AROMATIC ISOTHIOCYANATOPROPENONES AND THIOUREA DERIVATIVES. SYNTHESIS AND BIOLOGICAL PROPERTIES

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Rezumat

În prezenta lucrare se descriu metode de sinteză a unor propenone aromatice cu grupe tioamidice sau izotiocian și caracteristica lor biologică *in vitro*. Prin condensarea în cataliză acidă sau bazică a 4-(dimetilamino)benzaldehidei 4-Hidroxi-3-metoxibenzaldehidei și furan-2-carbaldehidei cu 3-(4-acetilfenil)-1,1dimetiltioureea au fost obținute propenone aromatice cu grupări –NHCSN(CH_3)$_2$, care la tratare termică sau în prezență de agenți cu caracter acid (HCl, H_2SO_4, (CH_3CO)$_2$O, CH_3COCl) elimină dimetilamină, transformîndu-se în izotiocianato-propenon cu randamentul de 54-92 %. La tratarea izo-

tiocianatopropenonelor cu amine primare au fost obținuti derivați cu grupări tioamidice -NHCSNH. Structura compușilor noi obținuți cu conținut de sulf a fost confirmată prin analiză elementală și spectrală IR, ^{1}H-, ^{13}C- RMN. Compușii sintetizați au fost testați ca inhibitori ai proliferării celulelor leucemice (HL-60), iar pentru produșii care au manifestat proprietăți mai ponunțate, a fost testată *in vitro* activitatea lor antibacteriană față de unele microorganisme gram-pozitive şi gram-negative: *Escherichia coli, Staphylococcus aureus, Shigella sonnei, Salmonella abony și Bacillus cereus.*

Cuvinte cheie: chalconă, izotiocianatopropenonă, propenonă, inhibitori ai proliferării, leucemie, activitate antibacteriană.

Depus la redacţie 07 mai 2014

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1. Introduction

Compounds with a wide spectrum of biological activities have been detected in the 1,3-diaryl- and 1,3-arylheterylpropenone series [20, 13, 1, 15, 23, 22, 16]. Licochalcone A, for example, extracted from Chinese licorice roots displays strong antileishmanial activity [2, 3, 26]. It has been demonstrated that some synthetic chalcones also display advanced properties against *Leishmania major* and *Leishmania donovani*. Licochalcone A also displays antimalarial activity [3] but 2,4-dimethoxy-4'-buthoxychalcone, its synthetic analogue, proved to be the most active of those studied [4]. Some 1,3-diphenylprop-2-en-1-ones with substitutes (F, Me, OMe, SO_2Me) in both nuclei display good *in vivo* anti-inflammatory activity [25]. The methyl and methoxy groups increase the inhibitory potential, but fluorine decreases it. It has been established that the propenonic structure is favourable to obtain inhibitors with high efficacy and selectivity.

The authors, by means of treating 2-chloro-2'-hydroxy-chalcone semicarbazone with o,o-dimethylchlorophosphate in the presence of pyridine, obtained an organophosphorus derivative with antifungal activity against some sugarcane pathogens [21]. This derivative displayed 75-85% mycelial growth inhibition at a concentration of 1000 ppm. The chalcones with hydroxyl groups in 2',3'-, 2,5'- and 3',4'- positions showed an excellent antioxidant activity (80 – 90 % at 50 μM concentration), that is comparable to that of ascorbic acid and α-tocopherol as reference substances [11], the hydroxyl groups in 2',4'- and 3',5'- positions caused a significant decrease in antioxidant activity. Recent investigations are devoted to anticancerigenic activity of chalcones [23, 23]. Other compounds with similar structure that, once again, demonstrate the increased efficacy of chalcones as compared to other substances, are studied in parallel. Thus, it was established that the replacing of methyl group with phenyl in dehydrozingerone increased the cytotoxic action (up to strong) for three types of carcinogenic cells [6]. Chalcones with hydroxyl groups in ortho - position are more active than those with hydroxyl groups in meta- or para- positions.

The anti-angiogenic properties of chalcones are reported in many sources [14, 17]. The antitumoural properties of curcumin have served as a reference point. It has been estimated that non-substituted chalcone is already more active than curcumin, but 2,6-dichloro-4'-methylchalcone is one of the most active. The properties of 1,3-

diphenylpropan-1-one have also been evaluated in order to prove the importance of propenonic fragment for different bioactivities of chalcones. Its low activity justifies the importance of enonic group presence in chalcone derivatives. The above mentioned chalcones were synthesized by condensing aromatic aldehydes (in base or acid medium) with substituted acetylarenes containing electron donor or electron acceptor groups or by modifying some active functional groups of final compounds. The 1,3-diaryl- and 1,3-arylheterylpropenones with thiourea or izothiocyanato groups [10, 7] are less studied and have become the object of our study.

2. Materials and methods

All commercially available reagents and chemicals were of analytical- or reagent-grade purity and used as received. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Bruker DRX 400 spectrometer at room temperature. All chemical shifts (^{1}H, ^{13}C) are given in ppm versus $SiMe_4$ using DMSO – d_6 as solvent. IR spectra were recorded on a Specord M 80 and are reported in cm^{-1}. Classic methods were applied for C, H, N and S elemental analyses, which were performed at Academy of Sciences of Moldova (Institute of Chemistry). TG/DT combined analyses were carried out using a SETARAM 92-1600 instrument. Each sample was deposited in a platinum crucible, which was heated ($60^{o}C\ h^{-}1$) in a current of air to allow evacuation of the products resulting from decomposition. The MS spectra were recorded on an analytical VG 7070E mass spectrometer using electron impact (EI) at 70eV as ionization technique. Melting points were determined by differential scanning calorimetry (DSC) with a Shimadzu DSC-50 instrument. Thin-layer chromatography (TLC) was performed using silica gel plates (60F254 Merck). Flash column chromatography was carried out on silica gel (70-240 mesh, G60 Merck).

