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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel benzoquinones as potential antimicrobial agents

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Abstract New series of 2,5-dihydroxyphenyl-1,3-thiazoles **4a–l** was synthesized by reacting 2,5-dihydroxyphenacyl bromide with various 4-aryl thiosemicarbazones **3a–l** that on oxidation with ferric chloride yielded the corresponding N^1-substituted benzylidene-N^2-[3-aryl-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazines **5a–l**. They were evaluated for antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *Candida albicans*. Almost all tested compounds were found to possess variable degrees of antimicrobial activity. The obtained data revealed that compounds **4b–h** and **5e, 5f** and **5l** exhibited promising antimicrobial activity against the tested organisms of which compound **4b** proved to be the most active.

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Introduction

The incidence of fungal and bacterial infections has dramatically increased. The widespread use of antifungal and antibacterial drugs together with resistance of fungal and bacterial infections to antimicrobial agents has led to serious health hazards. This has initiated discovery and modification of new antifungal and antibacterial drugs (Parmar *et al*., 2011; Kaplancikli *et al*., 2004).

Hydroquinones and quinones are of current interest because of their wide-spectrum bioactivity and chemotherapeutic value. Several quinone and hydroquinone derivatives exhibit antibacterial and antifungal activities (Tandon *et al*., 2005, 2006; Hammam *et al*., 2007; Batra *et al*., 2008; Tandon *et al*., 2009; Ryu *et al*., 2009). In addition, several naturally occurring quinones and hydroquinones such as avarol, avarone, hydroxysesamone, 2,3-epoxysesamone, and newbouldiaquinone were reported as potent antimicrobial agents (Cariello *et al*., 1982; Seibert *et al*., 1985; Kondracki and Guyot, 1989; Feroj Hasan *et al*., 2001; Machado *et al*., 2003; Eyong *et al*., 2006). Furthermore, kalafungin, grantican B, nanaomycins, and griseusins A and B are members of a family of naturally occurring antibiotics containing a quinone ring (Johnson and Dietz, 1968; Tsuji *et al*., 1976).

The 1,3-thiazole heterocycle is an interesting building block in a variety of natural and synthetic compounds found to possess good antibacterial potential (Sadek *et al*., 2011). In addition, literature survey revealed that thiazole ring systems have occupied a unique position in the design and synthesis of novel biologically active agents with

remarkable antimicrobial activity (Turan-Zitouni *et al*., 2002; Ryu *et al*., 2003; Bonde and Gaikwad, 2004; Shih and Ke, 2004; Bondock *et al*., 2007; Karegoudar *et al*., 2008). In view of the aforementioned properties and as a continuation of an ongoing program aiming at the synthesis of new quinones and hydroquinones with antimicrobial activity, in this article, we have reported the synthesis of some new hydroquinones and benzoquinones connected to thiazolines-bearing methoxy groups based on the fact that incorporation of alkoxy substituents would result in enhancement of several biological activities because of the magnification of compound's lipophilicity (Barboni *et al*., 2006; Lin *et al*., 2005).

Results and discussion

Chemistry

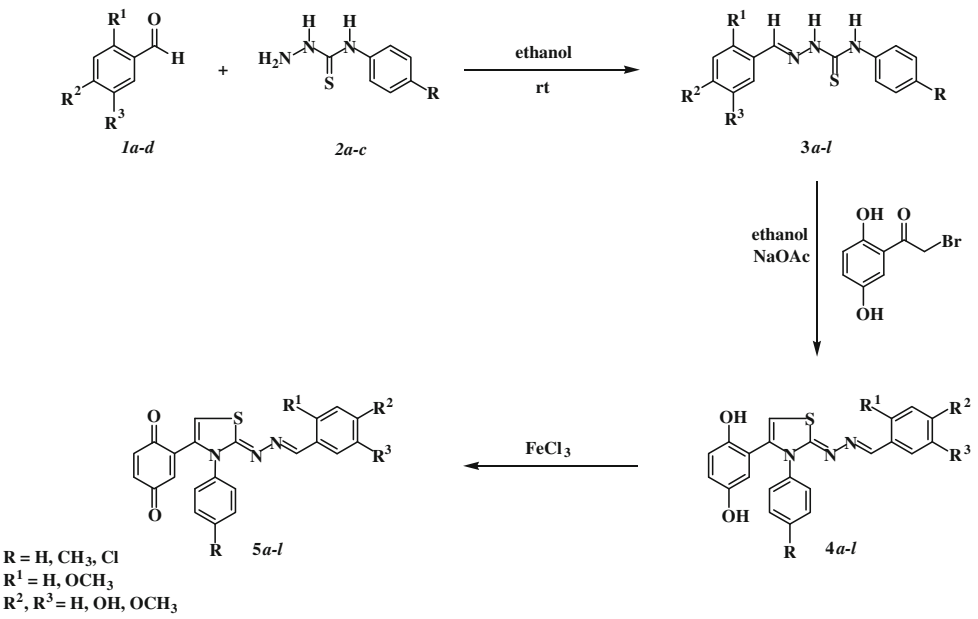
Synthesis of the intermediates and target compounds was accomplished according to the steps illustrated in Scheme 1. Acetylation of hydroquinone with acetic anhydride in the presence of drops of concentrated sulfuric acid afforded hydroquinone diacetate (Prichard, 1948). It was then subjected to Fries rearrangement using anhydrous aluminum chloride at 160–165 °C yielding 2,5-dihydroxyacetophenone (Amin and Shah, 1948). 2,5-Dihydroxyphenacyl bromide was prepared by reacting a suspension of 2,5-dihydroxyacetophenone with freshly prepared cupric bromide in ethyl acetate/chloroform mixture according to the reported method (King and Ostrum, 1964). The thiosemicarbazone derivatives **3a–l** were prepared by the condensation of equimolar amounts of the appropriate aldehyde **1a–d** and the appropriate aryl thiosemicarbazide **2a–c** in ethanol while stirring at room temperature. It is to be noted down that the thiosemicarbazones **3a–g** and **3k** were prepared following previously published reaction conditions (Das and Rout, 1955; Tišler, 1956; Chaaban *et al*., 2006; Cunha and da Silva, 2009; de Aquino *et al*., 2008). The 4-aryl-1-substituted benzylidene thiosemicarbazides **3a–l** were allowed to react with an equimolar amount of 2,5-dihydroxyphenacyl bromide in 99.9 % ethanol in the presence of anhydrous sodium acetate to afford the target thiazolines **4a–l**. Oxidation of the latter compounds with cold 20 % aqueous ferric chloride solution in dimethylformamide afforded the corresponding quinones **5a–l**.

