Supporting Information

SYNTHESIS AND ANTIMICROBIAL ACTIVITY EVALUATION OF NOVEL 4-THIAZOLIDINONES CONTAINING A PYRONE MOIETY

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Table of Contents:

1. General Methods	S-2
2. Synthesis of thiosemicarbazones (3)	S-2
3. General procedure for the synthesis of of 4-thiazolidinones (5a-d), characterization and ¹ H-NMR and ¹³ C-NMR spectrum	S-4
4. General procedure for synthesis of compounds (7a-d), characterization and ¹ H-NMR and ¹³ C-NMR spectrum	S-10
5. General method for the synthesis of compounds (9a-h), characterization and ¹ H-NMR and ¹³ C-NMR spectrum	S-15
6. Antimicrobial activity	S-28
7. Materials and methods	S-28
8. Microorganisms culture.	S-28
9. Well diffusion assay	S-28
10. Results of Antimicrobial activity of synthesized compounds (Table S-1).	S-29
11. Photos of the antimicrobial test	S-29

General Methods:

All melting points were measured on a Melting Point SMP 1 Stuart Scientific apparatus. The ^1H -NMR spectra (200 MHz) and ^{13}C -NMR (50 MHz) were obtained in dimethyl sulfoxide on a Bruker spectrometer, using TMS as an internal standard; chemical shifts are reported as δ units, and coupling constants (J) are reported in hertz (Hz). TLC developed was conducted on 0.2-mm silica gel plates. Mass spectra were recorded on GC-MS- QP2010S. IR spectra were collected on FT/ IR- 4100-

Synthesis of thiosemicarbazones (3)

Compound **3a** was prepared according to Satbir Mor. and all ^[34-36]

Thiosemicarbazone **3b** was prepared according to by M. Krasowska and all. ^[36]

1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl) éthan-1-one N-ethyl thiosemicarbazone (3c). A solution of (0.01 mol, 1.68 g) of dehydroacetic acid **1** and 0.01 mol of Ethyl thiosemicarbazide **2c** in 20 mL of EtOH was stirred for 2 hours at room temperature. The precipitate obtained was filtered, washed with ethanol.

White solid, yield 90%, mp 170-171 °C. IR (KBr, ν/cm^{-1}): 3257 (O-H), 3118 (N-H), 1718 (C=O), 1575 (C=N), 1150 (C=S). ^1H NMR (250 MHz, DMSO- d_6): δ 1.08 (t, 3H, $^3J_{\text{HH}}=7$ Hz, CH_2CH_3 (I, II)), 2.12 (s, 3H, CH_3 (I)), 2.04 (s, CH_3 (II)), 2.39 (s, 3H, NCCH_3 (I)), 2.18 (s, NCCH_3 (II)), 2.48 (q, 2H, $^3J_{\text{HH}}=7$ Hz, CH_2 (I, II)), 5.92 (s, 1H, CH(I)), 6.04 (s, CH(II)), 8.28 (t, 1H, $^3J_{\text{HH}}=7$ Hz, NHCH_2 (I, II)); 9.43 (s, 1H, NH(I)), 10.38 (s, NH(II)). ^{13}C NMR (63 MHz, DMSO- d_6): δ 14 (CH_3CH_2), 16.5 (NCCH_3), 19.3 ($\text{CH}_{3\text{pyr}}$), 39.4 (CH_2), 96.2 ($\text{C}=\text{COH}$), 104.2 (CH), 163.1 ($\text{N}=\text{CCH}_3$), 163.4

(C=C-O), 167 (C=O_{pyr}), 176.8 (COH), 179.9 (C=S). MS m/z (%)= 269 (M⁺, 41), 166 (100), 138 (15), 151 (25), 125 (22), 43(53). HRMS m/z calcd for C₁₁H₁₆N₃O₃S [M+H]⁺: 270.32010. Found: 270.32012.

1-(4-Hydroxy-6-methyle-2-oxo-2H-pyran-3-yl) éthan-1-one N-phényl thiosemicarbazone (3d) according to M Ulaganatha Raja and all.^[36, 37]

General procedure for the synthesis of 4-thiazolidinones (5a-d), characterization and ¹H-NMR and ¹³C-NMR spectrum

0.01 mol of thiosemicarbazones **3a-d** and 0.02 mol of ethyl 2-bromopropionate were refluxed in 30 mL of acetonitrile in the presence of (0.03 mol, 0.246 g) of anhydrous NaOAc and two drops of concentrated sulfuric acid for 1 to 3 hours. The solid formed was recuperated by filtration and recrystallized in acetonitrile (Method A).

The corresponding thiosemicarbazones **3a-d** (0.01 mol) and (0.02 mol, 3.62 g) of Ethyl 2-bromo propionate **4** were dissolved in 30 mL of acetonitrile. After addition of (0.12 mol %, 1 mg) of keggins heteropolyacid (H₃PW₁₂O₄, n H₂O) and (0.03 mol, 0.246 g) of anhydrous NaOAc. The reaction mixture was heated for 5-30 minutes. The precipitate was separated, washed and recrystallized in acetonitrile (Method B).

