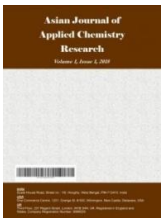

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Synthesis of New Thiosemicarbazone Derivatives Substituted with Heterocycles with Potential Antitumor Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JGL and PSB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DSSS, MDR and KMM managed the analyses of the study. Author TGS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To find new thiosemicarbazone derivatives with antitumor activity and good physico-chemical parameters.
Study Design: A preliminary SAR study with pyrrolidinyl, piperidinyl, and morpholinyl heterocycles as well as hydrogen, alkyl and phenyl groups in the thiosemicarbazone scaffold.
Place and Duration of Study: Department of Pharmaceutical Sciences and Department of Antibiotics, from Federal University of Pernambuco (Brazil), between January 2016 and February 2017.
Methodology: The thiosemicarbazones were synthesized through a condensation reaction between benzaldehyde substituted with heterocycles and several thiosemicarbazides, using a catalytic amount of hydrochloric acid. The new compounds were characterised using classical spectroscopic and spectrometric techniques. The cytotoxicity were evaluated using MTT assay

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against five cancer cell lines and doxorubicin as reference compound. The LogP was also predicted using the software MarvinSketch 17.4.3 and promising results were found.
Results: We found five new thiosemicarbazone derivatives with excellent cytotoxicity.
Conclusion: Thiosemicarbazone derivatives are promising compounds in medicinal chemistry field and certainly should be considered as scaffold for drug candidates.

Keywords: Cancer; cytotoxicity; thiosemicarbazones; heterocycles.

1. INTRODUCTION

Cancer is the uncontrolled growth and spread of cells and its represents one of the main causes of mortality worldwide [1]. Over the years, the number of successful therapies for cancer have increased. The modality of new drugs in cancer is changing and the development of small molecules lose ground to alternatives therapies, such monoclonal antibodies. However, small molecules remain the main way of generating new drugs [2]. Thiosemicarbazones (TSC) are an important class with wide pharmacological action due to its versatility as chelating agents, forming complexes with enzyme metals and participating in redox reactions [3]. For many years, thiosemicarbazone derivatives have provided an efficient resource for the discovery of potential anticancer agents [4,5].

In a previous work of our group, new TSC compounds were synthesized and showed cytotoxicity against the cell lines tested [6,7]. Continuing this work, we purpose new modifications expecting to find new active compounds. Therefore, in this paper, we describe the synthesis and preliminary anticancer studies of new TSC compounds (Scheme 1). To explore a primary structure-activity relationship we utilise the pyrrolidinyl, piperidinyl, and morpholinyl heterocycles as well as hydrogen, alkyl and phenyl groups in the thiosemicarbazone scaffold in order to identify the substituent features that are favourable to the cytotoxicity activity. In addition, the cyclic secondary amines have been added to the thiosemicarbazones to tune the hydrophobicity/ hydrophilicity of the whole molecule [8].

2. MATERIALS AND METHODS

2.1 General

All reagents were commercially available and were used without further purification. All melting points were determined using a Quimis model Q340S dry apparatus and are uncorrected. Infrared (IR) spectra (v, cm^{-1}) in KBr were recorded on a Shimadzu mini-UV model 1240. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained from Unity Plus, Varian, 300 and 75 MHz, respectively. The indicated chemical shifts (δ), expressed in ppm, were measured in relation to tetramethylsilane, used as internal reference. The multiplicities of the signals were designated as follows: singlet (s), doublet (d), triplet (t), quintuplet (q), and multiplet (m). The high-resolution mass spectrometry (HRMS) was obtained using a hybrid mass spectrometer, model micrOTOF-Q II (Bruker Daltonics), where the electrospray ionization (ESI) technique was used in the positive mode.

2.2 General Synthetic Procedure: Preparation of *p*-heteroaryl-substituted Benzaldehyde Derivatives 5-7

To a solution containing 0.2 mmol of K_2CO_3 and 20 mL of dimethylformamide (DMF), 0.15 mmol of the cyclic secondary amine **2-4** (pyrrolidine, piperidine and morpholine) and 0.1 mmol of 4-fluorobenzaldehyde **1** were added. The mixture was kept under constant stirring and heated to 90 °C until the end of the reaction, confirmed by TLC. Then, the final solution was cooled to room temperature. To obtain **5-6**, ice was added for precipitation, with further filtration. To obtain **7**, ethyl acetate extraction was carried out with subsequent rotavaporation. The obtained compounds were used in the next step without purification.