In vitro antibacterial and antifungal activities

Dimethylsulfoxide (DMSO) was used as blank and showed no activity. All the newly synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *Candida albicans*. Their inhibition zones were measured by the cup-diffusion technique (Jain and Kar, 1971) and further evaluation was carried out to determine their minimum inhibitory concentration (MIC) by the twofold serial dilution method (Scott, 1989). Ampicillin was used as standard antibacterial, while Clotrimazole was used as antifungal reference.

Antimicrobial screening of the thiazoline hydroquinones **4a–l** and their corresponding quinone derivatives

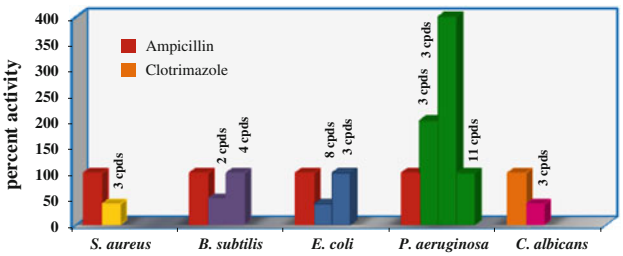
Scheme 1 Synthesis of intermediate and target compounds



5a–l revealed that most of them possess potent antimicrobial activity, whereas few show antifungal potency.

Compound **4a** (R, R^1, R^3 = H, R^2 = OCH_3) elicited equipotent activity to Ampicillin against *P. aeruginosa*, whereas its oxidation to the quinone **5a** maintained same activity against *P. aeruginosa* and increased activity against *E. coli*. Compound **4b** (R, R^2 = H, R^1, R^3 = OCH_3) was almost equipotent to Ampicillin against *B. subtilis* and *E. coli* as well as four times more potent against *P. aeruginosa*. Oxidation of **4b** to the corresponding quinone **5b** decreased the antimicrobial activity against *B. subtilis*, *E. coli*, and *P. aeruginosa*. Furthermore, compound **4c** (R, R^1 = H, R^2 = OH, R^3 = OCH_3) revealed nearly half the activity of reference drugs against *S. aureus* and *C. albicans*, whereas it showed nearly equal activity against *E. coli* and *P. aeruginosa*. Oxidation of **4c** to the corresponding quinone **5c** reduced activity against all organisms. The derivative **4d** (R, R^1 = H, R^2 = OCH_3, R^3 = OH) was equipotent to Ampicillin against *B. subtilis* and had four times the activity against *P. aeruginosa*, whereas oxidation to **5d** reduced antimicrobial activity. Furthermore, compound **4e** (R = CH_3, R^1, R^3 = H, R^2 = OCH_3) was almost equipotent to Ampicillin against Gram-negative bacteria, whereas its oxidation to the corresponding quinone **5e** decreased the activity twice against *E. coli* and increased it four times against *P. aeruginosa*. Derivative **4f** (R = CH_3, R^1, R^3 = OCH_3, R^2 = H) revealed almost half the activity against *S. aureus* and *E. coli*, equal activity against *B. subtilis* and double the activity against *P. aeruginosa* when compared with reference Ampicillin. Oxidation to quinone derivative **5f** maintained same activity against aforementioned Gram-positive and Gram-negative organisms besides increasing antifungal activity. Besides, compound **4g** (R = CH_3, R^1 = H, R^2 = OH, R^3 = OCH_3) showed moderate activity against *S. aureus* and equal activity to Ampicillin against *P. aeruginosa*, whereas its oxidation to **5g** diminished activity against *P. aeruginosa* and maintained it against *S. aureus*. The thiazoline **4h** (R = CH_3, R^1 = H, R^2 = OCH_3, R^3 = OH) had about half the activity of references against *B. subtilis*, *E. coli*, and *C. albicans*. It also elicited twice the activity of Ampicillin against *P. aeruginosa*. Converting to quinone **5h** reduced the antimicrobial activity against *P. aeruginosa* to be similar to that of Ampicillin, whereas it diminished activity against all other organisms. In addition, compounds **4i**, **4j** (R = Cl, R^1 = H, OCH_3, R^2 = OCH_3, H, R^3 = H, OCH_3) revealed about half the antimicrobial activity of Ampicillin against *E. coli* as well as equal activity against *P. aeruginosa*, whereas their oxidized forms **5i**, **5j** did not show any antimicrobial activity except moderate activity for compound **5j** against *S. aureus*. Compound **4k** (R = Cl, R^1 = H, R^2 = OH, R^3 = OCH_3) did not exhibit any antimicrobial activity, whereas its oxidation to **5k** increased the activity against *P. aeruginosa* to be equal to that of Ampicillin. Finally, compound **4l** (R = Cl, R^1 = H, R^2 = OCH_3, R^3 = OH) showed moderate activity against *S. aureus* and *C. albicans*, whereas oxidation to quinone **5l** increased the activity against *B. subtilis*, *E. coli*, and *C. albicans* to about half that of references also activity against *P. aeruginosa* increased to be equal to that of Ampicillin, whereas activity against *S. aureus* was reduced to its half.

