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Original article

Synthesis, antibacterial and antifungal activity of some new thiazolylhydrazone derivatives containing 3-substituted cyclobutane ring

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Abstract

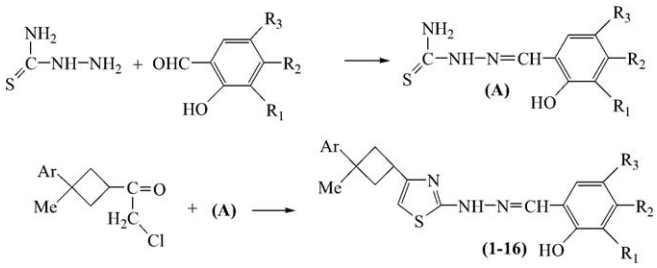
A series of Schiff bases, combining 2,4-disubstituted thiazole and cyclobutane rings, and hydrazone moieties in the same molecule, was synthesized, characterized and evaluated for screening antibacterial and antifungal activities on microorganisms, respectively, on four bacteria and *Candida tropicalis*. The structures of original compounds were confirmed by analytical and spectroscopic (FT-IR, ^{1}H NMR and ^{13}C NMR) methods and elemental analysis. Both the antibacterial and antifungal activities and MIC values of compounds were reported. Among the tested compounds, the most effective compound providing a MIC value of 16 μg ml^{-1} are **2** against *C. tropicalis* and *Bacillus subtilis* and **3** against *B. subtilis* only.

Keywords: Cyclobutane; Thiosemicarbazone; Antibacterial activity; Antifungal activity; MIC

1. Introduction

Since it is a common agreement of multidrug-resistant bacteria are the major cause of failure in the treatment of infectious diseases, the need for the synthesis of novel antibiotics is a reality. N^{1}-2-(thiazolyl)sulfanilamide (sulfathiazole) which contains 2-aminothiazole ring and exhibiting herbicidal and anti-inflammatory [1,2], antimicrobial [3] or antiparasitic activity [4] is a well-known compound was used as an early antibiotic agent since the late 1930s [5,6]. Thiazole itself and its derivatives are of importance in biological systems as anti-inflammatory, analgesic agents and inhibitors on lipoxygenase activities [7,8]. On the other hand, cyclobutane amino acids in different structures were described as highly potent l-glutamate, *N*-methyl-D-aspartate (NMDA) agonist, NMDA antagonists and anticonvulsive drugs [9–11]. The effective role of azomethine linkage in certain biological reactions [12] is well documented. Simple stable molecules containing the hydroxamic acid [13] functionality as well as methoxy-alkylthiazoles and 6-hydroxybenzothiazoles [14] were reported as LOs inhibitors.

Prompted by the observed biological activities of the above mentioned derivatives and in continuation of our ongoing studies on novel biologically active molecules, we have designed and synthesized some new hydrazone derivatives, bearing cyclobutane, thiazole and azomethine functionality in the same molecule, as potential antibacterial and antifungal agents.



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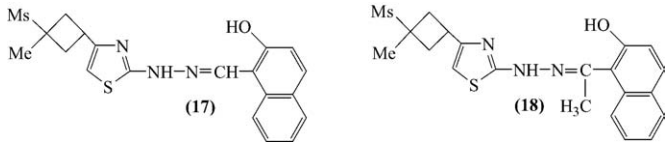
Substance	Ar	R_1	R_2	R_3
1	Phenyl (ph)	H	H	H
2	Phenyl	H	OH	H
3	Phenyl	H	H	Br
4	Phenyl	H	OCH_3	H
5	*p*-Xylyl (*p*-x)	H	H	H
6	*p*-Xylyl	H	OH	H
7	*p*-Xylyl	OCH_3	H	H
8	Mesityl (Ms)	H	H	H
9	Mesityl	H	OH	H
10	Mesityl	H	H	Br
11	Mesityl	OCH_3	H	H
12	Mesityl	H	H	OCH_3
13	Mesityl	H	H	NO_2
14	Mesityl	H	H	Cl
15	Mesityl	Cl	H	Cl
16	Mesityl	Br	H	Br

2. Chemistry

Synthetic process for the synthesis of the target compounds is outlined in Scheme 1. As known in multi-stage reactions, such as the synthesis of our compounds, the reaction sequences are important phenomenon. It is possible that the direct reaction of an α-haloketone with thiosemicarbazide affords several co-products and the separation of these substances is a tedious and time consuming process. Instead of this, in the first stage we have synthesized azomethine compounds (thiosemicarbazones), **A**. Subsequently, they have been converted into their hydrazone compounds by the reaction of α-haloketones. Such a modification of literature process produces no co-products and separations are almost not necessary. The application of the modified method is given in Section 3.