4-(pyrrolidin-1-yl)benzaldehyde **5**

Yellow powder. Reaction time: 8 h. Rf 0.53 (Hex./EtOAc 1:1). Mp: 83-84ºC. IR (KBr) v_{max} (cm^{-1}) 1669 (C=O), 1167 (C-N).

4-(piperidin-1-yl)benzaldehyde **6**

Yellow powder. Reaction time: 8 h. Rf 0.48 (Hex./EtOAc 7:3). Mp: 62-63ºC. IR v_{max} (cm^{-1}) 1664 (C=O), 1158 (C-N).

4-(morpholin-4-yl)benzaldehyde **7**

White powder. Reaction time: 22 h. Rf 0.59 (Hex./EtOAc 1:9). Mp 51-52ºC. IR v_{max} (cm^{-1}) 1658 (C=O), 1173 (C-N).

2.3 General Synthetic Procedure: Preparation of Thiosemicarbazones 12-23

To a solution containing 0.1 mmol of the respective thiosemicarbazide **8-11** in 20 mL of ethanol was added 0.11 mmol of benzaldehyde previously synthesized **5-7** and 2 drops of hydrochloric acid as catalyst. The mixture was kept under constant stirring and heated at reflux temperature until the end of the reaction, confirmed by TLC. The final solution was cooled to room temperature and the obtained precipitate was filtered and purified in an appropriated methodology. The data for compound **12-23** are described below:

(*E*)-2-(4-(pyrrolidin-1-yl)benzylidene)hydrazine-1-carbothioamide **12**

Yellow powder. Reaction time: 3 h. Rf 0.51 ($CHCl_3$/EtOH 10:1). Purification: Washing in $CHCl_3$. Yield: 87%. Mp: 211-212 ºC. IR (KBr) v_{max} (cm^{-1}) 3366, 3346, 3253 (N-H), 1596 (C=N), 1389 (C-N), 1182 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.95 (4H, t, CH_2), 3.26 (4H, t, CH_2), 6.54 (2H, d, Ar-H), 7.57 (2H, d, Ar-H), 7.73 (H, s, C-H), 7.92 (2H, s, NH_2), 11.14 (H, s, N-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 24.94, 47.21, 111.43, 120.70, 128.75, 143.57, 148.76, 176.87. HRMS ($[M+H]^+$ calcd for $C_{12}H_{17}N_4S^+$ ($[M+H]^+$) 249.1168, found 249.1168.

(*E*)-*N*-methyl-2-(4-(pyrrolidin-1-yl) benzylidene)hydrazine-1-carbothioamide **13**

Yellow powder. Reaction time: 3 h. Rf 0.59 ($CHCl_3$/EtOH 20:1). Purification: Washing in $CHCl_3$. Yield: 88%. Mp: 224-226 ºC. IR (KBr) v_{max} (cm^{-1}) 3272, 3110 (N-H), 1617 (C=N), 1255 (C-N), 1179 (C=S). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 2.02 (4H, q, CH_2), 3.24 (3H, d, CH_3), 3.33 (4H, t, CH_2), 6.54 (2H, d, Ar-H), 7.41 (H, s, C-H), 7.50 (2H, d, Ar-H), 7.69 (H, s, N-H), 9.32 (H, s, N-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 25.44, 31.07, 47.54, 111.56, 119.97, 128.92, 143.84, 149.37, 177.60. HRMS ($[M+H]^+$ calcd for $C_{13}H_{19}N_4S^+$ ($[M+H]^+$) 263.1325, found 263.1329.

(*E*)-*N*-ethyl-2-(4-(pyrrolidin-1-yl)benzylidene)hydrazine-1-carbothioamide **14**

Yellow powder. Reaction time: 4 h. Rf 0.60 ($CHCl_3$/EtOH 20:1). Purification: Recrystallization in EtOH. Yield: 81%. Mp: 214-216ºC. IR (KBr) v_{max} (cm^{-1}) 3309, 3173 (N-H), 1600 (C=N), 1383 (C-N), 1175 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.14 (3H, t, CH_3), 1.95 (4H, t, CH_2), 3.27 (4H, t, CH_2), 3.58 (2H, q, CH_2), 6.55 (2H, d, Ar-H), 7.58 (2H, d, Ar-H), 7.92 (H, s, C-H), 8.28 (H, t, N-H), 11.12 (H, s, N-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 14.76, 24.92, 38.10, 47.22, 111.39, 120.77, 128.68, 143.08, 148.71, 175.90. HRMS ($[M+H]^+$ calcd for $C_{14}H_{21}N_4S^+$ ($[M+H]^+$) 277.1481, found 277.1480.