The correlation between the activity of Ampicillin as well as Clotrimazole and the most potent investigated compounds against different tested organisms is illustrated in the following figure:



Conclusion

The objective of this study was to synthesize and investigate the antimicrobial activity of novel 2,5-dihydroxyphenyl-1,3-thiazoles and 1,4-benzoquinon-2-yl-1,3-thiazoles. These compounds were subjected to in vitro antibacterial and antifungal screening. The obtained data revealed that ten compounds, namely **4b–4h** and **5e, 5f**, and **5l,** exhibited promising antimicrobial activity against tested organisms. It is to be noted down that, among the tested hydroquinones, compound **4b** bearing two methoxy groups was found to be the most potent member. It showed a broad spectrum of antimicrobial activity with special high activity against *B. subtilis*, *E. coli*, and *P. aeruginosa*. On the other hand, compound **5f** comprising two methoxy groups was found to be the most potent member among the benzoquinone series. Further investigation of such compounds represents a fruitful matrix for the development of more potent antimicrobial agents (Table 1).

Statistical analysis

As illustrated in Tables 2, 3, and 4, standard deviation value was expressed in terms of ±SD. On the basis of calculated value by Mann–Whitney test, it was observed that differences less than 0.05 level were considered as statistically significant.

Experimental

Chemistry

Melting points of synthesized compounds were determined in open glass capillaries using a Griffin melting point apparatus and are all uncorrected. Infrared spectra (IR) were recorded using KBr disks using a Perkin-Elmer 1430 Infrared spectrophotometer. ^{1}H-NMR spectra were recorded using a Jeol-NMR 500 MHZ spectrophotometer, in the Faculty of Science, Alexandria University, and are reported as δ values (ppm) relative to tetramethylsilane as an internal reference. ^{13}C-NMR spectra were recorded using JNM-LA 400 FT NMR system (400 MHz), Faculty of Science, Assiut University. The type of signal was indicated by one of the following letters: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV) or on a gas chromatograph/mass spectrometer Shimadzu GCMS-Qp2010 Plus (70 eV), in the Faculty of Science, Cairo University. Elemental microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University or at Inspectorate International Ltd., Jubail technical centre, Jubail laboratory, Saudi Arabia. Reactions' progress was monitored by thin-layer chromatography on silica gel (60 GF254, Merck) using glass plates, and the spots were visualized by exposure to iodine vapor or to UV-lamp at λ 254 nm for few seconds.

