94 (bs, 1H, C<u>H</u>-OH), 7.17–7.37 (m, 5H, arom.). ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 9.16, 24.37 (2), 25.49, 33.13 (2), 33.37, 58.60, 61.29, 75.11, 126.06–128.20 (5), 142.01, 169.98. MS m/z (%): 332 (18.5, M^+). Analysis: calcd. for $C_{18}H_{24}N_2O_2S$: C, 65.03; H, 7.28; N, 8.43%; found: C, 64.76; H, 7.54; N, 8.77%.

7: Yield 74%; semisolid, IR (KBr, cm^{-1}): 3079 (CH arom.), 2981, 2836 (CH aliph.), 1710, 1688 (2C=O), 1618 (C=N), 786 (C-Cl).^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 1.08 (d, *J* = 6.2 Hz, 3H), 1.17–1.71 (m, 10H), 3.57 (d, *J* = 5.5 Hz, 1H, C<u>H</u>-NH l-norephedrine), 4.06 (m, 4H, O=C-C<u>H$_2$</u>-S and Cl-C<u>H$_2$</u>-C=O), 4.94 (bs, 1H, C<u>H</u>- cyclohexyl), 5.76 (bs, 1H, C<u>H</u>-OH), 7.17–7.30 (m, 5H, arom.). ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 17.13, 24.35 (2), 25.70, 32.37 (2), 33.47, 41.05, 53.10, 55.65, 61.33, 80.77, 127.62–128.81 (5), 136.44, 166.35, 171.61. MS m/z (%): 408 (15.2, M^+). Analysis: calcd. for $C_{20}H_{25}ClN_2O_3S$: C, 58.74; H, 6.16; N, 6.85%; found: C, 58.64; H, 6.51; N, 6.49%.

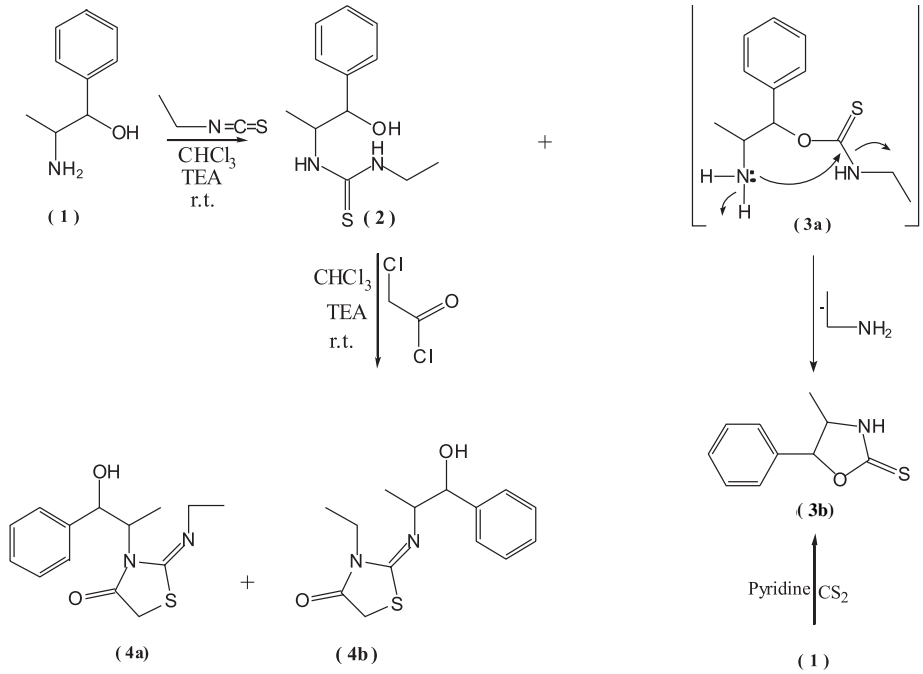
***In vitro* antitumor activity**

The cytotoxic activity was measured *in vitro* for the newly synthesized compounds using the sulforhodamine B stain (SRB) assay using the method of Skehan et al. (41). The *in vitro* anticancer screen-