2-[[1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl) ethylidene]hydrazinylidene]-5-methyl-1,3-thiazolidin-4-one (5a).

Yellow solid, yield 77%, mp 205 °C. IR (KBr, ν/cm^{-1}): 3443 (O-H), 1612 (C=O pyr),

1711 (C=O thiaz), 1349 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 1.53 (d, 3H, $^3J_{\text{HH}} = 7$ Hz, $\text{CH}_{3\text{thiaz}}$), 2.14 (s, 3H, $\text{CH}_{3\text{pyr}}$), 2.61 (s, 3H, $\text{CH}_3\text{-C=N}$), 4.43 (q, 1H, $^3J_{\text{HH}} = 7$ Hz, CH_{thiaz}), 5.96 (s, 1H, CH_{pyr}), 2.19 (s, 1H, NH). ^{13}C NMR (63 MHz, DMSO- d_6): δ 16.5 ($\text{CH}_{3\text{thiaz}}$), 18.4 (CH_3CN), 18.9 ($\text{CH}_{3\text{pyr}}$), 43.9 (CH_{thiaz}), 94.7 (C=COH), 104 (CH_{pyr}), 158.3 (C-O $_{\text{pyr}}$), 162 ($\text{H}_3\text{C-C=N}$), 163.1 (N=C=S), 168.7 (C=O $_{\text{pyr}}$), 175.9 (COH), 179.8 (C=O $_{\text{thiaz}}$). MS m/z (%) = 295 (M^+ , 5), 166 (30), 138 (100), 129 (24), 101 (42). HRMS m/z calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_4\text{S}$ [$\text{M}+\text{H}$] $^+$: 296.06995. Found: 296.06992.

2-{[1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene] hydrazinylidene}-3,5-dimethyl-1,3-thiazolidin-4-one (5b).

White solid, yield 86%, mp 203 °C. IR (KBr, ν/cm^{-1}): 3417 (O-H), 1654 (C=O pyr), 1718 (C=O thiaz), 1313 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 1.50 (d, 3H, $^3J_{\text{HH}} = 7$ Hz, $\text{CH}_3_{\text{thiaz}}$), 1.81 (s, 3H, CH_3_{pyr}), 2.00 (s, 3H, $\text{CH}_3\text{-C=N}$), 2.27 (s, 3H, NCH_3), 4.25 (q, 1H, $^3J_{\text{HH}} = 7$ Hz, CH_{thiaz}), 5.58 (s, 1H, CH_{pyr}). ^{13}C NMR (63 MHz, DMSO- d_6): δ 18.8 ($\text{CH}_3_{\text{thiaz}}$), 19.3 (CH_3CN), 22.1 (CH_3_{pyr}), 29.4 (CH_3N), 41.7 (CH_{thiaz}), 97.2 (C=COH), 106.7 (CH_{pyr}), 157.1 ($\text{C=CH}_3\text{=N}$), 159 (C-O), 163 (N=CS), 172.8 (C=O_{pyr}), 174.7 (COH), 178.1 (C=O_{thia}). MS m/z (%)= 309 (M^+ , 3), 166 (25), 138 (100), 129 (29), 101 (40). HRMS m/z calcd for $\text{C}_{13}\text{H}_{16}\text{N}_3\text{O}_4\text{S}$ [$\text{M}+\text{H}$] $^+$: 310.34090. Found: 310.34088.

3-ethyl-2-([1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl) ethylidene] hydrazinylidene)-5-methyl-1,3-thiazolidin-4-one (5c).

White solid, yield 88%, mp 150 °C. IR (KBr, ν/cm^{-1}): 3416 (O-H), 1656 (C=O pyr), 1712 (C=O thiaz), 1357 (NCS). ^1H NMR (250 MHz, CH_3NO_2): δ 1.30 (t, 3H, $^3J_{\text{HH}} = 7$ Hz, CH_2CH_3), 1.72 (d, 3H, $^3J_{\text{HH}} = 7$ Hz, CH_2), 4.36 (q, 1H, $^3J_{\text{HH}} = 7$ Hz, CH_{thiaz}), 5.90 (s, 1H, CH_{pyr}). ^{13}C NMR (63 MHz, CH_3NO_2): δ 10.9 (CH_3CH_2), 16 ($\text{CH}_3_{\text{thiaz}}$), 18.1 (CH_3CN), 18.8 (CH_3_{pyr}), 38.3 (CH_2CH_3), 43.1 (CH_{thiaz}), 95.5 ($\text{C}=\text{COH}$), 104 (CH_{pyr}), 156.9 ($\text{CCH}_3=\text{N}$), 162.6 (C-O), 164.1 ($\text{N}=\text{CS}$), 170.7 ($\text{C}=\text{O}_{\text{pyr}}$), 175.1 (COH), 181.2 ($\text{C}=\text{O}_{\text{thia}}$). MS m/z (%) = 323 (M^+ , 7), 166 (29), 138 (100), 129 (24), 101 (32), 43 (47). HRMS m/z calcd for $\text{C}_{14}\text{H}_{18}\text{N}_3\text{O}_4\text{S}$ [$\text{M}+\text{H}$] $^+$: 324.10125. Found: 324.10123.