The synthesis of the compounds **1**–**18** was performed in two steps as shown in Scheme 1. In the first step, desired thiosemicarbazones were obtained by heating thiosemicarbazide with appropriate aldehydes at 50–60 °C with continuous stirring in the presence of a few drops of *p*-toluenesulfonic acid as catalyst. In the second step, appropriate thiosemicarbazone was reacted with appropriate α-chloroketone to convert into its hydrazide derivatives containing 2,4-disubstituted thiazole and cyclobutane rings in the presence of $NaHCO_3$ or K_2CO_3 as neutralizing agent at 60–70 °C for about 5 min. Substances **2**, **6**, **15** and **16** are fully novel. The structures of the compounds synthesized here were supported by the spectral data of IR, ^{1}H NMR, ^{13}C NMR spectra and elemental analysis results which are in agreement with the proposed structures. Physical and spectral data of the compounds are given in Section 3.



Scheme 1. Synthesis and the structures of the compounds.

3. Experimental

3.1. Chemistry

Aldehydes and thiosemicarbazide were purchased from Merck (pure) and were used without further purification. α-Chloroketones, 1-phenyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane, 1-*p*-xylyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane and 1-mesityl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane were prepared according to the previously published procedure in [15]. Solvents were of analytical grade and purified by standard methods where necessary. Microanalyses were performed on a LECO CHNSO-932 auto elemental analysis apparatus. IR spectra were recorded on a Mattson 1000 (Unicam Ltd., Cambridge, UK) Fourier transform-infrared (FT-IR) Spectrometer using KBr pellets in the range 4000–400 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian-Gemini 200 MHz at 50.34 MHz spectrometer. Melting points were determined on a Gallenkamp melting point apparatus and checked by differential scanning calorimetry (DSC) and are uncorrected.

3.2. Synthesis of the compounds (*1*–*18*)

General method: To a solution of thiosemicarbazide (2.275 g, 25.0 mmol) and 0.05 g of *p*-toluenesulfonic acid as catalyst in 50 ml absolute EtOH, a solution of appropriate salicylaldehyde (25.0 mmol) in 20 ml absolute EtOH were added dropwise at 60–70 °C with continuous stirring. Expected solid product was formed about half an hour period. The course of the reaction was monitored by IR spectroscopy. After completing this reaction, the mixture of appropriate α-chloroketone (25.0 mmol) and K_2CO_3 (12.5 mmol) dissolved in anhydrous ethanol (50 ml) was added and heated at 60–70 °C with continuous stirring and monitoring the course of the reaction with IR. Monitoring the visibility of the carbonyl and $–CH_2–Cl$ groups of α-chloroketones is easily done and then it is very easy to determine when the reaction is complete. At the end of the reaction period and cooling to the room temperature the solution neutralized with 5% aqueous ammonia (if necessary), the solid product thus formed was filtered off, washed with copious water several times, dried in air and crystallized from aqueous EtOH to give compounds **1**–**18** [16–19].

3.2.1. 2-Hydroxybenzaldehyde [4-(3-methyl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (*1*)

Pale yellow crystals. Yield: 64%. M.p.: 194 °C (EtOH). IR (KBr, υ cm^{-1}): 3182 (–OH), 3131 (–NH–), 1625 (C=N azomethine). ^{1}H NMR ($CDCl_3$, TMS, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.47–2.51 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.57 (quint, $j = 8.9$ Hz, 1H, > CH– in cyclobutane ring), 6.06 (s, 1H, = CH–S in thiazole ring), 6.86–7.34 (m, 9H, aromatics), 8.12 (s, 1H, azomethine), 10.0–10.6 (br, 2H, –NH– plus –OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, TMS, δ ppm): 171.42, 159.82, 153.64, 153.20, 149.87, 132.97, 132.24, 130.30, 127.53. 126.57, 121.53, 120.05, 118.87, 101.63, 42.02,

40.97, 32.07, 31.73. Anal. calc. for $C_{21}H_{21}N_3OS$ (363.48); C: 69.39, H: 5.82, N: 11.56; found; C: 69.71, H: 6.03, N: 11.34.

3.2.2. 2,4-Dihydroxybenzaldehyde [4-(3-methyl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (2)

Orange crystals. Yield: 74%. M.p.: 173 °C (EtOH). IR (KBr, υ cm^{-1}): 3229 (*o*-OH), 3452 (*p*-OH), 3126 (–NH–), 1631 (C=N azomethine). ^1H NMR ($CDCl_3$, TMS, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.50 (d, j = 8.7 Hz, 4H, $–CH_2–$ in cyclobutane ring), 3.53 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane ring), 6.03 (s, 1H, = CH–S in thiazole ring), 6.50–7.30 (m, 9H, aromatics), 7.94 (s, 1H, azomethine), 9.96 (br, 1H, –NH–, D_2O exchangeable), 11.32 (s, 1H, *o*-OH, D_2O exchangeable), 7.43 (s, 1H, *p*-OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, TMS, δ ppm): 173.62, 158.72, 154.24, 153.61, 149.39, 133.07, 132.64, 129.30, 128.63. 127.07, 122.53, 120.45, 119.11, 101.55, 42.12, 41.37, 32.47, 31.64. Anal. calc. for $C_{21}H_{21}N_3O_2S$ (379.48); C: 66.47, H: 5.58, N: 11.07, S: 8.45; found; C: 66.03, H: 5.64, N: 10.87, S: 8.18.