3. Synthetic procedures

3.1. Synthesis of precursors 4 and 5. ***3-(4-Acetylphenyl)-1,1-dimethylthiourea (4).*** The mixture containing 1.68 g (0.012 mol) of 1-(4-aminophenyl)ethanone, 0.46 g (0.006 mol) of carbon disulphide and 7 mL of DMF is heated in a flask fitted with upward refrigerant at 90-95^{0}C. 2 g (0.0083 mol) of tetramethyltiuram disulphide (DTMT) is added in rates of 0.5 g during 4 hours. The mixture is heated 3 hours more and then the solvent is distilled under reduced pressure. From the crude solid of **4** and sulphur, the last is extracted with benzene and **4**, slightly soluble, is isolated (2.22 g). After filtering, the integral yield of **4** is 2.54 g (92%), m.p. 179-181°C [7].

1-(4-Isothiocyanatophenyl)ethanone (5). 1 g (0.01 mol) of concentrated sulphuric acid and 2.22 g (0.01 mol) of 3-(4-acetylphenyl)-1,1-dimethylthiourea (**4)** are added to 20 mL of dioxane with continuous stirring. The mixture is then heated at 80-90^{0}C for 2 hours. The dioxane is distilled under reduced pressure, the reaction product is extracted with benzene, the solvent is distilled, and the product is recrystallized from benzene with hexane addition. 1.52 g (86%) of 1-(4-isothiocyanatophenyl)ethanone (**5**) are obtained; m.p. 76-78°C [12].

3.2.Synthesis of thioureas and isothiocyanatopropenones

3-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-1,1-dimethylthiourea (6a). The mixture containing 0.75 g (0.005 mol) of 4-(dimethylamino)benzaldehyde, 1.1 g (0.005 mol) of 3-(4-acetylphenyl)-1,1-dimethylthiourea (**4**) and 3 mL of concentrated

hydrochloric acid is slowly heated till the temperature of 60^0C, then maintained at this temperature for 1 hour. The reaction mixture is neutralized, and the precipitate is then extracted with chloroform 1.68 g of propenone **6a,** with low impurities of isothiocyanatopropenone **7a**. IR (KBr), cm^{-1}: 1322 (m, C=S), 1639 (m, C=O), 3324 (s, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.03 (s, 6 H, $N(CH_3)_2$), 3.30 (s, 6 H, $SCN(CH_3)_2$), 6.74-8.07 (m, 10H, -C_6H_4 and =CH), 9.29 (s, 1H, NH). ^{13}C NMR (DMSO-d_6), ppm: 187.82 (C=O), 181.39 (C=S), 152.40 (C-N), 144.73 (-C_6H_4-**CH=)**, 145.75, 133.82, 123.90, 112.24, 123.79, 131.11, 128.84, 122.62, 41.65, 41.61, 40.44, 40.23.

3-(4-(3-(Furan-2-yl)acryloyl)phenyl)-1,1-dimethylthiourea (6c). 1 g (0.012 mol) of potassium hydroxide in 4 mL of ethanol is added by continuous stirring to a solution containing 2.22 g (0.01 mol) of 3-(4-acetylphenyl)-1,1-dimethylthiourea (**4)** and 6 mL of dimethylformamide, and then 1.15 g (0.012 mol) of furan-2-carbaldehyde in 4 mL of ethanol is dropped at 5-10^0C. The reaction mixture is left at room temperature for 12 hours, and then the solution is filtered of impurities and acidified with hydrochloric acid till weak acid medium 2.76 g of propenone **6c.** IR (KBr), cm^{-1}: 1322 (p, C=S), 1651 (m, C=O), 3326 (m, NH). ^{1}H- NMR (DMSO-d_6), ppm: 3.30 (s, 6H, $SCN(CH_3)_2$), 6.70-8.04 (m, 9H, -C_6H_4, **=CH**), 9.33 (s, 1H, NH). ^{13}C NMR (DMSO-d_6), ppm: 187.66 (C=O), 181.36 (C=S), 151.73 (2-furan 1-C=C), 146.31, 146.49, 113.58, 112.32, 132.87, 123.80, 130.34, 123.68, 129.02, 129.02, 119.30, 41.64, 41.65.

3-(4-(Dimethylamino)phenyl)-1-(4-isothiocyanatophenyl)prop-2-en-1-one (7a).

First method: The mixture containing 1.77 g (0.005 mol) of **6a**, 0.25 g (0,005 mol) of acetic anhydride and 6 mL of benzene is heated for 2 hours. The benzene solution is washed with aqueous solution of sodium hydrogenocarbonate and dried with Na_2SO_4. Some of the benzene is distilled, hexane is added (1:1) and the solution is chromatographed on silica gel (eluent-hexane). 1.42 g of isothiocyanatopropenone **7a** are obtained, (table 1).

Second method: 0.35 g (0.001 mol) of **6a** and 30 mL of p-xylene are introduced in a three necks flask. Through solution, heated at 125-135^0C, passes a current of CO_2 and the solvent is slowly distilled (3/4 of initial volume). The end of dimethylamine elimination is determined with the universal indicator. The remaining solvent is distilled under reduced pressure. The remaining product is dissolved in benzene and purified by chromatography to gas 0.21 g of isothiocyanatopropenone **7a**. IR (KBr), cm^{-1}: 1324 (m, C=S), 1641 (m, C=O), 2049 (p, NCS). ^{1}H-NMR (DMSO-d_6), ppm: 3.02 (s, 6H, $N(CH_3)_2$), 6.74-8.20 (m, 10H, =CH and C_6H_4). ^{13}C NMR (DMSO-d_6), ppm: 187.82 (C=O), 181.39 (C=S), 152.42 (C-N), 145.14 (-C_6H_4-**CH=**), 137.11 (-C_6H_4-**N=C=S**), 134.39, 122.59, 126.67, 131.45, 129.46, 111.54, 131.15, 40.65, 40.44, 145.14, 122.01.