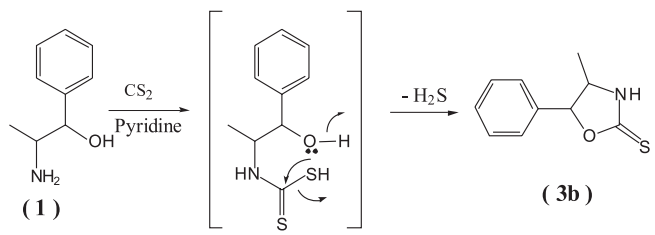
Table 1. *In vitro* anticancer screening of the newly synthesized compounds against human breast (MCF-7), liver (HEPG2), and colon (HCT 116) cancer cell lines.

Compd. No.	IC_{50} (μg/mL)[a]		
	MCF-7	HEPG2	HCT116
Doxorubicin	5.40	2.97	5.26
2	24.00	36.20	40.80
3b	43.00	37.40	41.80
4a	NA	NA	NA
4b	34.80	32.80	27.00
5	41.40	28.60	25.00
6	6.80	NA	3.20
7	2.60	2.80	2.60

[a]: IC_{50} value: Concentration causing 50% inhibition of cell viability. NA = No activity.



Scheme 1. Synthesis of oxazolidine and thiazolidinone derivatives



Scheme 2. Postulated mechanism for the formation of compound **3b**

ing was done at the Pharmacology Unit, the National Cancer Institute, Cairo University. Cells were plated in 96 multiwall microtiter plate (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (5, 12.5, 25 and 50 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an enzyme-linked immunosrbent assay ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time (41). The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1.

The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF-7), (HepG2) and (HCT 116). The response parameter calculated was IC_{50} value, which corresponds to the

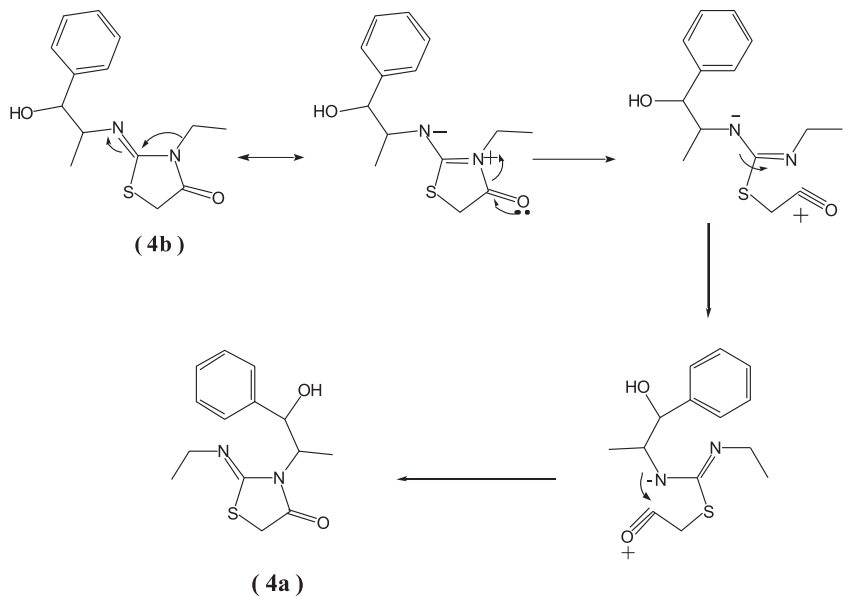
concentration required for 50% inhibition of cell viability.