3.2.3. 5-Bromo-2-hydroxybenzaldehyde [4-(3-methyl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (3)

Yellow crystals. Yield: 62%. M.p.: 201 °C (EtOH). IR (KBr, υ cm^{-1}): 3182 (–OH), 3131 (–NH–), 1625 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.50 (s, 3H, $–CH_3$), 2.41–2.62 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.54 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 6.05 (s, 1H, = CH– in thiazole ring), 6.91–7.27 (m, 8H, aromatics plus –NH–, D_2O exchangeable), 8.0 (s, 1H, N=CH–), 12.4 (s, 1H, –OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 167.10, 155.13, 152.70, 142.20, 131.41, 130.33, 127.06, 124.17, 123.80, 123.63, 119.87, 117.32, 109.87, 99.12, 39.00, 38.02, 37.55, 29.18. Anal. calc. for $C_{21}H_{20}N_3OSBr$ (442.38); C: 57.02, H: 4.56, N: 9.50; found; C: 56.69, H: 4.41, N: 9.67.

3.2.4. 2-Hydroxy-3-methoxybenzaldehyde [4-(3-phenyl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (4)

Yellow crystals. Yield: 68%. M.p.: 159 °C (EtOH). IR (KBr, υ cm^{-1}): 3416 (–OH), 3126 (–NH–), 1580 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.41 (s, 3H, $–CH_3$), 2.46–2.59 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.42 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 3.88 (s, 3H, $–OCH_3$), 5.98 (s, 1H, = CH– in thiazole ring), 6.81–7.37 (m, 8H, aromatics), 8.14 (s, 1H, N=CH–), 9.73 (br, 2H, –OH plus –NH–, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 171.07, 153.64, 149.11, 149.87, 147.16, 136.23, 130.30, 127.53, 126.57, 120.44, 120.37, 113.96, 113.81, 101.63, 57.68, 42.02, 40.97, 32.07, 31.73. Anal. calc. for $C_{22}H_{23}N_3O_2S$ (393.50); C: 67.2, H: 5.9, N: 10.7; found; C: 67.0, H: 6.1, N: 10.5.

3.2.5. 2,4-Dihydroxybenzaldehyde [4-(3-p-xylyl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (5)

Yellow crystals. Yield: 75%. M.p.: 168 °C (EtOH). IR (KBr, υ cm^{-1}): 3182 (–OH), 3113 (–NH–), 1619 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.19 (s, 3H, *p*-xylyl *o*-CH_3), 2.29 (s, 3H, *p*-xylyl *m*-CH_3), 2.55 (d, 4H, j = 9.0 Hz, $–CH_2–$ in cyclobutane ring), 3.54 (quint, j = 7.0 Hz, 1H, > CH– in cyclobutane), 6.07 (s, 1H, = CH– in thiazole ring), 6.79–7.28 (m, 6H, aromatics), 8.12 (s, 1H, N=CH–), 10.75–11.45 (br, 2H, –OH plus –NH–, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 171.02, 159.83, 153.39, 150.82, 149.83, 137.29, 133.26, 133.17, 132.96, 132.26, 129.81, 128.22, 121.51, 120.05, 118.86, 101.73, 43.06, 41.86, 32.14, 29.52, 22.99, 21.49. Anal. calc. for $C_{23}H_{25}N_3OS$ (391.54); C: 70.56, H: 6.44, N: 10.73; found; C: 70.85, H: 6.05, N: 10.89.

3.2.6. 2,4-Dihydroxybenzaldehyde [4-(3-p-xylxl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (6)

Orange crystals. Yield: 69%. M.p.: 174 °C (EtOH). IR (KBr, υ cm^{-1}): 3272 (*o*-OH), 3450 (*p*-OH), 3121 (–NH–), 1631 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.18 (s, 3H, *o*-CH_3), 2.25 (s, 3H, *m*-CH_3), 2.52 (d, j = 8.9 Hz, 4H, $–CH_2–$ in cyclobutane ring), 3.55 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 6.04 (s, 1H, = CH– in thiazole ring), 6.30–7.30 (m, 6H, aromatics), 10.02 (s, 1H, –NH–, D_2O exchangeable), 7.95 (s, 1H, N=CH–), 11.52 (s, 1H, *o*-OH, D_2O exchangeable), 7.20 (s, 1H, *p*-OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 166.13, 154.43, 151.77, 141.29, 130.41, 130.34, 127.46, 123.87, 123.70, 123.65, 120.87, 118.32, 110.27, 99.42, 38.77, 38.22, 37.44, 28.78. Anal. calc. for $C_{23}H_{25}N_3O_2S$ (407.54); C: 67.79, H: 6.18, N: 10.31, S: 7.87; found; C: 67.62, H: 6.15, N: 9.87, S: 8.02.