3-(4-Hydroxy-3-methoxyphenyl)-1-(4-isothiocyanatophenyl)prop-2-en-1-one (7b)

First method: 4.44 g (0.02 mol) of **4**, 3.04g (0,02 mol) of 4-hydroxy-3-methoxybenzaldehyde are added on cooling the solution obtained from 30 mL of dioxane and 2 g (0.02 mol) of sulphuric acid, and then the mixture is heated at 60-70^0C for 1.5 hours. The volume of dioxane (2/3) is distilled under reduced pressure, the reaction mixture is neutralized with a saturated solution of $NaHCO_3$ and the product is extracted with benzene and purified on silica gel (hexane eluent-benzene) to obtain 2.8 g of izothiocyanatopropenone **7b** (table 1).

Second method: 1.77 g (0.01 mol) of **5** and 1.52 g (0.01 mol) of 4-hydroxy-3-methoxybenzaldehyde are added to the solution obtained from dioxane and 1 g (0.01 mol) of sulphuric acid. The reaction mixture is treated and 1.61 g (72 %) of isothiocyanatopropenone **7b** are obtained. IR (KBr) cm^{-1}: 1652 (p, C=O), 2116 (p, NCS), 3162 (m, OH), 3455 (m, OH). ^{1}H-NMR (DMSO-d_6), ppm: 3.88 (s, 3H, OCH_3), 6.84-8.22 (m, 9H, =CH and C_6H_4), 9.78 (s, 1H, OH). ^{13}C NMR (DMSO-d_6), ppm: 187.99 (C=O), 181.39 (C=S), 150.43 (-C_6H_3-O-C), 146 (-C_6H_4-**CH=**), 137.17 (-C_6H_4-**N=C=S**), 148.46, 137.17, 135.82, 126.73, 112.28, 116.08, 126.66, 130,51, 124.88, 146.01, 118.72, 56.31.

3-(Furan-2-yl)-1-(4-isothiocyanatophenyl)prop-2-en-1-one (7c).

First method: The mixture containing 3 g (0.01 mol) of **6c**, 1.02 g (0.01 mol) of acetic anhydride and 20 mL of benzene is heated under reflux for 1 hours, and then treated with a saturated solution of $NaHCO_3$. The organic layer is dried with Na_2SO_4, the solvent is distilled and the reaction product is purified on silica gel (benzene-hexane 1:1). 1.78 g of isothiocyanatopropenone **7c** (table 1).

Second method: The mixture containing 3 g (0.01 mol) of **6c**, 0.78 g (0.01 mol) of acetyl chloride and 20 mL of benzene is refluxed for 2 hours, and then treated as above to provide 1.79 g of isothiocyanatopropenone **7c**. IR (KBr), cm^{-1}: 1659 (p, C=O), 2048 (p, NCS), 3122 (s, =CH, furan). ^{1}H-NMR (DMSO-d_6), ppm: 6.70-8.16 (m, 9H, =CH and C_4H_3). ^{13}C NMR (DMSO-d_6), ppm: 187.62 (C=O), 179.49 (C=S), 151.69 (2-furan 1-**C=C**), 136.99 (-C_6H_4-**N-C=S**), 119.18 (-C_4H_3O-**CH=**), 144.35, 113.58, 133.50, 131.38, 126.84.

1-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-3-(2-hydroxyethyl)thiourea (8a). 0.13 g (0.0022 mol) of monoethanolamine in methanol (1 mL) is added by stirring at room temperature to the solution obtained from 0.26 g (0.002 mol) of **7a** and 2 mL of diethylether and then heated for 10 minutes. On cooling, 0.78 g of **8a** obtained (recrystallized from ethanol). IR (KBr), cm^{-1}: 1041 (m, C-O), 1324 (m, C=S), 1636 (s, C=O), 1601 (p, C=O), 3319 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.01 (s, 6H, $N(CH_3)_2$, 3.58 (s, 4H, N-CH_2-CH_2O), 6.74-8.10 (m, 10H, =CH and -C_6H_4), 10.0 (s, 1H, **NH**-C_6C_4), 8.32 (s, 1H, **NH**-CH_2), 4.87 (s, 1H, OH). ^{13}C NMR (DMSO-d_6), ppm: 187.62 (C=O), 180.39 (C=S), 144.96 (-C_6H_4-**CH=**), 152.39 (C-N), 59.52 (C-O), 121.18, 133.57, 122.61, 112.24, 131.11, 129.54, 128.79, 40.63, 40.43.

1-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-3-(3-hydroxyphenyl) thiourea (8b). 0.22 g (0.002 mol) of 3-aminophenol in 1 mL of acetone are added to the solution obtained from 0.62 g (0.002 mol) of **7a** and 2 mL of acetone. The reaction mixture is heated at 50^0C for 10 minutes and then cooled. 0.76 g of thiourea **8b** was obtained (recrystallized from ethanol). IR (KBr), cm^{-1}: 1215 (m, C-O), 1326 (m, C=S), 1641 (s, C=O), 1601 (m, C=O), 3317 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.01 (s, 6H, $N(CH_3)_2$), 6.53-8.10 (m, 14H, =CH and -C_6H_4), 10.00 (s, 1H, **NH**-C_6H_4), 9.9 (s, 1H, **NH**-C_6H_4). ^{13}C NMR (DMSO-d_6), ppm: 187.73 (C=O), 180.03 (C=S), 145.02 (-C_6H_4-**CH=**), 155.52 (-C_6H_4-**OH**), 152.43 (C-N), 157.21 (-C_6H_4-**OH**), 144.26, 133.96, 122.61, 112.26, 126.40, 116.56, 131.12, 129.36, 114.26, 122.10, 40.66, 40.45.