Table 1 The inhibition zones (IZ) in mm diameter

Comp. no.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	S. *aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	14	15	14	14	12
4b	16	15	18	16	14
4c	15	16	14	15	15
4d	16	15	24	12	14
4e	15	13	15	16	15
4f	14	17	17	14	14
4g	15	15	14	16	12
4h	16	14	14	13	14
4i	15	14	14	12	15
4j	13	15	18	16	13
4k	16	16	15	14	14
4l	14	14	13	14	12
5a	15	15	16	15	15
5b	14	15	14	14	15
5c	15	15	15	14	12
5d	14	15	14	16	14
5e	16	14	16	15	14
5f	16	15	14	14	13
5g	14	15	15	15	15
5h	15	14	14	14	15
5i	14	15	16	16	14
5j	14	14	13	16	14
5k	16	14	16	15	15
5l	13	14	14	14	15

General method for the preparation of 4-aryl-1-substituted benzylidene thiosemicarbazides (**3a–l**)

A solution of the selected 4-aryl thiosemicarbazide **2a–c** (5 mmol) in 99.9 % ethanol (20 ml) was gradually added with stirring to a well-stirred solution of an equimolar amount of the selected aldehyde **1a–d** (5 mmol) in 99.9 % ethanol (4 ml). The reaction mixture was stirred for an additional hour and set aside at room temperature for an overnight whereupon the products separated out. The obtained products were filtered, washed with cold ethanol, air dried, and crystallized from ethanol.

Table 2 Inhibition zones (IZ) in mm diameter

Organism	Inhibition zone (mm)				Significance (*P*)
	Compounds **4a–l**		Compounds **5a–l**		
	Min–Max	Mean ± SD	Min–Max	Mean ± SD	
Gram-positive bacteria					
S. aureus	13–16	14.9 ± 0.9	13–16	14.7 ± 0.9	$Z = 0.664$ (0.507)
B. subtilis	13–17	14.9 ± 1.0	14–15	14.6 ± 0.5	$Z = 0.883$ (0.377)
Gram-negative bacteria					
E. coli	13–24	15.8 ± 3.1	13–16	14.8 ± 1.1	$Z = 0.482$ (0.63)
P. aeruginosa	12–16	14.3 ± 1.5	14–16	14.8 ± 0.8	$Z = 0.785$ (0.432)
Fungi					
C. albicans	12–15	13.7 ± 1.2	12–15	14.3 ± 0.9	$Z = 1.346$ (0.178)

±*SD* standard deviation, *Z* Mann–Whitney test

Table 3 Minimal inhibitory concentrations (MIC) in μg/ml

Organism	MIC (μg/ml)				Significance (*P*)
	Compounds **4a–l**		Compounds **5a–l**		
	Min–Max	Mean ± SD	Min–Max	Mean ± SD	
Gram-positive bacteria					
S. aureus	12.5–100	45.8 ± 29.4	12.5–100	53.1 ± 31.1	$Z = 0.608$ (0.543)
B. subtilis	12.5–50	38.5 ± 17.2	12.5–100	65.6 ± 32.5	$Z = 1.093$ (0.036)*
Gram-negative bacteria					
E. coli	12.5–100	40.6 ± 31.1	25–100	58.3 ± 32.6	$Z = 1.563$ (0.118)
P. aeruginosa	12.5–100	47.9 ± 28.6	12.5–100	65.6 ± 32.5	$Z = 1.357$ (0.175)
Fungi					
C. albicans	12.5–100	52.1 ± 32.3	12.5–100	51.0 ± 26.4	$Z = 0.093$ (0.926)

±*SD* standard deviation, *Z* Mann–Whitney test

* Significant at $P \leq 0.05$

Table 4 Minimal germicidal concentrations (MBC/MFC) in μg/ml

Organism	MBC/MFC (μg/ml)				Significance (*P*)
	Compounds **4a–l**		Compounds **5a–l**		
	Min–Max	Mean ± SD	Min–Max	Mean ± SD	
Gram-positive bacteria					
S. aureus	25–100	58.3 ± 26.8	25–100	60.4 ± 24.9	$Z = 0.302$ (0.763)
B. subtilis	25–100	47.9 ± 19.8	25–100	72.9 ± 29.1	$Z = 2.145$ (0.032)*
Gram-negative bacteria					
E. coli	25–100	56.3 ± 28.5	50–100	66.7 ± 24.6	$Z = 1.18$ (0.238)
P. aeruginosa	25–100	58.3 ± 26.8	25–100	72.9 ± 29.1	$Z = 1.242$ (0.214)
Fungi					
C. albicans	25–100	62.5 ± 29.2	25–100	64.6 ± 27.1	$Z = 0.258$ (0.797)