3.2.7. 2-Hydroxy-3-methoxybenzaldehyde [4-(3-p-xylyl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (7)

Light yellow crystals. Yield: 62%. M.p.: 152 °C (EtOH). IR (KBr, υ cm^{-1}): 3248 (–OH), 3184 (–NH–), 1574 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.17 (s, 3H, *p*-xylyl *o*-CH_3), 2.25 (s, 3H, *p*-xylyl *m*-CH_3), 2.51–2.57 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.79 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 3.81 (s, 3H, $–OCH_3$), 6.44 (s, 1H, = CH– in thiazole ring), 6.91–7.28 (m, 6H, aromatics), 8.21 (s, 1H, N=CH–), 9.48 (br, 1H, –NH–, D_2O exchangeable), 11.98 (br, 1H, –OH, D_2O exchangeable). ^{13}H NMR ($CDCl_3$, δ ppm): 169.76, 150.88, 149.73, 147.43, 136.25, 132.70, 132.59, 127.88, 127.73, 122.13, 120.93, 120.73, 120.37, 114.44, 114.37, 101.70, 57.69, 42.82, 32.07, 29.38, 22.55, 22.46, 20.93. Anal. calc. for $C_{24}H_{27}N_3O_2S$ (421.57); C: 68.4, H: 6.5, N: 10.0; found; C: 68.5, H: 6.2, N: 9.6.

3.2.8. 2-Hydroxybenzaldehyde [4-(3-mesityl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (8)

Pale yellow crystals. Yield: 82%. M.p.: 225 °C (EtOH). IR (KBr, υ cm^{-1}): 3285 (–OH), 3131 (–NH–), 1651 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.17 (s, 6H, *mesityl o*-CH_3), 2.41 (s, 3H, *mesityl p*-CH_3), 2.50–2.62 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.68 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 6.06 (s, 1H, = CH– in

thiazole ring), 6.86–7.34 (m, 5H, aromatics), 8.12 (s, 1H, N=CH–), 10.2 (br, 1H, –NH–, D_2O exchangeable), 10.11 (br, 1H, –OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 171.42, 159.82, 153.20, 148.38, 145.63, 136.99, 135.38, 132.97, 132.44, 132.24, 121.53, 120.05, 118.87, 101.63, 45.15, 42.88, 32.07, 26.45, 23.38, 22.42. Anal. calc. for $C_{24}H_{27}N_3OS$ (405.57); C: 71.2, H: 6.6, N: 10.4; found; C: 71.1, H: 6.7, N: 10.4.

*3.2.9. 2,4-Dihydroxybenzaldehyde [4-(3-mesityl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**9**)*

Light brown crystals. Yield: 65%. M.p.: 160 °C (EtOH). IR (KBr, υ cm^{-1}): 3416 (–OH), 3126 (–NH–), 1631 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.53 (s, 3H, $–CH_3$), 2.16 (s, 6H, *mesityl o*-CH_3), 2.45 (s, 3H, *mesityl p*-CH_3), 2.58–2.66 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.32 (quint, 1H, j = 8.9 Hz, > CH– in cyclobutane), 6.31 (s, 1H, = CH– in thiazole ring), 6.34–7.35 (m, 5H, aromatics), 8.09 (s, 1H, N=CH–), 9.85 (br, 1H, –NH–, D_2O exchangeable), 11.65 (br, 1H, –OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 169.58, 161.64, 159.71, 148.38, 145.98, 144.00, 136.43, 136.27, 135.72, 130.92, 109.55, 108.41, 104.28, 44.90, 42.62, 32.23, 26.38, 22.83, 22.70. Anal. calc. for $C_{24}H_{27}N_3O_2S$ (421.57); C: 68.5, H: 6.5, N: 9.7; found; C: 68.4, H: 6.5, N: 10.0.