1-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-3-(4-hydroxyphenyl) thiourea (8c). This compound is obtained from **7a** and 4-aminophenol. IR (KBr), cm^{-1}: 1222 (p, C-O), 1354 (p, C=S), 1628 (m, C=O), 3280 (m, NH). ^{1}H-RMN (DMSO-d_6),

ppm: 3.01 (s, 6H, $N(CH_3)_2$), 6.73-8.09 (m, 14H, =CH), 9.08 (s, 1H, **NH**-C_6H_4), 9.09 (s, 1H, **NH**-C_6H_4), 9.42 (s, 1H, OH). ^{13}C NMR (DMSO-d_6), ppm: 187.73 (C=O), 179.99 (C=S), 144.97 (-C_6H_4-**CH=**), 152.42 (C-N), 155.52 (-C_6H_4-**OH**), 130.14, 144.35, 133.89, 122.62, 115.57, 112.26, 126.61, 126.53, 131.1, 129.31, 116.63, 122.22, 40.67, 40.46.

1-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-3-(3-methoxyphenyl) thiourea (8d). The mixture obtained from 0.62 g (0.002 mol) of **7a**, 0.25 g (0.002 mol) of 4-methoxyaniline and 3 mL of acetone is heated at 50^{0}C for 20 minutes and then cooled. The reaction product is isolated and recrystallized from ethyl acetate. 0.78 g of thiourea **8d** is obtained. IR (KBr), cm^{-1}: 1231 (p, C-O), 1348 (p, C=S), 1635 (m, C=O), 3323 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.01 (s, 6H, $N(CH_3)_2$), 3.78 (s, 3H, OCH_3), 6.74-8.01 (m, 14H,-C_6H_4 and =CH), 9.92 (s, 1H, **NH**-C_6H_4), 10.02 (s, 1H, **NH**-C_6H_4). ^{13}C NMR (DMSO, d_6), ppm: 187.72 (C=O), 179.43 (C=S), 145.07 (-C_6H_4-**CH=**), 152.41 (C-N), 54.88 (-O-**C**$\mathbf{H_3}$), 157.99, 131.15, 144.23, 134.02, 122.59, 114.40, 110.76, 128.79, 129.77, 122.59, 40.62, 40.42.

1-(4-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)phenyl)3-(2-hydroxylethyl) thiourea (9a). This compound is obtained (see the synthesis of thiourea **8a**) from 0.62 g (0.002 mol) of **7b** and 0.13 g (0.0022 mol) of monoethanolamine. 0.70 g of thiourea **9a** is obtained. IR (KBr), cm^{-1}: 1033 (p, C-O), 1315 (m, C=O), 3294 (p, NH, OH.). ^{1}HNMR (DMSO-d_6), ppm: 3.85 (s, 4H, N-CH_2O), 3.94 (s, 3H, OCH_3), 4.07 (s, 1H, OH), 6.89-8.27 (m, 9H, -C_6H_3 and =CH), 9.51 (s, 1H, **NH**-CH_2), 9.39 (s, 1H, **NH**-C_6H_4). ^{13}C NMR (DMSO-d_6), ppm: 187.82 (C=O), 181.39 (C=S), 144.73 (-C_6H_3-**CH=**), 56.42 (C_6H_3-O-**C**$\mathbf{H_3}$), 59.92 (C-O), 150.09, 148.48, 144.65, 133.44, 112.06, 128.78, 130.74.

1-(4-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)phenyl)3-(3-hydroxyphenyl) thiourea (9b). This compound is obtained (see **8b**) from 0.26 g (0.002 mol) of **7b** and 0.22 g (0.002 mol) of 3-aminophenol. 0.71 g of thiourea **9b** are obtained. IR (KBr), cm^{-1}: 1213 (p, C-O), 1331 (m, C=S), 1644 (m, C=O), 3247 (m, OH...OCH_3), 3328 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.88 (s, 3H, OCH_3), 6.76-8.14 (m, 13H, -C_6H_3 and =CH), 9.43 (s, 1H, **NH**-C_6H_4), 9.69 (s, 1H, **NH**-C_6H_4), 9.92 (s, 1H, OH). ^{13}C NMR (DMSO-d_6), ppm: 187.93 (C=O), 179.97 (C=S), 144,73 (-C_6H_3-**CH=**), 56.32 (-C_6H_3-O-**C**$\mathbf{H_3}$), 155.53 (-C_6H_4-OH), 150.09, 148.48, 155.53, 144.65, 133.44, 126.87, 119.11, 116.07, 115.58, 122.17, 130.74, 129.53.

1-(4-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)phenyl)3-(4-hydroxyphenyl) thiourea (9c). This compound is obtained from **7b** and 4-aminophenol. IR (KBr), cm^{-1}: 1216 (p, C-O), 1248 (p, C-N), 1327 (m, C=S), 1643 (m, C=O), 3248 (m, OH...OCH_3), 3314 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.88 (s, 3H, OCH_3), 6.54-8.15 (m, 13H, -C_6H_3 and =CH), 9.51 (s, 1H, **NH**-C_6H_4), 9.71 (s, 1H, **NH**-C_6H_4), 10.02 (s, 1H, OH). ^{13}C NMR (DMSO, d_6), ppm: 187.96 (C=O), 179.45 (C=S), 144.53 (-C_6H_3-**CH=**), 56.33 (-C_6H_3-O-**C**$\mathbf{H_3}$), 157.90 (-C_6H_4-OH), 150.11, 148.49, 157.99, 129.77, 144.53, 133.60, 116.07, 112.01, 122.22.