(*E*)-*N*-phenyl-2-(4-(pyrrolidin-1-yl)benzylidene)hydrazine-1-carbothioamide **15**

Yellow powder. Reaction time: 4.5 h. Rf 0.64 ($CHCl_3$/EtOH 20:1). Purification: Recrystallization in $CHCl_3$. Yield: 58%. Mp: 206-208ºC. IR (KBr) v_{max} (cm^{-1}) 3288, 3152 (N-H), 1589 (C=N), 1386 (C-N), 1179 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.96 (4H, t, CH_2), 3.28 (4H, t, CH_2), 6.57 (2H, d, Ar-H), 7.18 (H, d, Ar-H), 7.35 (2H, d, Ar-H), 7.62 (2H, d, Ar-H), 7.69 (2H, d, Ar-H), 8.04 (H, s, C-H), 9.90 (H, s, N-H), 11.56 (H, s, N-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 24.94, 47.22, 111.40, 120.45, 124.91, 125.37, 127.94, 129.17, 139.21, 144.20, 148.91, 174.73. HRMS ($[M+H]^+$ calcd for $C_{18}H_{21}N_4S^+$ ($[M+H]^+$) 325.1481, found 325.1481.

(*E*)-2-(4-(piperidin-1-yl)benzylidene)hydrazine-1-carbothioamide **16**

Pink powder. Reaction time: 4 h. Rf 0.55 (Hex./EA 1:1). Purification: Washing in $CHCl_3$. Yield: 60%. Mp: 197-200ºC. IR (KBr) v_{max} (cm^{-1}) 3349, 3278, 3174 (N-H), 1695 (C=N), 1355 (C-N), 1249 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.69 (2H, s, CH_2), 1.98 (4H, s, CH_2), 3.50 (4H, t, CH_2), 7.79 (2H, s, Ar-H), 7.93 (2H, d, Ar-H), 8.08 (2H, s, NH_2), 8.26 (H, s, CH), 11.52 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 24.39, 25.38, 33.37, 48.74, 110.00, 114.84, 123.58, 129.46, 153.12, 156.57, 174.60. HRMS ($[M+H]^+$ calcd for $C_{12}H_{19}N_4S^+$ ($[M+H]^+$) 263.1325, found 263.1125.

(*E*)-*N*-methyl-2-(4-(piperidin-1-yl)benzylidene) hydrazine-1-carbothioamide **17**

Pink powder. Reaction time: 4 h. Rf 0.51 (Hex./EA 3:2). Purification: Washing in $CHCl_3$. Yield: 80%. Mp: 203-204ºC. IR (KBr) v_{max} (cm^{-1}) 3249, 3115 (N-H), 1533 (C=N), 1293 (C-N), 1239 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.65 (2H, s, CH_2), 1.95 (4H, s, CH_2), 3.01 (3H, d, CH_3), 3.47 (4H, t, CH_2), 7.77 (2H, s, Ar-H), 7.93

(2H, d, Ar-H), 8.04 (H, s, C-H), 8.56 (H, d, NH), 11.53 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 21.34, 23.16, 30.78, 54.32, 120.50, 121.06, 128.36, 140.19, 144.99, 177.71. HRMS ($[M+H]^+$ calcd for $C_{14}H_{23}N_4S^+$ ($[M+H]^+$) 277.1481, found 277.1497.

(*E*)-*N*-ethyl-2-(4-(piperidin-1-yl)benzylidene) hydrazine-1-carbothioamide **18**

Pink powder. Reaction time: 5 h. Rf 0.57 (Hex./EA 3:2). Purification: Washing in $CHCl_3$. Yield: 80%. Mp: 203-204ºC. IR (KBr) ν_{max} (cm^{-1}) 3255, 3122 (N-H), 1527 (C=N), 1307 (C-N), 1188 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.13 (3H, t, CH_3), 1.63 (2H, s, CH_2), 1.92 (4H, s, CH_2), 3.45 (4H, t, CH_2), 3.58 (2H, q, CH_2), 7.72 (2H, s, Ar-H), 7.90 (2H, d, Ar-H), 8.02 (H, s, CH), 8.56 (H, t, NH), 11.44 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 15.05, 21.93, 23.74, 38.74, 54.62, 120.26, 120.83, 128.92, 140.89, 146.07, 177.15. HRMS ($[M+H]^+$ calcd for $C_{15}H_{25}N_4S^+$ ($[M+H]^+$) 291.1638, found 291.1645.