±*SD* standard deviation, *Z* Mann–Whitney test

* Significant at $P \leq 0.05$

1-(3-Hydroxy-4-methoxybenzylidene)-
4-(4-tolyl)thiosemicarbazide **3h**

Yield 91 %. M.p. 184–186 °C. IR (KBr, υ cm^{-1}): 3517 (OH), 3308, 3151 (υNH), 1610 (C=N), 1543 (δNH), 1543, 1272, 1128, 981 (N–C=S thioamide I, II, III, IV bands), 1201, 1072 (C–O–C). ^{1}H-NMR (CD_3COCD_3, δ ppm): 2.36 (s, 3H, C_6H_4–CH_3); 3.92 (s, 3H, OCH_3); 6.85 (d, 1H, *J:* 8.4 Hz, 3-hydroxy-4-methoxyphenyl-C_5-H); 7.09 (dd, 1H, *J:* 8.4, 1.55 Hz, 3-hydroxy-4-methoxyphenyl-C_6-H); 7.20 (d, 2H, *J:* 8.4 Hz, *p*-tolyl-$C_{3,5}$-H); 7.37 (d, 1H, *J:* 1.55 Hz, 3-hydroxy-4-methoxyphenyl-C_2-H); 7.44 (d, 2H, *J:* 8.4 Hz, *p*-tolyl-$C_{2,6}$-H); 7.92 (s, 1H, CH=N); 9.12, 9.84, 10.60 (three s, each 1H, 2NH and OH, D_2O-exch.). Anal. calcd. (%) for $C_{16}H_{17}N_3O_2S$: C 60.93, H 5.43, N 13.32. Found: C 60.97, H 5.80, N 13.41.

1-(4-Methoxybenzylidene)-
4-(4-chlorophenyl)thiosemicarbazide **3i**

Yield 87 %. M.p. 190–192 °C. IR (KBr, υ cm^{-1}): 3323, 3150 (υNH), 1605 (C=N), 1543 (δNH), 1543, 1273, 1168, 942 (N–C=S thioamide I, II, III, IV bands), 1247, 1076 (C–O–C). Anal. calcd. (%) for $C_{15}H_{14}ClN_3OS$: C 56.33, H 4.41, N 13.14. Found: C 56.52, H 4.60, N 13.28.

1-(2,5-Dimethoxybenzylidene)-
4-(4-chlorophenyl)thiosemicarbazide **3j**

Yield 88 %. M.p. 188–190 °C. IR (KBr, υ cm^{-1}): 3279, 3111 (υNH), 1589 (C=N), 1543 (δNH), 1543, 1263, 1134, 962 (N–C=S thioamide I, II, III, IV bands), 1215, 1044 (C–O–C). Anal. calcd. (%) for $C_{16}H_{16}ClN_3O_2S$: C 54.93, H 4.61, N 12.01. Found: C 54.57, H 4.20, N 11.48.

1-(3-Hydroxy-4-methoxybenzylidene)-4-(4-chlorophenyl)thiosemicarbazide **3l**

Yield 99 %. M.p. 196–198 °C. IR (KBr, υ cm^{-1}): 3435 (OH), 3316, 3180 (υNH), 1603 (C=N), 1537 (δNH), 1581, 1276, 1154, 984 (N–C=S thioamide I, II, III, IV bands), 1247, 1051 (C–O–C). Anal. calcd. (%) for $C_{15}H_{14}ClN_3O_2S$: C 53.65, H 4.20, N 12.51. Found: C 53.66, H 5.02, N 12.80.

N^1-substituted benzylidene-N^2-[3-aryl-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazines (**4a–l**)

A solution of 2,5-dihydroxyphenacyl bromide (1.39 g, 6 mmol) in 99.9 % ethanol (20 ml) was gradually added to a suspension of the aryl thiosemicarbazones **3a–l** (6 mmol) in 99.9 % ethanol (20 ml) containing anhydrous sodium acetate (0.49 g, 6 mmol). The reaction mixture was heated under reflux for 18 h, concentrated, cooled, poured onto crushed ice while stirring, and set aside for an overnight. The formed precipitates were filtered, washed several times with water, air dried, and crystallized.

N^1-benzylidene-N^2-[3-(4-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4a**

Crystallized from ethyl acetate. Yield 88 %. M.p. 212–214 °C. IR (KBr, υ cm^{-1}): 3288 (OH), 1603 (C=N), 1251, 1166 (C–S–C), 1219, 1029 (C–O–C). ^{1}H-NMR (DMSO-d_6, δppm): 3.74 (s, 3H, OCH_3); 6.33 (s, 1H, thiazoline-C_5-H); 6.41–6.49 (m, 3H, dihydroxyphenyl-$C_{3,4,6}$-H); 6.94 (d, 2H, *J:* 8.4 Hz, methoxyphenyl-$C_{3,5}$-H); 7.16–7.28 (m, 5H, phenyl-H); 7.58 (d, 2H, *J:* 8.4 Hz, methoxyphenyl-$C_{2,6}$-H); 8.04 (s, 1H, CH=N); 8.77, 8.87 (two s, each 1H, OH, D_2O-exch.). Anal. calcd. (%) for $C_{23}H_{19}N_3O_3S$: C 66.17, H 4.59, N 10.07. Found: C 66.48, H 4.64, N 10.17.

N^1-benzylidene-N^2-[3-(2,5-dimethoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4b**

Crystallized from ethyl acetate. Yield 97 %. M.p. 220–222 °C (with decomposition). IR (KBr, υ cm^{-1}): 3280 (OH), 1598 (C=N), 1271, 1160 (C–S–C), 1215, 1033 (C–O–C). Anal. calcd. (%) for $C_{24}H_{21}N_3O_4S$: C 64.41, H 4.73, N 9.39. Found: C 64.98, H 4.40, N 9.52.