2-[[1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl) ethylidene]hydrazinylidene]-5-methyl-3-phenyl-1,3-thiazolidin-4-one (5d).

Yellow solid, yield 90%, mp 195 °C. IR (KBr, ν/cm^{-1}): 3448 (O-H), 1697 (C=O pyr), 1710 (C=O thiaz), 1254 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 1.58 (d, 3H, $^3J_{\text{HH}} = 7$ Hz, $\text{CH}_{3\text{thiaz}}$), 1.79 (s, 3H, $\text{CH}_{3\text{pyr}}$), 1.98 (s, 3H, $\text{CH}_3\text{-C=N}$), 4.38 (q, 1H, $^3J_{\text{HH}} = 7$ Hz, CH_{thiaz}), 5.58 (s, 1H, CH_{pyr}), 7.34-7.54 (m, 5H, CH_{Ar}). ^{13}C NMR (63 MHz, DMSO- d_6): δ 17.5 ($\text{CH}_{3\text{thiaz}}$), 18.7 (CH_3CN), 19.7 ($\text{CH}_{3\text{pyr}}$), 41.8 (CH_{thiaz}), 96.6 (C=COH), 106.7 (CH_{pyr}), 128- 128.8- 129- 134.1- 134.7 (C_{Ar}), 159.6 ($\text{C=CH}_3\text{=N}$), 162.9 (C-O), 166 (C_2 thiaz), 173.2 (C=O_{pyr}), 174.1 (COH), 177.8 (C=O_{thia}). MS m/z (%)= 371 (M^+ , 5), 294 (54), 166 (21), 138 (100), 77 (59). HRMS m/z calcd for $\text{C}_{18}\text{H}_{18}\text{N}_3\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$: 372.41028. Found: 372.41026.

General procedure for the synthesis of 4-thiazolidinones (7a-d), characterization and ^1H -NMR and ^{13}C -NMR spectrum

0.01 mol of thiosemicarbazones **3a-d** and 0.02 mol of phenyl bromoacetate **6** were refluxed in 30 mL of acetonitrile in the presence of (0.03 mol, 0.246 g) of anhydrous NaOAc and two drops of concentrated sulfuric acid for 1 to 3 hours. The solid formed was recuperated by filtration and recrystallized in acetonitrile (Method A).

2-{2-[1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl) ethylidene] hydrazinyl}-1, 3-thiazolidin-4-one (7a).

White solid, yield 88%, mp 210 °C. IR (KBr, ν/cm^{-1}): 3414 (O-H), 1614 (C=O pyr), 1704 (C=O thiaz), 1258 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 2.13 (s, 3H, CH_3_{pyr}), 2.59 (s, 3H, $\text{CH}_3\text{-C=N}$), 4.43 (s, 2H, CH_2), 5.96 (s, 1H, CH_{pyr}), 12.2 (s, 1H, NH). ^{13}C NMR (63 MHz, DMSO- d_6): δ 16.6 (CH_3CN), 19.3 (CH_3_{pyr}), 34.4 (CH_2), 94.9 (C=COH), 104.2 (CH_{pyr}), 160 (C-O), 161.8 ($\text{CCH}_3\text{=N}$), 163.8 (N=C=S), 168.5 (C=O_{pyr}), 173.1 (C-OH), 180 (C=O_{thia}). MS m/z (%)= 281 (M^+ , 7), 166 (58), 138 (100), 115 (30). HRMS m/z calcd for $\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$: 282.28774. Found: 282.28771.

2-{2-[1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl) ethylidene] hydrazinyl}-3-methyl-1,3-thiazolidin-4-one (7b).

White solid, yield 91%, mp 256-257 °C. IR (KBr, ν / cm^{-1}): 3427 (O-H), 1658 (C=O pyr), 1710 (C=O thiaz), 1316 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 2.17 (s, 3H, $\text{CH}_{3\text{pyr}}$), 2.69 (s, 3H, $\text{CH}_3\text{-C=N}$), 3.16 (s, 3H, NCH_3), 4.17 (s, 2H, CH_2), 6.01 (s, 1H, CH_{pyr}). ^{13}C NMR (63 MHz, DMSO- d_6): δ 15.8 (CH_3CN), 19.1 ($\text{CH}_{3\text{pyr}}$), 28.6 (CH_3NH), 33.6 (CH_2), 94.6 (C=COH), 104 (CH_{pyr}), 159.5 (C-O), 161.5 ($\text{CCH}_3\text{=N}$), 164.1 (N=CS), 168.8 (C=O_{pyr}), 173.4 (C-OH), 180.1 (C=O_{thia}). MS m/z (%)= 295 (M^+ , 17), 166 (49), 138 (100). HRMS m/z calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$: 296.31432. Found: 296.31429.