(*E*)-*N*-phenyl-2-(4-(piperidin-1-yl)benzylidene)hydrazine-1-carbothioamide **19**

Pink powder. Reaction time: 7 h. Rf 0.47 (Hex./EA 3:2). Purification: Washing in $CHCl_3$. Yield: 76%. Mp: 184-186ºC. IR (KBr) ν_{max} (cm^{-1}) 3285, 3077 (N-H), 1530 (C=N), 1322 (C-N), 1183 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.69 (2H, s, CH_2), 1.95 (4H, s, CH_2), 3.51 (4H, t, CH_2), 7.25 (H, t, Ar-H), 7.42 (2H, t, Ar-H), 7.59 (2H, t, Ar-H), 7.74 (2H, s, Ar-H), 8.02 (2H, d, Ar-H), 8.19 (H, s, CH), 10.16 (H, s, NH), 11.90 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 21.57, 23.36, 38.93, 53.89, 119.99, 125.37, 125.92, 128.05, 128.90, 139.04, 141.65, 146.00, 175.99. HRMS ($[M+H]^+$ calcd for $C_{19}H_{25}N_4S^+$ ($[M+H]^+$) 339.1638, found 339.1647.

(*E*)-2-(4-morpholinobenzylidene)hydrazine-1-carbothioamide **20**

Yellow powder. Reaction time: 3 h. Rf 0.59 (Hex./EA 1:1). Purification: Washing in $CHCl_3$. Yield: 78%. Mp: 196-198ºC. IR (KBr) ν_{max} (cm^{-1}) 3384, 3245, 3157 (N-H), 1607 (C=N), 1340 (C-N), 1252 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 3.24 (4H, t, CH_2), 3.78 (4H, t, CH_2), 7.09 (2H, d, Ar-H), 7.70 (2H, d, Ar-H), 7.99 (2H, s, NH_2), 8.13 (H, s, CH), 11.37 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 47.05, 48.81, 66.03, 113.67, 115.76, 129.00, 143.25, 151.25. HRMS ($[M+H]^+$ calcd for $C_{12}H_{17}N_4OS^+$ ($[M+H]^+$) 265.1118, found 265.1125.

(*E*)-*N*-methyl-2-(4-morpholinobenzylidene) hydrazine-1-carbothioamide **21**

Purple powder. Reaction time: 3 h. Rf 0.57 (Hex./EA 1:1). Purification: Washing in $CHCl_3$. Yield: 84%. Mp: 207-208ºC. IR (KBr) ν_{max} (cm^{-1}) 3301, 3228 (N-H), 1542 (C=N), 1410 (C-N), 1242 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 3.01 (3H, d, CH_3), 3.27 (4H, t, CH_2), 3.80 (4H, t, CH_2), 7.15 (2H, d, Ar-H), 7.72 (2H, d, Ar-H), 7.97 (H, s, CH), 8.42 (H, d, NH), 11.34 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 31.22, 49.15, 65.93, 66.23, 116.11, 128.83, 142.17, 150.84, 117.80. HRMS ($[M+H]^+$ calcd for $C_{13}H_{19}N_4OS^+$ ($[M+H]^+$) 279.1274, found 279.1279.

(*E*)-*N*-ethyl-2-(4-morpholinobenzylidene) hydrazine-1-carbothioamide **22**

White powder. Reaction time: 4 h. Rf 0.47 (Hex./EA 3:2). Purification: Washing in MeOH. Yield: 78%. Mp: 209-210ºC. IR (KBr) ν_{max} (cm^{-1}) 3304, 3135 (N-H), 1538 (C=N), 1379 (C-N), 1238 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 1.14 (3H, t, CH_3), 3.19 (4H, t, CH_2), 3.58 (2H, q, CH_2), 3.71 (4H, t, CH_2), 6.96 (2H, d, Ar-H), 7.65 (2H, d, Ar-H), 7.95 (H, s, CH), 8.39 (H, t, NH), 11.22 (H, s, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 15.17, 38.62, 47.96, 66.39, 114.70, 124.92, 128.85, 142.70, 152.46, 176.65. HRMS ($[M+H]^+$ calcd for $C_{14}H_{21}N_4OS^+$ ($[M+H]^+$) 293.1431, found 293.1430.