1-(4-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)phenyl)3-(4-methoxyphenyl) thiourea (9d). This compound is obtained (see **8d**) from 0.26 g (0.002 mol) of **7b** and 0.25 g (0.002 mol) of 4-methoxyaniline. 0.74 g of thiourea **9d** is obtained. IR (KBr), cm^{-1}: 1212 (p, C-O), 1329 (m, C=S), 1642 (m, C=O), 3232 (m, OH...OCH_3), 3324 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 3.81 (s, 3H, OCH_3), 6.89-8.26 (m, 13H, -C_6H_3

and =CH), 9.17 (s, 1H, **NH**-C_6H_4), 9.21 (s, 1H, **NH**-C_6H_4). ^{13}C NMR (DMSO, d_6), ppm: 187.82 (C=O), 181.39 (C=S), 144.50 (-C_6H_3-**CH=**), 56.20 (-C_6H_3-O-**CH_3**), 54.94 (-O-**CH_3**), 150.20, 160.01, 129.82, 144.35, 115.04, 116.38, 128.61, 112.29, 122.39, 133.76, 132.61.

1-(4-(3-(Furan-2-yl)acryloyl)phenyl)-3-(2-hydroxyethyl)thiourea (10a). This compound is obtained (see **8a**) from 0.51 g (0.002 mol) of **7c** and 0.13 g of monoethanolamine. 0.56 g of thiourea **10a** are obtained. IR (KBr), cm^{-1}: 1018 (m, C-O), 1233 (p, C-N), 1325 (m, C=S), 1644 (p, C=O), 3322 (p, NH, OH...OH). ^{1}H-RMN (DMSO-d_6), ppm: 3.71 (s, 4H, NH-CH_2), 6.65-8.10 (m, 9H,-C_4H_3 and =CH), 9.63 (s, 1H, **NH**-C_6H_4), 9.37 (s, 1H, **NH**-CH_2). ^{13}C NMR (DMSO, d_6), ppm: 187.11 (C=O), 180.44 (C=S), 119.93 (-C_4H_3O-**CH=**), 60.02 (C-O), 151.89, 145.35, 114.09, 112.69, 144.09, 133.47, 129.24, 46.93.

1-(4-(3-(Furan-2-yl)acryloyl)phenyl)-3-(3-hydroxyphenyl)thiourea (10b). This compound is obtained (see **8b**) from 0.51 g (0.002 mol) of **7c** and 0.22 g (0.002 mol) of 3-aminophenol, 0.62 g of thiourea **10b** are obtained. IR (KBr), cm^{-1}: 1018 (m, C-O), 1233 (p, C-N), 1327 (m, C=S), 1649 (p, C=O), 3207 (m, OH...OH), 3344 (m, NH). ^{1}H-NMR (DMSO-d_6), ppm: 6.55-8.07 (m, 13H, -C_6H_3 and =CH), 10.05 (s,1H, **NH**-C_6H_4), 10.13 (s, 1H, **NH**-C_6H_4). ^{13}C NMR (DMSO, d_6), ppm: 187.82 (C=O), 181.39 (C=S), 119.25 (-C_4H_3O-**CH=**), 158.53 (-C_6H_4-OH), 151.71, 144.84, 114.26, 113.57, 157.23, 133.01, 132.96, 126.41, 117.23, 129.55, 115.26, 130.04, 128.31.

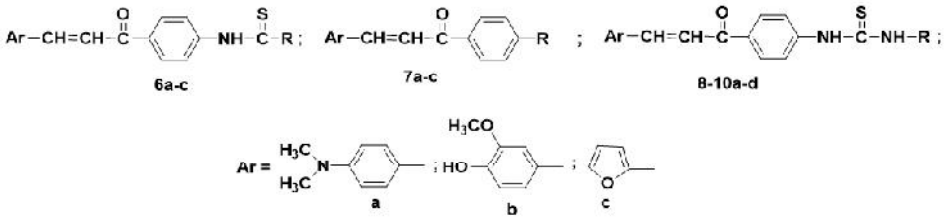
1-(4-(3-(Furan-2-yl)acryloyl)phenyl)-3-(4-hydroxyphenyl)thiourea (10c). This compound is analogically obtained from **7c** and 4-aminophenol. IR (KBr), cm^{-1}: 1222 (p, C-O), 1332 (m, C=S), 1655 (m, C=O), 3203 (p, NH, OH...OH). ^{1}H-NMR (DMSO-d_6), ppm: 6.70-8.07 (m, 13H, -C_6H_3 and =CH), 9.94 (s, 1H, **NH**-C_6H_4), 10.05 (s, 1H, **NH**-C_6H_4). ^{13}C NMR (DMSO, d_6), ppm: 187.23 (C=O), 180.44 (C=S), 119.21 (-C_4H_3O-**CH=**), 157.23 (-C_6H_4-OH), 151.82, 142.72, 11278, 113.48, 154.72, 129.86, 144.08, 133.78, 115.92, 126.72, 131.45, 126.64.

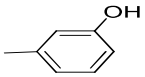
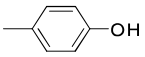
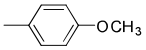
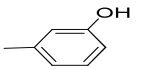
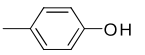
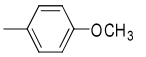
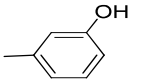
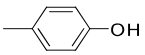
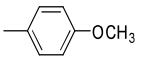
1-(4-(3-(Furan-2-yl)acryloyl)phenyl)-3-(4-methoxyphenyl)thiourea (10d). This compound is obtained (see 8d) from 0.51 g (0.002 mol) of **7c** and 0.25 g (0.002 mol) of 4-methoxyaniline; 0.62 g of thiourea **10d** are obtained. IR (KBr), cm^{-1}: 1221 (p, C-O), 1327 (m, C=S), 1655 (m, C=O), 3310 (m, NH). ^{1}HNMR (DMSO-d_6), ppm: 3.94 (s, 3H, OCH_3), 6.65-8.09 (m, 14H, -C_6H_4 and =CH), 9.22 and 9.25 (2s, 1H, 2***NH**-C_6H_4). ^{13}C NMR (DMSO-d_6), ppm: 187.82 (C=O), 181.39 (C=S), 119.38 (-C_4H_3O-**CH=**), 54.88 (-O-CH_3), 157.95, 145.39, 114.08, 112.72, 157.95, 129.95, 113.05, 133.86, 115.93, 126.72, 131.45, 128.95. Additional characteristics of propenones **8a-d**, **9a-d** and **10a-d** are given in Table 1.