95.1 (C=COH), 104.1 (CH_{pyr}), 158.9 (CCH₃=N), 161.8 (C-O), 163.9 (N=C=S), 169.1 (C=O_{pyr}), 171.1 (COH), 179.8 (C=O_{thia}). MS *m/z* (%)= 309 (M⁺, 15), 166 (43), 138 (100), 43 (52). HRMS *m/z* calcd for C₁₃H₁₆N₃O₄S [M+H]⁺: 310.08560. Found: 310.08560.

2-{2-[1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene] hydrazinyl}-3-phenyl-1, 3-thiazolidin-4-one (7d).

White solid, yield 78%, mp 250 °C. IR (KBr, ν/cm^{-1}): 3415 (O-H), 1656 (C=O pyr), 1710 (C=O thiaz), 1219 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 2.13 (s, 3H, CH_3_{pyr}), 2.35 (s, 3H, $\text{CH}_3\text{-C=N}$), 4.27 (s, 2H, CH_2), 5.99 (s, 1H, CH_{pyr}), 7.35-7.56 (m, 5H, CH_{Ar}). ^{13}C NMR (63 MHz, DMSO- d_6): δ 16.5 (CH_3CN), 19.3 (CH_3_{pyr}), 33.6 ($\text{CH}_2_{\text{thiaz}}$), 95 (C=COH), 104 (CH_{pyr}), 128- 128.8-129- 134.5 (C_{Ar}), 160.2 ($\text{CCH}_3\text{=N}$), 161.6 (C-O), 164.1 (N=C=S), 169.3 (C=O_{pyr}), 171 (COH), 179.8 ($\text{C=O}_{\text{thiaz}}$). MS m/z (%)= 357 (M^+ , 5), 280 (40), 166 (28), 138 (100), 77 (68).). HRMS m/z calcd for $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_4\text{S}$ ($\text{M}+\text{H}$) $^+$: 358.08560. Found: 358.08563.

General method for the synthesis of compounds (9a-h)

A mixture of thiosemicarbazones **3a** or **3c** (0.01 mol), appropriate maleimide **8a-d** (0.01 mol) and two drops of concentrated sulfuric acid (12 N) in 30 mL of acetonitrile was refluxed for 3-4 hours. The reaction mixture was then cooled at room temperature and the solid precipitated was filtered, abundantly washed with acetonitrile and ethanol and then recrystallized, or the precipitate was purified by column chromatography using Ethyl acetate as eluent.

2-(3-Ethyl-2-{2-[1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl) ethylidene] hydrazinyl}-4-oxo-1,3-thiazolidin-5-yl)acetamide (**9a**).

White solid, yield 48%, mp 255-256 °C. IR (KBr, ν/cm^{-1}): 3397 (O-H), 3298; 3179 (NH_2), 1673 ($\text{C}=\text{O}$ pyr), 1704 ($\text{C}=\text{O}$ thiaz), 1583 ($\text{NC}=\text{O}$), 1344 (NCS). ^1H NMR (250 MHz, DMSO-d_6): δ 1.18 (t, 3H, $^3J_{\text{HH}} = 7$ Hz, CH_3CH_2), 2.17 (s, 3H, CH_3pyr), 2.68 (s, 3H, $\text{N}=\text{CCH}_3$), 2.77 (dd, H_a , $^3J_{\text{HH}} = 9$ Hz, $^2J_{\text{HH}} = 17$ Hz), 3.07 (dd, H_b , $^3J_{\text{HH}} = 4$ Hz, $^2J_{\text{HH}} = 17$ Hz), 3.75 (q, 2H, $^3J_{\text{HH}} = 7$ Hz, CH_2), 4.58 (dd, H_x , $^3J_{\text{HH}} = 4$ and 9 Hz), 5.98 (s, 1H, CH_{pyr}), 7.09, 7.58 (s, 2H, NH_2). ^{13}C NMR (63 MHz, DMSO-d_6): δ 11.4 (CH_3CH_2), 17.1 ($\text{N}=\text{CCH}_3$), 19.4 (CH_3pyr), 37.1 (CH_2CH_3), 38.2 (CH_2CONH_2), 47.2 (CH_{thiaz}), 94.4 ($\text{HOC}=\text{C}$), 104 (CH_{pyr}), 159.2 ($\text{N}=\text{CCH}_3$), 162.1 (CCH_3), 163.4 ($\text{C}=\text{O}_{\text{pyr}}$), 169.1 ($\text{N}=\text{CS}$), 169.9 (COH), 173 (CONH_2), 180.1 ($\text{C}=\text{O}_{\text{thia}}$). MS m/z (%) = 366 (M^+ , 12), 308 (36), 166 (100), 151 (50), 58 (37). HRMS m/z calcd for $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 367.10707. Found: 367.10690.