N^1-benzylidene-N^2-[3-(4-hydroxy-3-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4c**

Crystallized from methylene chloride. Yield 96 %. M.p. 150–152 °C (with decomposition). IR (KBr, υ cm^{-1}): 3376 (OH), 1584 (C=N), 1265, 1121 (C–S–C), 1206, 1025 (C–O–C). Anal. calcd. (%) for $C_{23}H_{19}N_3O_4S$: C 63.73, H 4.42, N 9.69. Found: C 63.82, H 4.50, N 9.53.

N^1-benzylidene-N^2-[3-(3-hydroxy-4-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4d**

Crystallized from ethanol. Yield 99 %. M.p. 162–164 °C. IR (KBr, υ cm^{-1}): 3415 (OH), 1588 (C=N), 1266, 1178 (C–S–C), 1213, 1033 (C–O–C). Anal. calcd. (%) for $C_{23}H_{19}N_3O_4S$: C 63.73, H 4.42, N 9.69. Found: C 63.03, H 4.00, N 9.55.

N^1-4-methylbenzylidene-N^2-[3-(4-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4e**

Crystallized from benzene. Yield 93 %. M.p. 172–174 °C. IR (KBr, υ cm^{-1}): 3300 (OH), 1606 (C=N), 1244, 1168 (C–S–C), 1196, 1026 (C–O–C). Anal. calcd. (%) for $C_{24}H_{21}N_3O_3S$: C 66.80, H 4.91, N 9.74. Found: C 66.75, H 4.99, N 9.60.

N^1-4-methylbenzylidene-N^2-[3-(2,5-dimethoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4f**

Crystallized from benzene. Yield 94 %. M.p. 172–174 °C. IR (KBr, υ cm^{-1}): 3415 (OH), 1588 (C=N), 1266, 1178 (C–S–C), 1213, 1033 (C–O–C). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.22 (s, 3H, C_6H_4–CH_3); 3.70, 3.71 (two s, each 3H, OCH_3); 6.33 (s, 1H, thiazoline-C_5-H); 6.43–6.50 (m, 3H, dihydroxyphenyl-$C_{3,4,6}$-H); 6.91 (dd, 1H, *J:* 9.2, 3.05 Hz, dimethoxyphenyl-C_4-H); 6.95 (d, 1H, *J:* 9.2 Hz, dimethoxyphenyl-C_3-H); 7.04–7.10 (m, 4H, *p*-tolyl-H); 7.33 (d, 1H, *J:* 3.05 Hz, dimethoxyphenyl-C_6-H); 8.23 (s, 1H, CH=N); 8.77, 8.87 (two s, each 1H, OH, D_2O-exch.). Anal. calcd. (%) for $C_{25}H_{23}N_3O_4S$: C 65.06, H 5.02, N 9.10. Found: C 64.90, H 5.10, N 9.10.

N^1-4-methylbenzylidene-N^2-[3-(4-hydroxy-3-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4g**

Crystallized from ethyl acetate. Yield 92 %. M.p. 240–242 °C. IR (KBr, υ cm^{-1}): 3371(OH), 1594 (C=N), 1272, 1116 (C–S–C), 1204, 1031 (C–O–C). Anal. calcd. (%) for $C_{24}H_{21}N_3O_4S$: C 64.41, H 4.73, N 9.39. Found: C 64.77, H 4.46, N 9.35.

N^1-4-methylbenzylidene-N^2-[3-(3-hydroxy-4-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4h**

Crystallized from benzene. Yield 92 %. M.p. 240–242 °C. IR (KBr, υ cm^{-1}): 3290 (OH), 1606 (C=N), 1269, 1121 (C–S–C), 1205, 1022 (C–O–C). ^{1}H-NMR (CD_3COCD_3, δ ppm): 2.22 (s, 3H, C_6H_4–CH_3); 3.74 (s, 3H, OCH_3); 6.29 (s, 1H, thiazoline-C_5-H); 6.40–6.50 (m, 3H, dihydroxyphenyl-$C_{3,4,6}$-H); 6.88 (d, 1H, *J:* 8.4 Hz, 3-hydroxy-4-methoxyphenyl-C_5-H); 7.02–7.08 (m, 4H, *p*-tolyl-H); 7.19 (dd, 1H, *J:* 8.4, 1.55 Hz, 3-hydroxy-4-methoxyphenyl-C_6-H); 7.33 (d, 1H, *J:* 1.55 Hz, 3-hydroxy-4-methoxyphenyl-C_2-H); 7.92 (s, 1H, CH=N); 8.76, 8.86, 9.17 (three s, each 1H, OH, D_2O-exch.). Anal. calcd. (%) for $C_{24}H_{21}N_3O_4S$: C 64.41, H 4.73, N 9.39. Found: C 64.82, H 5.10, N 9.39.