*3.2.10. 5-Bromo-2-hydroxybenzaldehyde [4-(3-mesityl-3-phenylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**10**)*

Pale yellow crystals. Yield: 87%. M.p.: 230 °C (EtOH). IR (KBr, υ cm^{-1}): 3285 (–OH), 3131 (–NH–), 1600 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.49 (s, 3H, $–CH_3$), 2.14 (s, 6H, *mesityl o*-CH_3), 2.39 (s, 3H, *mesityl p*-CH_3), 2.51–2.59 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.31 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 5.96 (s, 1H, = CH– in thiazole ring), 6.70–7.37 (m, 5H, aromatics), 8.02 (s, 1H, N=CH–), 10.10 (br, 1H, –NH–, D_2O exchangeable), 10.25 (br, 1H, –OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 171.26, 158.80, 152.37, 148.35, 145.63, 136.99, 136.92, 135.38, 134.19, 132.44, 121.80, 120.73, 113.05, 101.68, 45.15, 42.88, 32.24, 26.45, 23.38, 22.42. Anal. calc. for $C_{24}H_{26}N_3OSBr$ (484.46); C: 59.5, H: 5.4, N: 8.7; found; C: 59.5, H: 5.2, N: 8.7.

*3.2.11. 2-Hydroxy-3-methoxybenzaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**11**)*

Yellow crystals. Yield: 62%. M.p.: 121 °C (EtOH). IR (KBr, υ cm^{-1}): 3256 (–OH), 3121 (–NH–), 1612 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.51 (s, 3H, $–CH_3$), 2.17 (s, 6H, *mesityl o*-CH_3), 2.47 (s, 3H, *mesityl p*-CH_3), 2.60–2.70 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.33 (quint, j = 8.9 Hz, 1H, > CH– in cyclobutane), 3.80 (s, 3H, $–OCH_3$), 6.44 (s, 1H, = CH– in thiazole ring), 6.73–7.20 (m, 5H, aromatics), 8.21 (s, 1H, N=CH–), 9.51 (br, 1H, –NH–, D_2O exchangeable), 11.93 (br, 1H, –OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 159.71, 149.66, 147.39, 146.44, 145.99, 135.49, 132.00, 131.68, 131.27, 122.13, 120.94, 120.32, 114.00, 102.77, 57.67, 44.96, 42.64, 32.27, 26.40, 22.84, 22.76. Anal. calc. for $C_{25}H_{29}N_3O_2S$ (435.60); C: 68.9, H: 6.7, N: 9.7; found; C: 69.3, H: 6.6, N: 9.5.

*3.2.12. 2-Hydroxy-5-methoxybenzaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**12**)*

Orange crystals. Yield: 94%. M.p.: 231 °C (EtOH). IR (KBr, υ cm^{-1}): 3310 (–OH), 3125 (–NH–), 1584 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.53 (s, 3H, $–CH_3$), 2.17 (s, 9H, *mesityl o*-CH_3 + *mesityl p*-CH_3), 2.51–2.62 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.39 (quint, 1H, j = 7.0 Hz, > CH– in cyclobutane), 3.71 (s, 3H, $–OCH_3$), 6.42 (s, 1H, = CH– in thiazole ring), 6.72–7.11 (m, 5H, aromatics), 8.17 (s, 1H, N=CH–), 9.65 (br, 1H, –NH–, D_2O exchangeable), 11.12 (br, 1H, –OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 167.54, 155.74, 154.02, 151.64, 145.98, 138.43, 135.50, 131.87, 131.45, 121.97, 118.76, 118.64, 112.10, 109.20, 57.15, 42.57, 41.51, 40.06, 26.36, 22.84, 21.85. Anal. calc. for $C_{25}H_{29}N_3O_2S$ (435.58); C: 47.99, H: 4.92, N: 18.65; found; C: 48.11, H: 5.02, N: 18.27.

*3.2.13. 2-Hydroxy-5-nitrobenzaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**13**)*

Yellow crystals. Yield: 81%. M.p.: 222 °C (EtOH). IR (KBr, υ cm^{-1}): 3265 (–OH), 3114 (–NH–), 1619 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.52 (s, 3H, $–CH_3$), 2.15 (s, 6H, *mesityl o*-CH_3), 2.18 (s, 3H, *mesityl p*-CH_3), 2.47–2.61 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.43 (quint, 1H, j = 8.9 Hz, > CH– in cyclobutane), 6.45 (s, 1H, = CH– in thiazole ring), 6.71 (s, 2H, aromatics on mesityl), 7.05 (d, j = 9.0 Hz, 1H aromatics), 8.13 (dd, j_o = 9.0 Hz; j_m = 2.9 Hz, 1H, aromatics), 8.48 (d, j = 2.9, 1H aromatics), 8.21 (s, 1H, N=CH–), 12.11 (br, 2H, –OH plus –NH–, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 169.72, 153.09, 145.95, 141.82, 136.43, 136.11, 135.50, 131.88, 131.67, 127.27, 123.27, 123.18, 123.03, 110.41, 44.54, 42.55, 40.04, 28.34, 22.82, 21.84. Anal. calc. for $C_{24}H_{26}N_4O_3S$ (450.55); C: 64.0, H: 5.8, N: 12.4; found; C: 64.1, H: 5.7, N: 12.5.