2-(3-Ethyl-2-{2-[1-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethyldene]hydrazinyl}-4-oxo-1,3-thiazolidin-5-yl)-N-methylacetamide (9b).

White solid, yield 52%, mp 239-240 °C. IR (KBr, ν/cm^{-1}): 3302 (O-H), 3111 (N-H), 1634 (C=O pyr), 1718 (C=O thiaz), 1581 (NC=O), 1341 (NCS). ^1H NMR (250 MHz,

DMSO-d₆): δ 1.19 (t, 3H, $^3J_{HH} = 7$ Hz, $\underline{\text{CH}_3\text{CH}_2}$), 2.17 (s, 3H, CH_3pyr), 2.32 (d, 3H, $^3J_{HH} = 4$ Hz, NCH_3), 2.68 (s, 3H, $\text{N}=\underline{\text{CCH}_3}$), 2.79 (dd, H_a , $^3J_{HH} = 9$ Hz, $^2J_{HH} = 17$ Hz), 3.04 (dd, H_b , $^3J = 4$ Hz, $^2J_{HH} = 17$ Hz), 3.77 (q, 2H, $^3J_{HH} = 7$ Hz, $\text{CH}_3\underline{\text{CH}_2}$), 4.59 (dd, H_x , $^3J_{HH} = 4$ and 9 Hz), 5.99 (s, 1H, CH_{pyr}), 8.07 (q, 1H, $^3J_{HH} = 4$ Hz, NH). ^{13}C NMR (63 MHz, DMSO-d₆): δ 11.7 ($\underline{\text{CH}_3\text{CH}_2}$), 16.4 ($\text{N}=\underline{\text{CCH}_3}$), 19.7 ($\underline{\text{CH}_3}$), 25.8 ($\underline{\text{CH}_3\text{NH}}$), 36.7 ($\underline{\text{CH}_2\text{CH}_3}$), 38.2 ($\underline{\text{CH}_2\text{CONH}_2}$), 45.1 (CH_{thiaz}), 94.4 ($\text{HOC}=\underline{\text{C}}$), 104.8 (CH_{pyr}), 158.5 ($\text{N}=\underline{\text{CCH}_3}$), 162.1 ($\underline{\text{CCH}_3}$), 164.2 ($\text{C}=\text{O}_{\text{pyr}}$), 168.4 ($\text{N}=\underline{\text{CS}}$), 169.8 (COH), 173.6 (CONH_2), 180.4 ($\text{C}=\text{O}_{\text{thia}}$). MS m/z (%) = 380 (M^+ , 10), 166 (100), 151 (50), 126 (41), 72 (43). HRMS m/z calcd for $\text{C}_{16}\text{H}_{21}\text{N}_4\text{O}_5\text{S}$ [$\text{M}+\text{H}$] $^+$: 381.12272. Found: 381.12274.

N-Ethyl-2-(3-ethyl-2-{2-[1-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene] hydrazinyl}-4-oxo-1,3-thiazolidin-5-yl)acetamide (9c**).**

White solid, yield 50%, mp 260 °C. IR (KBr, ν/cm^{-1}): 3310 (O-H), 3092 (N-H), 1643 (C=O pyr), 1710 (C=O thiaz), 1589 (NC=O), 1397 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 1.00 (t, 3H, $^3J_{\text{HH}} = 7$ Hz, CH_3CH_2), 1.18 (t, 3H, $^3J_{\text{HH}} = 7$ Hz, CH_3CH_2), 2.17 (s, 3H, CH_3_{pyr}), 2.68 (s, 3H, $\text{N}=\text{CCH}_3$), 2.76 (dd, H_a , $^3J_{\text{HH}} = 9$ Hz, $^2J_{\text{HH}} = 17$ Hz), 3.66 (dd, H_b , $^3J_{\text{HH}} = 4$ Hz, $^2J_{\text{HH}} = 17$ Hz), 3.74 (q, 2H, $^3J_{\text{HH}} = 7.47$ Hz, CH_3CH_2), 4.59 (dd, H_x , $^3J_{\text{HH}} = 4$ and 9 Hz), 5.99 (s, 1H, CH_{pyr}), 8.10 (t, 1H, $^3J_{\text{HH}} = 5$ Hz, NH). ^{13}C NMR (63 MHz, DMSO- d_6): δ 12 ($\text{CH}_3\text{CH}_2\text{NH}$), 14.1 (CH_3CH_2), 17 ($\text{N}=\text{CCH}_3$), 19.5 (CH_3), 33.2 ($\text{CH}_3\text{CH}_2\text{NH}$), 94.1 ($\text{HOC}=\text{C}$), 103.9 (CH_{pyr}), 158.9 ($\text{N}=\text{CCH}_3$), 161.8 (CCH_3), 164.2 ($\text{C}=\text{O}_{\text{pyr}}$), 167.4 ($\text{N}=\text{CS}$), 169.8 (COH), 173 (CONH_2), 180.7 ($\text{C}=\text{O}_{\text{thia}}$). MS m/z (%) = 394 (M^+ , 14), 166 (100), 151 (46), 86 (40). HRMS m/z calcd for