N^1-4-chlorobenzylidene-N^2-[3-(4-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4i**

Crystallized from ethyl acetate. Yield 99 %. M.p. 228–230 °C. (with decomposition). IR (KBr, υ cm^{-1}): 3286 (OH), 1604 (C=N), 1251, 1166 (C–S–C), 1219, 1024 (C–O–C). Anal. calcd. (%) for $C_{23}H_{18}ClN_3O_3S$: C 61.13, H 4.01, N 9.30. Found: C 61.80, H 3.91, N 9.43. EI MS, *m/z* (%)**:** 453 (30.6) ($M^{+\cdot}$+2), 451 (76.4) ($M^{+\cdot}$), 120 (100).

N^1-4-chlorobenzylidene-N^2-[3-(2,5-dimethoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4j**

Crystallized from ethanol/water 9:1. Yield 85 %. M.p. 174–176 °C. IR (KBr, υ cm^{-1}): 3271 (OH), 1597 (C=N), 1269, 1160 (C–S–C), 1215, 1026 (C–O–C). ^{1}H-NMR (CD_3COCD_3, δ ppm): 3.76, 3.77 (two s, each 3H, OCH_3); 6.30 (s, 1H, thiazoline-C_5-H); 6.54 (d, 1H, *J:* 9.2 Hz, dihydroxyphenyl-C_3-H); 6.62 (dd, 1H, *J:* 9.2, 3.05 Hz, dihydroxyphenyl-C_4-H); 6.68 (d, 1H, *J:* 3.05 Hz, dihydroxyphenyl-C_6-H); 6.90 (dd, 1H, *J:* 9.2, 3.05 Hz, dimethoxyphenyl-C_4-H); 6.93 (d, 1H, *J:* 9.2 Hz, dimethoxyphenyl-C_3-H); 7.27–7.33 (m, 4H, *p*-chlorophenyl-H); 7.52 (d, 1H, *J:* 3.05 Hz, dimethoxyphenyl-C_6-H); 8.03, 8.04 (two s, each 1H, OH, D_2O-exch.); 8.40 (s, 1H, CH=N). Anal. calcd. (%) for $C_{24}H_{20}ClN_3O_4S{\cdot}½H_2O$: C 58.71, H 4.31, N 8.56. Found: C 58.89, H 3.82, N 8.56.

N^1-4-chlorobenzylidene-N^2-[3-(4-hydroxy-3-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4k**

Crystallized from methylene chloride. Yield 86 %. M.p. 186–188 °C. IR (KBr, υ cm^{-1}): 3384 (OH), 1596 (C=N), 1273, 1120 (C–S–C), 1203, 1027 (C–O–C). ^{1}H-NMR (CD_3COCD_3, δ ppm): 3.86 (s, 3H, OCH_3); 6.26 (s, 1H, thiazoline-C_5-H); 6.54 (d, 1H, *J:* 8.4 Hz, dihydroxyphenyl-C_3-H); 6.61 (dd, 1H, *J:* 8.4, 3.05 Hz, dihydroxyphenyl-C_4-H); 6.67 (d, 1H, *J:* 3.05 Hz, dihydroxyphenyl-C_6-H); 6.82 (d, 1H, *J:* 8.4 Hz, 4-hydroxy-3-methoxyphenyl-C_5-H); 7.12 (dd, 1H, *J:* 8.4, 1.55 Hz, 4-hydroxy-3-methoxyphenyl-C_6-H); 7.26–7.31 (m, 4H, *p*-chlorophenyl-H); 7.37 (d, 1H, *J:* 1.55 Hz, 4-hydroxy-3-methoxyphenyl-C_2-H); 8.00 (s, 1H, CH=N); 8.02, 8.04 (two s, each 1H, OH, D_2O-exch.). Anal. calcd. (%) for $C_{23}H_{18}ClN_3O_4S$: C 59.04, H 3.88, N 8.98. Found: C 59.00, H 3.80, N 9.20. EI MS, *m/z* (%)**:** 469 (31) ($M^{+\cdot}$+2), 467 (56) ($M^{+\cdot}$), 209 (100).

N^1-4-chlorobenzylidene-N^2-[3-(3-hydroxy-4-methoxyphenyl)-4-(2,5-dihydroxyphenyl)-1,3-thiazol-2-ylidene]hydrazine **4l**