3.3. HL-60 antiproliferative bioassay

Cell culture. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing L- glutamine (2 nM), antibiotics (100 IU/mL penicillin, 100 mg/mL streptomycin) and supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37^{0}C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS.

Table 1. Characteristics of isothiocyanatopropenones and thioureas.



Comp No.	R	Formula	Found/Calculated, %			M. P., ^{0}C	Yield, %
			C	H	N		
6a	—$N(CH_3)_2$	$C_{20}H_{23}N_3OS$	67.98/67.96	6.59/6.56	11.90/11.89	136-138	95
6b	—$N(CH_3)_2$	$C_{16}H_{16}N_2O_2S$	64.01/63.98	5.33/5.37	9.38/9.33	163-165	91
7a	—NCS	$C_{18}H_{16}N_2OS$	70.26/70.10	5.28/5.23	9.26/9.08	136-138	72-92
7b	—NCS	$C_{17}H_{13}NO_3S$	65.64/65.58	4.26/4.21	4.68/4.50	124-126	54-72
7c	—NCS	$C_{14}H_9NO_2S$	65.98/65.87	3.64/3.55	5.58/5.49	82-83	63-82
8a	- CH_2CH_2OH	$C_{20}H_{23}N_3O_2S$	65.12/65.01	6.31/6.27	11.47/11.37	178-179	95
8b		$C_{24}H_{23}N_3O_2S$	69.18/69.04	5.46/5.55	10.23/10.06	175-176	91
8c		$C_{24}H_{23}N_3O_2S$	69.12/69.04	5.49/5.55	10.28/10.06	164-165	86
8d		$C_{25}H_{25}N_3O_2S$	69.52/69.58	5.94/5.84	9.83/9.74	154-155	92
9a	- CH_2CH_2OH	$C_{19}H_{20}N_2O_4S$	61.23/61.27	5.44/5.41	7.66/7.52	153-154	95
9b		$C_{23}H_{20}N_2O_4S$	65.74/65.70	4.82/4.79	6.78/6.66	176-177	84
9c		$C_{23}H_{20}N_2O_4S$	65.68/65.70	4.84/4.79	6.76/6.66	154-155	82
9d		$C_{24}H_{22}N_2O_4S$	66.26/66.34	5.18/5.10	6.56/6.45	158-159	87
10a	- CH_2CH_2OH	$C_{16}H_{16}N_2O_3S$	60.85/60.74	5.12/5.10	8.98/8.85	172-173	88
10b		$C_{20}H_{16}N_2O_3S$	65.98/65.92	4.47/4.43	7.82/7.69	169-170	85
10c		$C_{20}H_{16}N_2O_3S$	65.80/65.92	4.49/4.43	7.81/7.69	152-153	82
10d		$C_{21}H_{18}N_2O_3S$	66.78/66.65	4.89/4.79	7.57/7.40	179-180	81

Cell proliferation assay. The cell proliferation assay for compounds and ligands was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 10,000 cells in a total of 100 mL medium in 96-well microtiter plates (Becton, Dickinson and Company, Lincoln Park, NJ, USA) were incubated for 24 hours, 5% CO_2, at 37^{0}C. Compounds were dissolved in ethanol to prepare the stock solution of 1 J 1022 M. These compounds and doxorubicin (Novapharm, Toronto, Canada), as a positive control, were diluted at multiple concentrations (1 and 10 μM) with culture media, added to each well and incubated for 3 days. Following each treatment, MTS (20 μL) was added to each well and the mixture incubated for 4 hours. MTS is converted to water-soluble colored formazan by dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

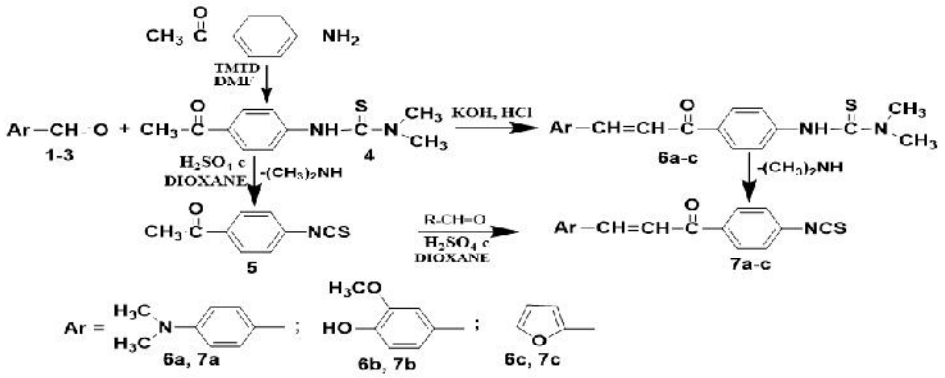
3.4. Antibacterial bioassay. The antibacterial activity of compounds and also of their prototype Furaciline was determined under liquid nutritive environment [2% of peptonate bullion (pH 7.0)] using successive dilution method [18, 9]. *Escherichia coli ATSS 25922, Staphylococcus aureus (ATSS 2512), Shigella sonnei, Salmonella abony GISK 03/03* and *Bacillus cereus GISK 8035* were used as reference strains for *in vitro* experiment. The dissolution of studied substances in dimethylformamide, cultivation of microorganisms, obtaining of suspension, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried out according to the method previously reported [19].