¹H NMR spectrum (DMSO-d₆) of the compound. The chemical structure is shown above the spectrum. The spectrum displays peaks in the aromatic region (6.8-8.2 ppm), a methine proton (5.8 ppm), a methylene group (3.8 ppm), and an ethyl group (1.0-1.4 ppm). Integration values are provided below the baseline, and chemical shifts are listed above the peaks.

Chemical structure: CCNC(=O)CSC1=NC(=C2C(=C1)C(=O)OC(C)=C2O)N

¹H NMR (DMSO-d₆) peaks (ppm): 8.0 (1H), 6.9 (1H), 5.8 (1H), 3.8 (2H), 3.4 (1H), 3.2 (1H), 3.0 (1H), 2.8 (1H), 2.5 (3H), 2.2 (2H), 1.3 (3H), 1.0 (3H).

White solid, yield 47%, mp 240 °C. IR (KBr, ν / cm^{-1}): 3310 (O-H), 3146 (N-H), 1645 (C=O_{pyr}), 1699 (C=O thiaz), 1588 (NC=O), 1315 (NCS). ¹H NMR (250 MHz,

DMSO-d₆): δ 1.21 (t, 3H, $^3J_{HH} = 7$ Hz, CH₃CH₂), 2.11 (s, 3H, CH₃pyr), 2.69 (s, 3H, N=CCH₃), 3.09 (dd, H_a, $^3J_{HH} = 9$ Hz, $^2J_{HH} = 17$ Hz), 3.34 (dd, H_b, $^3J_H = 4$ Hz, $^2J_{HH} = 17$ Hz), 3.78 (q, 2H, $^3J_{HH} = 8$ Hz, CH₂CH₃), 4.71 (dd, H_x, $^3J_{HH} = 4$ and 9 Hz), 5.98 (s, 1H, CH_{pyr}), 6.94-7.62 (m, 5H, CH_{Ar}), 10.21 (s, 1H, NH). ¹³C NMR (63 MHz, DMSO-d₆): δ 11.4 (CH₃CH₂), 16.5 (N=CCH₃), 19.2 (CH₃), 44.2 (CH_{thiaz}), 94.8 (HOC=C), 103.7 (CH_{pyr}), 118.5-123.4-128-137.9 (C₆H₅), 158.7 (N=CCH₃), 162 (CCH₃), 163.6 (C=O_{pyr}), 167.5 (N=CS), 169.9 (COH), 173.1 (CONH₂), 179.8 (C=O_{thia}). MS m/z (%) = 442 (M⁺, 11), 166 (100), 151 (45), 77 (67). HRMS m/z calcd for C₂₁H₂₃N₄O₅S [M+H]⁺: 443.48818. Found: 443.48820.

2-(2-{2-[1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl) ethylidene]hydrazinyl}-4-oxo-1,3-thiazolidin-5-yl) acetamide (9e).

White solid, yield 54%, mp 256-257 °C. IR (KBr, ν/cm^{-1}): 3385 cm^{-1} (O-H), 3201; 3090 (NH_2), 1687 ($\text{C}=\text{O}$ pyr), 1730 ($\text{C}=\text{O}$ thiaz), 1599 ($\text{NC}=\text{O}$), 1357 (NCS). ^1H NMR (250 MHz, DMSO-d_6): δ 2.13 (s, 3H, CH_3_{pyr}), 2.68 (s, 3H, $\text{N}=\text{CCH}_3$), 2.74 (dd, H_a , $^3J_{\text{HH}} = 9$ Hz, $^2J_{\text{HH}} = 17$ Hz), 3.00 (dd, H_b , $^3J_{\text{HH}} = 4$ Hz, $^2J_{\text{HH}} = 17$ Hz), 4.53 (dd, H_x , $^3J_{\text{HH}} = 4$ and 9 Hz), 5.93 (s, 1H, CH_{pyr}), 7.13, 7.56 (s, 2H, NH_2), 12.14 (s, 1H, OH). ^{13}C NMR (250 MHz, DMSO-d_6): δ 16.4 ($\text{N}=\text{CCH}_3$), 19.2 (CH_3), 37 (CH_2CONH_2), 45.8 (CH_{thiaz}), 94.4 ($\text{HOC}=\text{C}$), 104.2 (CH_{pyr}), 159.8 ($\text{N}=\text{CCH}_3$), 161.5 (CCH_3) 163.5 ($\text{C}=\text{O}_{\text{pyr}}$), 168.4 ($\text{N}=\text{CS}$), 170.5 (COH), 174.9 (CONH_2), 180.3 ($\text{C}=\text{O}_{\text{thia}}$). MS m/z (%)= 338 (M^+ , 15), 280 (68), 166 (40), 151 (100), 58 (57). HRMS m/z calcd for $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}_5\text{NaS}$ [$\text{M}+\text{Na}$] $^+$: 361.05771. Found: 361.05774.