*3.2.14. 2-Hydroxy-5-chlorobenzaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**14**)*

Pink crystals. Yield: 83%. M.p.: 240 °C (EtOH). IR (KBr, υ cm^{-1}): 3283 (–OH), 3115 (–NH–), 1619 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.53 (s, 3H, $–CH_3$), 2.12 (s, 6H, *mesityl o*-CH_3), 2.16 (s, 3H, *mesityl p*-CH_3), 2.47–2.87 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.40 (quint, 1H, j = 8.9, > CH– in cyclobutane), 6.44 (s, 1H, = CH– in thiazole ring), 6.72 (s, 2H, aromatics on mesityl), 6.89 (d, j = 8.7, 1H, aromatics), 7.21 (dd, j_o = 8.7 Hz; j_m = 2.7 Hz, 1H aromatics), 7.58 (d, j = 2.9 Hz, 1H, aromatics), 8.14 (s, 1H, N=CH–), 10.45 (br, 1H, –OH D_2O exchangeable), 12.06 (br, 1H, –NH–, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 164.13, 156.40, 123.82, 131.88, 145.86, 144.45, 136.51, 136.17, 135.51, 131.38, 130.63, 124.94, 119.66, 102.69, 42.55, 42.13, 40.45, 26.35, 22.85, 21.86. Anal. calc. for $C_{24}H_{26}ClN_3OS$ (440.0); C: 65.5, H: 6.0, N: 9.6; found; C: 65.6, H: 6.1, N: 9.5.

*3.2.15. 2-Hydroxy-3,5-dichlorobenzaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**15**)*

Pink crystals. Yield: 83%. M.p.: 228 °C (EtOH). IR (KBr, υ cm^{-1}): 3280 (–OH), 3115 (–NH–), 1614 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.53 (s, 3H, $–CH_3$), 2.18 (s, 6H, *mesityl o*-CH_3), 2.16 (s, 3H, *mesityl p*-CH_3), 2.42–2.59 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.38 (quint, 1H, $j = 8.9$, > CH– in cyclobutane), 6.41 (s, 1H, = CH– in thiazole ring), 6.72 (s, 2H, aromatics mesityl), 7.52 (d, $j_m = 2.0$ Hz, 1H), 7.56 (d, $j_m = 2.1$, 1H) 8.23 (s, 1H, N=CH–), 10.20 (br, 2H, –NH– plus –OH, D_2O exchangeable). Characteristic ^{13}C NMR ($CDCl_3$, TMS, δ ppm): 123.38 (C_1), 145.78 (C_2), 114.24 (C_3), 128.12 (C_4), 117.24 (C_5), 125.15 (C_6), 135.52 (C_7), 152.71 (C_8), 103.21 (C_9), 136.39 (C_{10}), 40.71 (C_{11}), 41.91 (C_{12}), 44.64 (C_{13}), 26.29 (C_{14}), 131.70 (C_{15}), 128.18 (C_{16}), 124.31 (C_{17}), 130.75 (C_{18}), 21.86 (C_{19}), 22.84 (C_{20}). Anal. calc. for $C_{24}H_{25}Cl_2N_3OS$ (474.45); C: 60.76, H: 5.31, N: 8.86, S: 6.76; found; C: 60.51, H: 4.94, N: 9.02, S: 6.89.

*3.2.16. 2-Hydroxy-3,5-dibromobenzaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**16**)*

Light yellow crystals. Yield: 83%. M.p.: 237 °C (EtOH). IR (KBr, υ cm^{-1}): 3265 (–OH), 3115 (–NH–), 1614 (C=N azomethine). ^1H NMR ($CDCl_3$, δ ppm): 1.47 (s, 3H, $–CH_3$), 2.12 (s, 6H, *mesityl o*-CH_3), 2.21 (s, 3H, *mesityl p*-CH_3), 2.53 (d, $j = 8.7$ Hz, 4H, $–CH_2–$ in cyclobutane ring), 3.26 (quint, 1H, $j = 8.9$ Hz, > CH– in cyclobutane), 5.93 (s, 1H, = CH– in thiazole ring), 6.69 (s, 2H, aromatics mesityl), 7.19 (d, $j_m = 2.3$, 1H), 7.63 (d, $j_m = 2.2$ Hz, 1H) 8.03 (s, 1H, N=CH–), 10.20 (br, 2H, –NH– plus –OH, D_2O exchangeable). Characteristic ^{13}C NMR ($CDCl_3$, TMS, δ ppm): 122.47 (C_1), 151.22 (C_2), 112.95 (C_3), 133.30 (C_4), 113.57 (C_5), 132.85 (C_6), 145.39 (C_7), 155.55 (C_8), 101.39 (C_9), 149.78 (C_{10}), 31.92 (C_{11}), 42.89 (C_{12}), 45.03 (C_{13}), 28.42 (C_{14}), 137.72 (C_{15}), 136.89 (C_{16}), 132.46 (C_{17}), 137.06 (C_{18}), 22.46 (C_{19}), 23.38 (C_{20}). Anal. calc. for $C_{24}H_{25}Br_2N_3OS$ (563.35); C: 51.17, H: 4.47, N: 7.46, S: 5.69; found; C: 51.40, H: 4.58, N: 7.35, S: 5.53.