RESULTS AND DISCUSSION

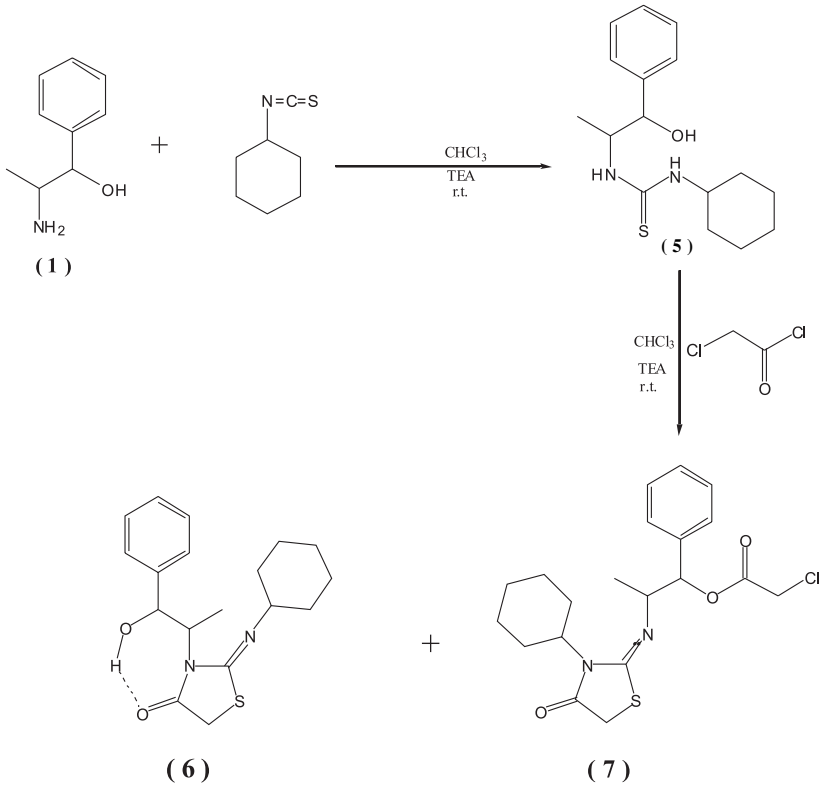
Chemistry

The compounds were designed in order to explore their anticancer activity. The behavior of the natural alkaloid l-norephedrine **1** with both nitrogen and oxygen nucleophiles toward isothiocyanate was studied. Thus, when **1** (2-amino-1-phenylpropan-1-ol) was allowed to react with ethyl isothiocyanate in chloroform in the presence of triethylamine as catalyst at room temperature for 5 min, the corresponding 1-ethyl-3-(1-hydroxy-1-phenylpropan-2-yl)-thiourea **(2)** and 4-methyl-5-phenyloxazolidine-2-thione **3b** (42) were obtained (Scheme 1). Compound **3b** was produced *via* initial formation of intermediate **3a** followed by intermolecular cyclization with elimination of ethylamine (Scheme 1, 2). Also, **3b** was obtained in one step under different conditions *via* reaction of **1** with carbon disulfide in pyridine (m.p. = 90–92°C as reported) (Scheme 1). The structures of **2** and **3b** were elucidated from elemental analysis and spectral data. The IR spectra of **2** showed the absence of NH_2 bands and the presence of characteristic bands for OH, NH and C=S. Its ^{1}H-NMR spectrum showed $\underline{CH_3}$-CH_2-NH of ethyl isothiocyanate, which appeared at δ_H 1.07 (t) ppm, δ_C 14.42 ppm, CH_3-$\underline{CH_2}$-NH δ_H 3.40 (m) ppm, δ_C 38.18, CH_3-CH_2-$\underline{NH}$ δ_H 7.46 (t) ppm. These assignments were based on COSY, HSQC and HMBC experiments. Further support for the structure of **2** was the carbon signal at δ_C 181.49 ppm assigned for the C=S carbon. The secondary alcohol group of 2-amino-1-phenylpropan-1-ol showed signals at δ_H 4.87 (bs) ppm and δ_C 73.44 ppm in ^{1}H- and ^{13}C NMR, respectively. The hydroxyl proton was assigned to the signal at δ_H 5.47 (bs) ppm based on the COSY correlation with the C$\underline{H}$-O proton at δ_H 4.87 ppm. The oxazolidine-2-thione moiety in **3b** was evident from signals at δ_H 6.01 (d); δ_C 84.87 (CH-O-), δ_H 4.42 (bs); δ_C 54.89 ($\underline{CH}$-NH) and δ_H 10.20 (bs) ppm (CH-$\underline{NH}$) and the disappearance of the OH signal.