2-(2-{2-[1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl) ethylidene] hydrazinyl}-4-oxo-1,3-thiazolidin-5-yl)-*N*-methylacetamide (9f).

White solid, yield 48%, mp 192-193 °C. IR (KBr, ν/cm^{-1}): 3312 (O-H), 3085 (N-H), 1657 (C=O pyr), 1737 (C=O thiaz), 1556 (NC=O), 1362 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 2.13 (s, 3H, $\text{CH}_{3\text{pyr}}$); 2.25 (d, 3H, $^3J_{\text{HH}} = 4\text{ Hz}$, NCH_3), 2.60 (s, 3H,

N=CCH₃), 2.75 (dd, H_a, ³J_{HH}= 9 Hz, ²J_{HH}= 17 Hz), 3.01 (dd, H_b, ³J_{HH}= 4 Hz, ²J_{HH}= 17 Hz), 4.54 (dd, H_x, ³J_{HH}= 4 and 9 Hz), 5.94 (s, 1H, CH_{pyr}), 8.04 (q, 1H, ³J_{HH}= 4 Hz, NH), 12.14 (1, s, OH). ¹³C NMR (63 MHz, DMSO-d₆): 15.7 (N=CCH₃), 19.8 (CH₃), 25.2 (CH₃NH), 36.9 (CH₂CONH₂), 45.7 (CH_{thiaz}), 94.2 (HOC=C), 104.2 (CH_{pyr}), 159.4 (N=CCH₃), 162.1 (CCH₃), 163.6 (C=O_{pyr}), 168.2 (N=CS), 169.3 (COH), 174.9 (CONH₂), 180 (C=O_{thia}). MS *m/z* (%)= 352 (M⁺, 13), 280 (51), 166 (48), 151 (100), 73 (62). HRMS *m/z* calcd for C₁₄H₁₇N₄O₅S [M+H]⁺: 353.36564. Found: 353.36562.

***N*-Ethyl-2-(2-{2-[1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethyldene]hydrazinyl}-4-oxo-1, 3-thiazolidin-5-yl) acetamide (**9g**).**

White solid, yield 46%, mp 210°C; IR (KBr, ν cm^{-1}): 3309 (O-H), 3093 (N-H), 1634 (C=O_{pyr}), 1713 (C=O_{thiaz}), 1556 (NC=O), 1363 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 1.02 (t, 3H, $^3J_{\text{HH}} = 7\text{ Hz}$, CH₃CH₂), 2.13 (s, 3H, CH₃_{pyr}), 2.61 (s, 3H, N=CCH₃), 2.81 (dd, H_a, $^3J_{\text{HH}} = 9\text{ Hz}$, $^2J_{\text{HH}} = 17\text{ Hz}$), 2.94 (dd, H_b, $^3J_{\text{HH}} = 4\text{ Hz}$, $^2J_{\text{HH}} = 17\text{ Hz}$), 3.09 (q, 2H, $^3J_{\text{HH}} = 7\text{ Hz}$, CH₂CH₃), 4.52 (dd, H_x, $^3J_{\text{HH}} = 4$ and 9 Hz), 5.94 (s, H, CH_{pyr}), 8.09 (t, 1H, $J_{\text{HH}} = 5\text{ Hz}$, NH), 12.15 (s, 1H, OH). ^{13}C NMR (63 MHz, DMSO- d_6): δ 14 (CH₃CH₂), 16.2 (N=CCH₃), 18.9 (CH₃), 38 (CH₂CONH₂), 45.9 (CH_{thiaz}), 95.2 (HOC=C), 104.5 (CH_{pyr}), 159.5 (N=CCH₃), 163.7 (C=O_{pyr}), 167.4 (N=C_S), 168.1 (COH), 174.7 (CONH₂), 180.5 (C=O_{thiaz}). MS m/z = 366 (M^+ , 9): 280 (61), 166 (43), 151 (100), 87 (71). HRMS m/z calcd for C₁₅H₁₉N₄O₅S ($\text{M}+\text{H}$)⁺: 367.39222. Found: 367.39219.