*3.2.17. 2-Hydroxy-1-naphthaldehyde [4-(3-mesityl-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**17**)*

Yellow crystals. Yield: 94%. M.p.: 221 °C (EtOH). IR (KBr, υ cm^{-1}): 3430 (–OH), 3151 (–NH–), 1624 (C=N azomethine). ^1H NMR (DMSO-d_6, δ ppm): 1.53 (s, 3H, $–CH_3$), 2.17 (s, 9H, *mesityl o*-CH_3 + *mesityl p*-CH_3), 2.45–2.66 (m, 4H, $–CH_2–$ in cyclobutane ring), 3.45 (quint, 1H, $j = 7.0$, > CH– in cyclobutane), 6.42 (s, 1H, = CH– in thiazole ring), 6.72 (s, 2H, aromatics on mesitylene), 7.20–7.87 (m, 5H, aromatics), 8.56 (d, 1H aromatics), 8.93 (s, 1H, N=CH–), 11.35 (br, 1H, –OH, D_2O exchangeable), 12.02 (br, 1H, –NH–, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 169.64, 158.25, 149.81, 145.88, 138.17, 136.43, 135.53, 133.42, 132.87, 131.71, 130.55, 129.85, 129.30, 125.02, 123.02, 120.13, 111.62, 101.84, 44.79, 41.70, 31.98, 26.31, 22.85, 21.68. Anal. calc. for $C_{28}H_{29}N_3OS$ (455.62); C: 73.81, H: 6.42, N: 9.22, S: 7.04; found; C: 74.12, H: 6.63, N: 9.36, S: 7.07.

*3.2.18. (1E)-1-(2-Hydroxy-1-naphthyl)ethanone [4-(3-amino-3-methylcyclobutyl)-1,3-thiazol-2-yl]hydrazone (**18**)*

Brown crystals. Yield: 81%. M.p.: 169 °C (EtOH). IR (KBr, υ cm^{-1}): 3429 (–OH), 3129 (–NH–), 1635 (C=N azomethine). ^1H NMR (DMSO-d_6, δ ppm): 1.51 (s, 3H, $–CH_3$), 2.15 (s, 9H, *mesityl o*-CH_3 + *mesityl p*-CH_3), 2.40–2.63 (m, 7H, $–CH_2–$ in cyclobutane ring plus $–CH_3$ on azomethine), 3.45 (quint, 1H, $j = 7.0$, > CH– on cyclobutane), 6.27 (s, 1H, = CH– in thiazole ring), 6.71 (s, 2H, aromatics on mesitylene), 7.37–7.86 (m, 6H, aromatics and D_2O exchangeable –NH–), 8.31 (m, 1H aromatics), 11.41 (br, 1H, –OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 169.87, 157.68, 150.30, 145.81, 136.39, 136.25, 135.71, 131.72, 129.14, 129.01, 127.07, 126.53, 126.42, 124.55, 124.44, 119.68, 114.62, 99.36, 44.26, 41.73, 31.05, 26.18, 22.85, 21.85, 16.03. Anal. calc. for $C_{29}H_{31}N_3OS$ (469.64); C: 74.17, H: 6.65, N: 8.95, S: 7.83; found; C: 74.22, H: 6.72, N: 9.11, S: 7.63.

3.3. Microbiology

The compounds were tested against two gram-positive (*Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633) and two gram-negative bacteria (*Salmonella typhimurium* NRRL B 4420, *Escherichia coli* ATCC 25922). The antifungal activities of compounds were evaluated in vitro against a yeast-like fungi such as *Candida tropicalis* ATCC 13803.

The cultures were obtained from Müller–Hinton broth (Difco) for all the bacterial strains after 24 h of incubation at 37 °C. The yeast was maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 °C.

Antibacterial activities of compounds were determined by using agar dilution method by the National Committee for Clinical Laboratory Standards (NCCLS). Müller–Hinton broth and Sabouraud dextrose broth were used as medium to prepare micro dilution. The final inoculum densities were $1–3 \times 10^4$ CFUs ml^{-1} for bacteria and fungi, respectively. The stock solutions were prepared in dimethyl formamide (DMF) and DMF had no effect on the microorganism in the concentrations studied.