2.5 Cytotoxicity: Percentage of Inhibition

The percentage of inhibition of cellular growth were evaluated using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma Aldrich Co., St. Louis, MO/USA) assay against the following human cancer cells lines: NCI-H292 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), MOLT-4 (human T lymphoblast), HT-29 (human colon adenocarcinoma) and HEp-2 (human laryngeal carcinoma) [9,10]. All cancer cells were maintained in RPMI 1640 or DMEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin at 37°C with 5% CO_2 [11]. The cytotoxicity of all compounds was tested using the MTT reduction assay. For all experiments, tumor cells were plated in 96-well plates (105 cells/mL for NCI-H292, MCF-7, HT-29, and Hep-2 or 3 × 105 cells/mL for MOLT-4). Tested Compounds (25 µg/mL) dissolved in DMSO 0.1% were added to each well and incubated for 72 h. Control

groups received the same amount of DMSO. After 69 h of treatment 25 µL of MTT (5 mg/mL) was added, three hours later, the MTT formazan product was dissolved in 100 µL of DMSO, and absorbance was measured at 595 nm in plate spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Chemical

Scheme 1 shows the synthetic route used in this study. The benzaldehyde intermediates **5-7**, previously described in the literature, were prepared through nucleophilic substitution between 4-fluorobenzaldehyde **1** and cyclic secondary amines, namely pyrrolidine **2**, piperidine **3** and morpholine **4**, with satisfactory yields (66-86%) and their structures were confirmed by infrared [12].

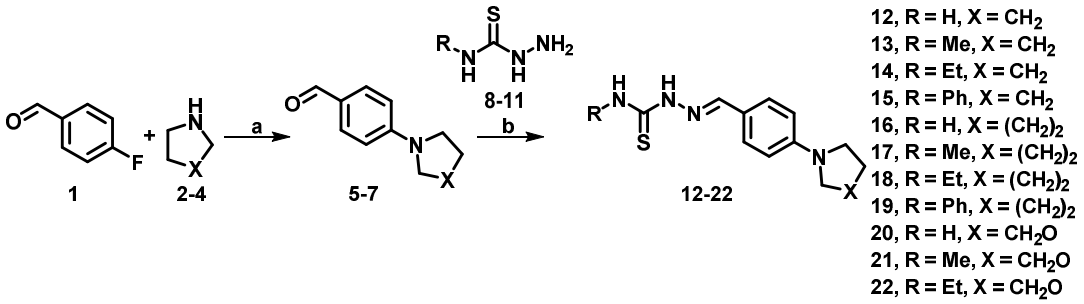
The condensation between benzaldehyde intermediates **5-7** and substituted thiosemicarbazides **8-11** in catalytic amount of HCl gave the TSC derivatives **12-22** in good to excellent yields (58-88%). In this reaction, the product TSC is formed faster at pH 4–5, at pH < 4 there is the protonation of amino nitrogen from thiosemicarbazide, reducing its nucleophilia, already at pH > 5 there is a deficit in protonation of carbonyl group, what makes a slow reaction. Thus, the use of acid catalysis in these reactions is normal [13,14]. The compounds **12-22** were characterized using IR, ^{1}H and ^{13}C NMR and HRMS. In IR spectroscopy, these compounds showed bands in 3384-3077 cm^{-1} relative to the axial stretching of NH, in 1527-1695 cm^{-1} relative to the axial stretching of C=N, in 1293-1410 cm^{-1} referring to the axial stretching of C-N, and in 1175-1252 cm^{-1} referring to the axial stretching of C=S, proving the condensation between benzaldehyde intermediates **5-7** and thiosemicarbazides **8-11**. Another strong indication is the absence of absorption in the axial stretching region of carbonyl (1710-1685 cm^{-1}). The analysis of the ^{1}H NMR spectra allowed observing singlets with chemical displacement in 9.32-11.90 ppm, integrating to a hydrogen, corresponding to the hydrogen of nitrogen. The presence of the singlet in this region means that there is no coupling to hydrogen bound to another hydrogen of another vicinal atoms, confirming once again the formation of the C=N bond between carbonyl and hydrazine group. ^{13}C NMR spectra allowed observing peaks in 151.25-177.80, referring to C=S.