2-(2-{2-[1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl) ethylidene] hydrazinyl}-4-oxo-1,3-thiazolidin-5-yl)-*N*-phenylacetamide (9h).

White solid, yield 51%, mp 190-191 °C. IR (KBr, ν/cm^{-1}): 3308 (O-H), 3147 (N-H), 1613 (C=O pyr), 1698 (C=O thiaz), 1557 (NC=O), 1357 (NCS). ^1H NMR (250 MHz, DMSO- d_6): δ 2.12 (s, 3H, CH_3_{pyr}), 2.61 (s, 3H, $\text{N}=\text{CCH}_3$), 3.09 (dd, H_a , $^3J_{\text{HH}} = 9$ Hz, $^2J_{\text{HH}} = 17$ Hz), 3.06 (dd, H_b , $^3J_{\text{HH}} = 4$ Hz, $^2J_{\text{HH}} = 17$ Hz), 4.67 (dd, H_x , $^3J_{\text{HH}} = 4$ and 9 Hz),

5.90 (s, 1H, CH_{pyr}), 6.97, 7.56 (m, 5H, H_{Ar}), 10.20 (s, 1H, NH), 12.25 (1s, 1H_x, OH).
¹³C NMR (63 MHz, DMSO-d₆): 11.4 (N=CCH₃), 19.23 (CH₃), 30.9 (CH₂CONH₂),
 45.2 (CH_{thiaz}), 95 (HOC=C), 103.6 (CH_{pyr}), 128- 128.8- 129-134.5 (C_{Ar}), 159.9
 (N=CCH₃), 161.6 (CH₃), 163.6 (C=O_{pyr}), 167.7 (N=CS), 168.1 (COH), 174.7
 (CONH₂), 180 (C=O_{thia}). MS *m/z* (%) = 414 (M⁺, 9), 280 (59), 166 (48), 15 (100), 77
 (87). HRMS *m/z* calcd for C₁₉H₁₉N₄O₅S [M+H]⁺: 415.43502. Found: 415.43505.

Antimicrobial activity

Materials and methods

The synthesized chemicals compound **3a-d**, **5a-d**, **7a-d** and **9a-h** were subjected of *in vitro* evaluation of their anti microbial activity against five human pathogens microorganisms.

Microorganisms culture

Bacterial and Yeast strains were derived from MUSTAPHA BACHA hospital of Algiers (Algeria), namely: *Escherichia coli* ATCC 25992, *Pseudomonas aeruginosa* ATCC 27852, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923 and *Candida albicans*. All the strains were seed in nutrient broth and incubated at 37°C for 24 h.

Well diffusion assay

Agar diffusion assay is used to determine the anti microbial activity of molecules cited above. The technique works well with defined inhibitors. Mueller-Hinton agar (Difco) prepared was poured in the Petri dish and 24 h old cultures of *Escherichia coli* ATCC 25992, *Pseudomonas aeruginosa* ATCC 27852, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923 and the Yeast *Candida albicans* were swabbed on it. The wells (4 mm diameter) were made by using the shank of Pasteur pipette. Thereafter, 50 μ l of each synthesized compound solution (concentration 0.5 mg/ml in DMSO) were added in the respective wells. The DMSO was served as control. The plates were then incubated at 37°C for 24 h. The diameter of the inhibition was measured.

Results of Antimicrobial activity of synthesized compounds (Table S-1).

Table S-1. Antimicrobial activity of synthesized compounds (diameter zones in mm).

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi
	Staphylococcus aureus (ATCC-43300)	Staphylococcus aureus (ATCC-25923)	Escherichia coli (ATCC-25992)	Pseudomonas aeruginosa (ATCC-27852)	Candida albicans
3a	Bactericidal activity	-	12	-	20
3b	Bactericidal activity	-	-	20	18
3c	-	-	-	17	-
3d	-	-	-	20	18
5a	-	-	-	-	-
5b	-	-	-	-	-
5c	-	-	-	-	-
5d	-	-	-	-	-
7a	-	20	-	-	-
7b	17	-	-	-	14
7c	Bactericidal activity	28	13	25	-
7d	26	22	-	13	22
9a	Bactericidal activity	-	14	15	-
9b	Bactericidal activity	-	-	10	12
9c	Bactericidal activity	-	15	18	-
9d	Bactericidal activity	-	10	-	-
9e	Bactericidal activity	-	-	25	-
9f	15	-	-	-	14
9g	Bactericidal activity	-	-	-	-
9h	Bactericidal activity	-	10	-	-

Photos of the antimicrobial test

3a corresponding to the reference number 9,

3b corresponding to the reference number 12

3c corresponding to the reference number 10

3d corresponding to the reference number 11

Compound	Reference of biological test
5a	1
5b	4
5c	2
5d	3
7a	13
7b	16
7c	14
7d	15
9a	5
9b	6
9c	7
9d	8
9e	17
9f	18
9g	19
9h	20