Subsequent synthesis of 2-imino-4-thiazolidinones **4a,b** was performed by condensation of thiourea **2** with chloroacetyl chloride in the presence of triethylamine in $CHCl_3$ at room temperature. The reaction provided a mixture of two isomers: ethylimino **4a** and hydroxyphenylpropaneimino **4b** in about 1 : 3 ratio after 15 min. The ratio was maintained when the reaction was left overnight and monitored by TLC. They conceivably originated from the condensation of chloroacetyl chloride with the sulfur atom of two different intermediate thiols generated from **2** by delocalization of the lone pairs of the two different nitrogen atoms on the adjacent thiocarbonyl group. The synthesis of the isomer **4b** was favored by the intermediate thiol involving the NH group adjacent to the electron releasing group.



Scheme 3. Rearrangement of **4b** into **4a**



Scheme 4. Formation of thiourea and thiazolidinone derivatives

On the other hand, when the reaction was performed in MeOH at reflux, both isomers were present with notable decrease of **4b** by time based on TLC study. After 24 h, the most stable isomer **4a** was the only product indicating that **4b** converted to **4a**. The suggested mechanism of the conversion of **4b** into **4a** is depicted in Scheme 3. The extended electronic delocalization of amidine system gave rise to the cleavage of the cyclic amide bond. A possible low barrier around the C-S σ bond and the subsequent cyclization accounted for the intermolecular rearrangement providing the most stable compound **4a**.

The structures of **4a,b** were supported based on elemental analysis, IR, ^{1}H-, ^{13}C-NMR and mass spectral data. The IR spectrum of **4a** exhibited the absence of NH band and the presence of characteristic bands for OH and C=O. In both compounds, the groups adjacent to the newly formed C=N resonate at different chemical shifts due to the increased deshielding effect generated by the extended electronic delocalization of the N lone pair. The ^{1}H- and ^{13}C-NMR spectra of **4a** indicated that the resonances of the –$\underline{CH_2}$-N- group of ethyl isothiocyanate was shifted to δ_H 3.88 (q) and δ_C 46.62 ppm. In **4b,** the shift was more evident at the 2-amino-1-phenylpropan-1-ol moiety. The =N-CH(CH_3)-CH-OH signals appeared at δ_H 3.45; δ_C 63.16 (=N-CH), δ_H 0.98 (d); δ_C 12.28 (CH_3), δ_H 4.61 (bs); δ_C 77.13 (CH-OH) ppm. On the other hand, when **1** was allowed to react with cyclohexyl isothiocyanate in $CHCl_3$ in the presence of triethylamine as catalyst at room temperature for 15 min, the corresponding 1-cyclohexyl-3-(1-1-hydroxy-1-phenylpropan-2-yl) thiourea **5** was obtained in good yield (Scheme 4). Structure of compound **5** was supported on the basis of elemental analysis, IR, ^{1}H-, ^{13}C-NMR and mass spectral data. Its IR spectrum exhibited the absence of NH_2 band, and the presence of a characteristic bands for NH, OH and C=S. ^{1}H-NMR showed signals at d_H 7.21–7.42 ppm corresponding for five aromatic protons and δ_H 1.16–1.86 ppm assigned to ten cyclohexyl protons. The $\underline{CH}$- of cyclohexyl appeared at δ_H 4.01 (bs); δ_C 51.48 ppm, while that of 2-amino-1-phenylpropan-1-ol appeared at δ_H 4.47 (bs); δ_C 54.57 ppm in both ^{1}H- and ^{13}C NMR. The CH- of cyclohexyl appeared at δ_H 7.34, while that of 2-amino-1-phenylpropan-1-ol appeared at δ_H 7.23 ppm. These assignments were based on COSY, HSQC and HMBC experiments.