3.2 Cytotoxicity

Synthesized TSC analogues **12-22** were evaluated to in vitro cytotoxicity by MTT assay in human cancer cell lines HEp-2 (laryngeal carcinoma), HT-29 (colon adenocarcinoma), MCF-7 (breast cancer), MOLT-4 (leukemia) and NCI-H292 (lung cancer). The MTT assay is currently the most widely used in vitro cytotoxicity assay. It is based on the ability of viable cells to reduce MTT in formazan, another compound having purple staining and maximum absorbance in 570 nm. Dead cells have no ability to reduce MTT, so colour change is used as a marker for viable cells [9].

The substitutions with pyrrolidinyl, piperidinyl and morpholidinyl groups as well as hydrogen, alkyl (R= Me, Et) and phenyl groups in the TSC derivatives were used to observe their effects on the cytotoxicity activities. Table 1 shows the % inhibition of tumour growth of tested TSC compounds **12-19** and doxorubicin as reference compound, except for morpholinyl series **20-22** that showed no activity. The initial optimization efforts were focused on the R substituents of pyrrolidinyl derivatives **12-15**. Replacing the hydrogen substituent with a methyl **13** or ethyl **14** group provided the loss of activity, with % inhibition values between 3.5-47.2 and 8.2-31.8, respectively. In addition, these substitutions increased LogP from 1.99 to 2.21 and 2.57, respectively. Among derivatives substituted with the pyrrolidinyl group **12-15**, compounds with no substitution **12** and substituted with phenyl group **15** showed moderate inhibition against all cell lines tested, except HT-29. The compound **12** presented excellent activity against the cell line MOLT-4 and have the smallest LogP among this series. LogP is an important parameter to evaluate druglikeness of new chemical compounds with certain pharmacological activity and should be < 5 [15].

Derivatives substituted with piperidinyl group **16-19** showed excellent activity against MOLT-4, but the replacement of hydrogen by alkyl or aryl groups caused the decrease of activity, just as pyrrolidinyl series **12-15**. Besides that, piperidinyl series **16-19** showed moderate activity against HEp-2 and MCF-7. The compound **16** presented excellent activity against the cell line MOLT-4 and was the best result between the both series.



Scheme 1. Synthetic route to obtain the new thiosemicarbazone derivatives. (a) K_2CO_3 (2 eq.), DMF, 90°C; (b) HCl catalyst, EtOH, reflux

Table 1. Percentual of inhibition of tumor growth of thiosemicarbazone derivatives

Compound	X	R	% inhibition of growth cancer cell lines					LogP*
			HEp-2	HT-29	MCF-7	MOLT-4	NCI-H292	
12	CH_2	H	50.2 ± 0.3	23.9 ± 0.3	46.4 ± 0.2	86.0 ± 1.4	30.2 ± 0.4	1.99
13	CH_2	Me	39.2 ± 1.5	3.5 ± 0.1	21.8 ± 0.4	47.2 ± 1.0	9.9 ± 0.8	2.21
14	CH_2	Et	12.2 ± 0.9	31.8 ±0.05	18.1 ±0.05	26.9 ± 0.5	8.2 ± 0.7	2.57
15	CH_2	Ph	19.5 ± 0.5	9.1 ± 0.5	50.1 ± 0.1	52.4 ± 1.4	51.9 ± 1.8	3.87
16	$(CH_2)_2$	H	76.6 ± 3.3	32.2 ± 1.9	69.4 ± 2.1	94.9 ± 0.3	38.9 ± 1.9	2.44
17	$(CH_2)_2$	Me	45.7 ± 4.3	24.4 ± 0.05	53.5 ± 0.7	88.9 ± 1.0	13.4 ± 1.0	2;66
18	$(CH_2)_2$	Et	64.2 ± 2.3	18.3 ± 1.5	52.8 ± 4.9	79.6 ± 1.1	25.3 ± 1.5	3.02
19	$(CH_2)_2$	Ph	56.1 ± 2.6	24.0 ± 1.3	46.2 ± 1.7	79.6 ± 1.9	23.8 ± 0.9	4.32
Dox.	-	-	79.4 ± 2.6	75.3 ± 1.3	74.8 ± 2.1	96.6 ± 0.9	94.9 ± 1.9	-

*Dox. – Doxorubicin. Compounds that show inhibition ≥ 75% are considered actives, between 50 and 75% are moderately actives and ≤ 50% are inactives. *LogP were calculated using software MarvinSketch 17.4.3.*

4. CONCLUSION

The TSC analogues were obtained in satisfactory yields and had their structures elucidated by classical spectroscopic and spectrometric techniques. Among the tested series, the compounds showed moderate to good activity, especially piperidinyl series **16-19** that showed the best percentage of inhibition as well as derivative **16** against MOLT-4.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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