The doubling concentrations used for both of them were 1024–4 μg ml^{-1}. Ampicillin (Mustafa Nevzat) and fluconazole (Pfizer) were used as antibiotic reference powders for bacteria and fungi, respectively. The antibacterial activity was measured after 18 h of incubation at 37 °C and after incubation for 48 h for the antifungal assay.

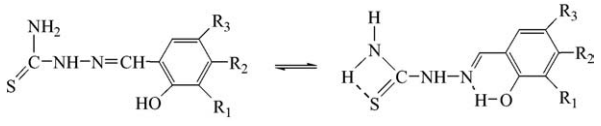
The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compound and yield no visible turbidity and growth on the plate.

4. Results and discussion

The structure of all compounds synthesized were identified by ^1H NMR, ^{13}C NMR, FT-IR and elemental analysis.

Table 1
The in vitro activities of the compounds and the reference drugs (MIC values in μg ml^{-1})

Sample	*S. aureus*	*B. subtilis*	*S. typhimurium*	*E. coli*	*C. tropicalis*
1	256	256	128	32	32
2	32	16	128	32	16
3	512	16	128	32	128
4	512	64	64	128	256
5	512	256	512	256	256
6	64	64	128	64	64
7	256	256	256	256	256
8	256	256	256	256	256
9	128	128	256	128	256
10	256	128	256	128	256
11	256	256	256	128	256
12	512	256	512	512	512
13	128	256	64	128	128
14	256	256	256	256	256
15	256	512	256	256	256
16	128	256	128	128	258
17	256	512	128	128	256
18	256	128	128	128	256
Ampicillin	2	2	2	2	–
Fluconazole	–	–	–	–	8



Scheme 2. Intramolecular hydrogen bonding illustration of hydrazones, **A**.

The ^1H NMR spectra of the starting substances **A** and ligands were recorded in DMSO-d_6 or $CDCl_3$ solvents and assignments are given in Section 3. As expected from the structures of starting substances **A**, only aromatic, –NH–, $–NH_2$, –N=CH– and –OH peaks were observed. These are all singlets and each one indicates one proton intensity, according to the integral of the spectra at 200 MHz NMR. This result implies that the thioamide part of the molecule has a hydrogen bonding (H···S), shown in Scheme 2. Two of them are broad singlets supporting our hydrogen bonding interpretation. All of these protons, except azomethine proton, are D_2O exchangeable protons. –OH group signals for both compounds are broad singlets. This is also a result of the presence of intramolecular hydrogen bonding [20]. Phenolic –OH groups of both compounds exhibit the downfield signals, according to –OH of a free phenol. Since the molecules have electron-attracting groups, this is an expected result. A more detailed spectral investigation of a similar cyclobutane compound, synthesized and published by the same authors, can be found in literature [16].

The antibacterial and antifungal activities of the substances **1**–**18** were assayed using the agar dilution method against gram-positive bacteria *S. aureus* ATCC 6538P and *B. subtilis* ATCC 6633, gram-negative bacteria *S. typhimurium* NRRL B 4420 and *E. coli* ATCC 25922, and yeast-like fungi *C. tropicalis* ATCC 13803. Ampicillin and fluconazole were used as antibiotic reference compounds for bacteria and fungi, respectively. As it can be seen in Table 1, the compounds inhibited the bacteria and fungi with MICs between 16 and 512 μg ml^{-1}. Among the tested compounds, **2** and **3** are the most effective compounds with MIC 16 μg ml^{-1} against *B. subtilis*. The compound **2** is also most effective substance with MIC 16 μg ml^{-1} against yeast-like fungi, *C. tropicalis*. This effectiveness is very close to the effect of fluconazole against *C. tropicalis*. First tree substances are very effective against *E. coli* providing a MIC value of 32 μg ml^{-1}. The compound **6** has a moderate effect among the other compounds used against all the bacteria and fungi in comparison to the reference drugs, but it did not show noteworthy activity against *S. typhimurium*. The lowest effective substance is **12** against all the microorganisms studied. Interestingly, despite all the substances have very similar structures, their antibacterial and antifungal activities are very different. Most of them demonstrate weak activity against gram-positive and gram-negative bacteria and fungi in comparison to the reference drugs. As mentioned in Section 1, Suzuki et al. [3] and Holla et al. [8] have investigated the antibacterial and antifungal properties of similar compounds to that of ours. Their MIC results, especially Holla et al.'s are in good agreement with our results, since Holla et al. synthesized and investigated similar thiazolylhydrazone compounds. The detailed observed data on the antibacterial and antifungal activities of the compounds and the control drugs are given in Table 1.

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