Further support for the thiourea derivative **5** was the carbon signal at δ_C 181.57 ppm assigned for the C=S carbon. One of the characteristic groups of the structure is the secondary alcohol group of 2-amino-1-phenylpropan-1-ol showed signals at d_H 4.87 (bs); δ_C 73.36 ppm in both ^{1}H- and ^{13}C-NMR spectra. The hydroxyl proton was assigned to the signal at δ_H 5.50 (bs) ppm based on the COSY correlation with the C<u>H</u>-O proton at δ_H 4.87 ppm. Subsequent synthesis of 2-imino-4-thiazolidinones **6, 7** was performed by condensation of thiourea **5** with chloroacetyl chloride in chloroform in the presence of triethylamine at room temperature (Scheme 4). The structure of isomers **6** and **7** was assessed by elemental analysis, IR, ^{1}H- and ^{13}C-NMR. In compound **6**, the resonances of the -<u>CH</u>-NH of cyclohexyl was shifted to δ_H 3.08 (bs); δ_C 60.23 ppm. The hydroxyl proton appears at δ_H 4.94 (bs) based on its COSY correlation with the C<u>H</u>-O proton at δ_H 4.71 ppm. In compound **7**, the shifts were more evident at the 2-amino-1-phenylpropan-1-ol moiety. The =N-CH(CH_3)-CH-OH signals appeared at δ_H 3.57; δ_C 61.33 (=N-CH), δ_H 1.08 (d); δ_C 17.13 (CH_3), δ_H 5.57 (bs); δ_C 80.77 (CH-OH) ppm. In both compounds the groups adjacent to the newly formed C=N resonate at different chemical shift due to the increased deshielding effect generated by the extended electronic delocalization of the N lone pair. In the spectrum of **7** the disappearance of the OH proton, the down field shift of the C<u>H</u>-OH, the additional CH_2 signal at δ_H 4.06; δ_C 41.05 ppm and the carbonyl signal at δ_C 166.31 ppm were diagnostic for further acylation of the OH group. The inability of the hydroxyl group in **6** to react with chloroacetyl chloride under the same conditions as compound **7** is most likely due to the formation of hydrogen boding with the carbonyl of thiazolidinone ring (Scheme 4).

In vitro antitumor activity

The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line (MCF-7), human liver cancer cell line (HEPG2) and human colon cancer cell line cell line (HCT 116). Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of cancer cell lines. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the *in vitro* cytotoxic activity of the synthesized compounds, where the thiazolidinone derivative **7** having the cyclohexyl moiety at 3-position with 1-hydroxyl-1-phenylpropane-imino moiety at 2-position was the most active compound against all cancer cell lines with IC_{50} values (2.60, 2.80, 2.60 µg/mL) compared with the doxorubicin with IC_{50} value (5.40, 2.97, 5.26 µg/mL). On the other hand, thiazolidinone derivative **6** carrying the cyclohexylimino moiety at 2-position with 1-hydroxy-1-phenylpropane at 3-position exhibited higher activity against the HCT 116 with IC_{50} value (3.20 µg/mL) compared with doxorubicin with IC_{50} value (5.26 µg/mL) and moderate activity less than doxorubicin with IC_{50} value (6.80 µg/mL) against MCF-7.

CONCLUSION

The objective of the present study was to semi-synthesize and investigate the anticancer activity of some novel thiourea and thiazolidinone derivatives carrying the biologically active cyclohexyl and cyclohexylimino moieties. Compounds **7** showed promising anticancer activity higher than that of doxorubicin as reference drug against all the tested cancer cell lines, while compound **6** exhibited higher activity against colon cancer cell line and more active than that of doxorubicin. Also, compound **6** is nearly as active as doxorubicin as positive control against breast cancer cell line.

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