4. Results and discussion

4.1. Chemistry

This paper is devoted to methods for obtaining the mentioned compounds with NHCS and NCS groups from 4-(dimethylamino)benzaldehyde (**1)**, 4-hydroxy-3-methoxybenzaldehyde (**2)** and furan-2-carbaldehyde (**3)** by condensing them with 3-(4-acetylphenyl)-1,1-dimethylthiourea (**4)** according to the following scheme 1:

Scheme 1.



Acetylphenylthiourea **4** [7] has been obtained by heating 1-(4-aminophenyl) ethanone with tetramethyltiuram disulphide (TMTD) in dimethylformamide at 90^{0}C.

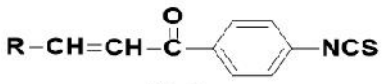
It has been established that carbon disulphide, added to the reaction mixture, reduces time reaction up to 7 and provides a product yield of 88%. The catalytic role of carbon disulphide is fully consistent with the mechanism of thiocarbonylation of aromatic amines [8]. Thus, adding DTMT in low rates has a favourable effect, which contributed to more efficient consumption of the reagent.

Condensation of aldehydes (**1)** and (**2**) with 3-(4-acetylphenyl)-1,1-dimethylthiourea (**4)** occurs unsatisfactory under base catalysis (KOH) conditions, where to when furan-2-carbaldehyde (**3)** is condensed in molar ratio of reagents - aldehyde (**3**): thiourea (**4**): KOH = 1:1.2:1.8, the yield of **6c** reaches 92% within 12 hours.

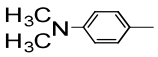
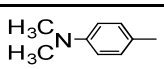
Condensation of 4-(dimethylamino)benzaldehyde (**1)** with 3-(4-acetylphenyl)-1,1-dimethylthiourea (**4)** occurs in acid catalysis, the aldehyde being activated by double protonation to nitrogen and carbonyl group. When using concentrated hydrochloric acid, the reaction ends within 1-1.5 hours in the temperature range of 60-70^{0}C. The propenone (**6a)** simultaneously eliminates dimethylamine, diminishing the final product (**7a)** yield with rise in temperature and reaction time. The final product contains isothiocyanatopropenone (**7a).** 4-Hydroxy-3-methoxybenzaldehyde (**2)**, being disabled by two electron donor groups, condenses by heating (70^{0}C) with sulphuric acid in dioxane. Under such conditions, the propenone **6b** intermediate eliminates dimethylamine and turns into **7b** (54%). The deamination of **4** in **5** and its condensation with **2** are also possible. This possibility has been confirmed by direct condensation of **2** with **5**, when the propenone **7b** is obtained with a yield of 72%. The low yields of **6b** and **7b** are caused by secondary reaction between reagent NCS and CH=O group, which becomes the main reaction when the reaction mixture is heated to reflux.

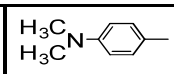
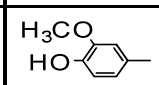
In the case of **7a** and **7c,** different ways of obtaining have been studied, including the elimination of dimethylamine from propenones **6a** and **6c** under specific action of some acid agents. Acetic anhydride is the most suitable reagent in the synthesis of **7a**, and in the case of **7c** the deamination of **6c** occurs more effectively by treating it with sulphuric acid in dioxane. Thioureas **6a** and **6c** heated (125-135^{0}C) in xylene slowly eliminate dimethylamine, turning into isothiocyanatopropenones **7a** and **7c.** The yield increases when a current of carbon dioxide passes through the reaction mixture, but is less than in the case of other reagents (Table 2).

Table 2. Optimization of isothiocyanatopropenones yields



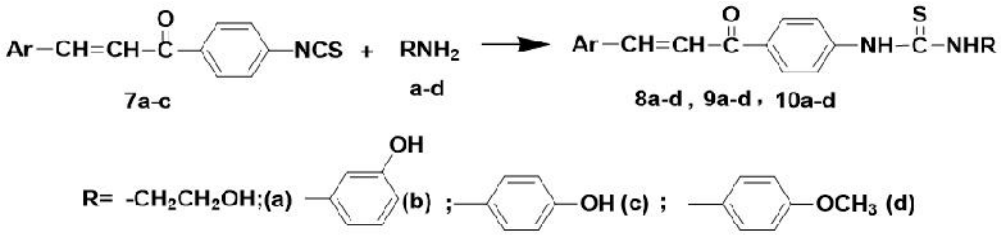
7a-c

Compound No.	R	Reagent	Solvent	Reaction time,τ^{-3}_{10}s	Temperature ^{0}C	Yield, %
7a		$(CH_3CO)_2O$	benzene	7.2	reflux	92
7a		CH_3COCl	benzene	7.2	reflux	91

7a		CO_2	xylene	3.6	125-135	72
7b		H_2SO_4	dioxane	5.4	60-70	54
7c		$(CH_3CO)_2O$	benzene	7.2	reflux	68
7c		H_2SO_4	dioxane	3.6	80	82
7c		CH_3COCl	benzene	3.6	reflux	70
7c		CO_2	xylene	3.6	125-135	63

Propenones **8-10 (a-d)** with thiourea groups have been obtained by addition of an amine to **7a, 7b, 7c** according the following schema 2:

Scheme 2.



Adding amines to NCS group occurs at room temperature or at a slight heating, without affecting the propenone group. The end of reaction is determined by TLC following the consumption of respective isothiocyanatopropenones. The structure of compounds **6, 7a-c** and **8**, **9, 10a-d** has been confirmed by means of elemental and spectral analyses (IR and NMR) and via reactions of elimination or addition to functional groups.

4.2.Antileukemia activity

All compounds were tested as inhibitors of HL-60 cells proliferation (Table 3). These human promyelocytic leukemia cells were incubated for three days in the presence of synthetic compounds (thioureas and isothiocyanatopropenones) and the number of viable cells was measured using the MTS assay. The results are expressed as the percentage of cell growth inhibition at two concentrations of 10 and 1 μM (Tatsuzaki et al., 2006). Some thioureas and isothiocyanatopropenones have a significant inhibitory activity at 10 μM but less at 1 μM. The nature of substituents in thioureas appears to modulate the cell inhibition. The substances **6a, 8a, 9c and 10b** are remarkable among all tested compounds and can effectively inhibit the HL-60 cell proliferation at 10 μM. The activity of compounds is influenced by the nature of the substituents: furan, benzene, dimethylamine and hydroxo group in different meta-, ortho- and para-positions.

Table 3. Antiproliferative activity of thioureas on human leukemia (HL-60) cells at two concentrations.

Compound, No	Inhibition of cell proliferation, (%)	
	1μM	**10μM**
6a	0	78
8a	0	69
8b	0	30
8c	9	37
9b	0	0
9c	2	81
9d	7	12
10a	0	0
10b	18	91
10c	0	28
10d	0	12
Dox	100	100

[a] SEM < ± 4% of a single experiment in triplicate, **Dox –** doxorubicin

4.3. Antibacterial activity

Twelve thioureas and isothiocyanatopropenones have been screened for their *in vitro* antibacterial activity. Experimental results obtained from the study of antimicrobial activity are given in Table 4. As it could be seen, they display bacteriostatic activity towards gram-positive and gram-negative bacteria in 4.61 – 300 μg/mL concentration range. For comparison, we also presented the antimicrobial data characteristic for Furaciline, a bactericide used in medical practice.

The experimental data prove that compounds **7a**, **8c** and **8d** display similar antimicrobial activity as Furaciline towards gram-negative *Salmonella abony* microorganism. At the same time the compounds **7a**, **7b**, **8d** and **9c** are active towards gram-positive bacteria *Staphylococcus aureus*. The minimum bactericidal concentration for all gram-positive and gram-negative strains *Escherichia coli, Staphylococcus aureus, Shigella sonnei, Salmonella abony* and *Bacillus cereus* is higher than 300 μg/mL.

Table 4. Antibacterial activity (MIC* / MBC) (μg/mL) of some sulphur containing compounds.**

Stem		Compounds											
		6a	**7a**	**8a**	**8c**	**8b**	**8d**	**6c**	**7c**	**10a**	**7b**	**9c**	**Fura-ciline**
***Escherichia coli*, ATSS 25922,(G -)**	CMI	18.75	4.69	37.5	18.75	18.75	37.5	37.5	37.5	> 300	> 300	9.37	2.34
	CMB	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	37.5	9.37

***Staphylococcus aureus,* ATSS 25923 (*G.* +)**	CMI	18.75	9.37	37.5	37.5	37.5	9.37	75.0	37.5	18.75	9.37	9.37	2.34
	CMB	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	37.5	9.37
***Shigella sonnei* (G -)**	CMI	37.5	18.75	37.5	9.37	18.75	> 300	> 300	37.5	> 300	> 300	> 300	2.34
	CMB	> 300	300.0	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	4.68
***Salmonella abony* GISK 03/03,(G -)**	CMI	37.5	9.37	75.0	9.37	18.75	9.37	18.75	37.5	> 300	> 300	> 300	4.68
	CMB	> 300	> 300	300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	4.68
***Bacillus cereus* GISK 8035,(G+)**	CMI	37.5	18.75	18.75	> 300	18.75	18.75	37.5	> 300	> 300	9.37	9.37	4.68
	CMB	300.0	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300	37.5	4.68

**MIC – minimum inhibitory concentration. **MBC – minimum bactericidal concentration.*

5. Conclusion

We have synthesized a novel series of aromatic propenones containing thioamidic or isothiocyano groups. Aromatic propenones with $NHCSN(CH_3)_2$-group have been obtained by catalytic condensation of 4-(dimethylamino)benzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and furan-2-carbaldehyde with 3-(4-acetylphenyl)-1,1-dimethylthiourea in acid or base medium. These aromatic propenones eliminated dimethylamine and turned into isothiocyanatopropenones with 54-92% yields by means of thermal treatment or in the presence of acid agents (HCl, H_2SO_4, $(CH_3CO)_2O$, CH_3COCl). For better thermostability, propenona **7a** is acetic anhydride, and sulfuric acid in dioxane for propenona **7b**. Derivatives with NHCSNH thioamidic group have been obtained by treating isothiocyanatopropenones with primary amines (81-95%). All compounds were tested as inhibitors of HL-60 leukemia cells proliferation and the most potent of them have also been tested for their *in vitro* antibacterial activity against some gram-positive and gram-negative microorganisms: *Escherichia coli, Staphylococcus aureus, Shigella sonnei, Salmonella abony and Bacillus cereus.* Among all tested compounds **6a, 8a, 8b, 8c, 9d 10b, 10c** and **10d** efficiently inhibited the HL-60 cell proliferation at 10 μM concentration and **7a, 8c, 8d** and **9c** display a significant antimicrobial activity towards gram-negative *Escherichia coli* and *Salmonella abony* microorganisms.

Acknowledgments. *The authors wish to thank the CHUL Research Center in Quebec City (Canada) and State University of Medicine and Pharmacy "N.Testemitanu" of Chisinau (Moldova) for their help in carrying out biological studies.*

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