Crystallized from methylene chloride. Yield 86 %. M.p. 162–164 °C. IR (KBr, υ cm^{-1}): 3267 (OH), 1605 (C=N), 1272, 1121 (C–S–C), 1211, 1020 (C–O–C). ^{1}H-NMR (CD_3COCD_3, δ ppm): 3.83 (s, 3H, OCH_3); 6.27 (s, 1H, thiazoline-C_5-H); 6.53 (d, 1H, *J:* 8.4 Hz, dihydroxyphenyl-C_3-H); 6.61 (dd, 1H, *J:* 8.4, 3.05 Hz, dihydroxyphenyl-C_4-H); 6.67 (d, 1H, *J:* 3.05 Hz, dihydroxyphenyl-C_6-H); 6.93 (d, 1H, *J:* 8.4 Hz, 3-hydroxy-4-methoxyphenyl-C_5-H); 7.06 (dd, 1H, *J:* 8.4, 2.3 Hz, 3-hydroxy-4-methoxyphenyl-C_6-H); 7.26-7.30 (m, 4H, *p*-chlorophenyl-H); 7.32 (d, 1H, *J:* 2.3 Hz, 3-hydroxy-4-methoxyphenyl-C_2-H); 7.84, 8.02 (two s, each 1H, OH, D_2O-exch.); 7.79 (s, 1H, CH = N). ^{13}C-NMR (DMSO-d_6, δ ppm): 55.6, 101, 111.8, 112.3, 116.1, 117.3, 118.1, 120.1, 128.1, 128.2, 128.8, 130, 131.6, 136.4, 137.1, 146.6, 147.6, 149.3, 149.4, 151.4, 168.2. Anal. calcd. (%) for $C_{23}H_{18}ClN_3O_4S$: C 59.04, H 3.88, N 8.98. Found: C 58.99, H 3.80, N 8.90. EI MS, *m/z* (%): 469 (14.7) ($M^{+\cdot}$+2), 467 (52.3) ($M^{+\cdot}$), 209 (100).

N^1-substituted benzylidene-N^2-[3-aryl-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazines (**5a–l**)

A cold solution of the appropriate hydroquinone **4a–l** (1 mmol) in DMF (5 ml) was gradually treated with cold 20 % ferric chloride in water/DMF 1:1 (15 ml). Stirring was continued for 30 min and then the reaction mixture was diluted with water (30 ml) whereupon blue or green precipitates were formed. The formed colored precipitates were filtered, washed with water several times till free from ferric ions, air dried, and crystallized from the proper solvent as blue or green crystals.

N^1-benzylidene-N^2-[3-(4-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5a**

Crystallized from *n*-hexane. Yield 74 %. M.p. 110–112 °C. IR (KBr, υ cm^{-1}): 1649 (C=O), 1608 (C=N), 1296, 1159 (C–S–C), 1215, 1072 (C–O–C). Anal. calcd. (%) for $C_{23}H_{17}N_3O_3S$: C 66.49, H 4.12, N 10.11. Found: C 66.50, H 4.21, N 10.14.

N^1-benzylidene-N^2-[3-(2,5-dimethoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5b**

Crystallized from methanol. Yield 80 %. M.p. 164–166 °C (with decomposition). IR (KBr, υ cm^{-1}): 1658 (C=O), 1597 (C=N), 1278, 1160 (C–S–C), 1215, 1041 (C–O–C). ^{1}H-NMR (CH_3COCH_3, δ ppm): 3.71 (s, 3H, OCH_3); 6.69 (s, 1H, thiazoline-C_5-H); 6.67 (d, 1H, *J:* 2.3 Hz, benzoquinonyl-C_6-H); 6.77 (d, 1H, *J:* 8.4 Hz, benzoquinonyl-C_3-H); 6.90–6.98 (m, 3H, benzoquinonyl-C_4-H and dimethoxyphenyl-$C_{3,4}$-H); 7.27–7.35 (m, 3H, dimethoxyphenyl-C_6-H and phenyl-$C_{2,6}$-H); 7.36–7.42 (m, 3H, phenyl-$C_{3,4,5}$-H); 8.29 (s, 1H, CH=N). Anal. calcd. (%) for $C_{24}H_{19}N_3O_4S$: C 64.71, H 4.30, N 9.43. Found: C 64.91, H 4.22, N 9.22.

N^1-benzylidene-N^2-[3-(4-hydroxy-3-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5c**

Crystallized from benzene. Yield 85 %. M.p. 200 °C (with decomposition). IR (KBr, υ cm^{-1}): 3309 (OH), 1652 (C=O), 1607 (C=N), 1276, 1167 (C–S–C), 1243, 1051 (C–O–C). Anal. calcd. (%) for $C_{23}H_{17}N_3O_4S$: C 64.03, H 3.97, N 9.74. Found: C 63.75, H 4.77, N 9.58.

N^1-benzylidene-N^2-[3-(3-hydroxy-4-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5d**

Crystallized from benzene. Yield 95 %. M.p. 180–182 °C (with decomposition). IR (KBr, υ cm^{-1}): 3397 (OH), 1652 (C=O), 1596 (C=N), 1286, 1161 (C–S–C), 1231, 1072 (C–O–C). Anal. calcd. (%) for $C_{23}H_{17}N_3O_4S$: C 64.03, H 3.97, N 9.74. Found: C 64.06, H 3.62, N 9.21.

N^1-4-methylbenzylidene-N^2-[3-(4-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5e**

Crystallized from ethanol. Yield 84 %. M.p. 178–180 °C (with decomposition). IR (KBr, υ cm^{-1}): 1665 (C=O), 1604 (C=N), 1296, 1164 (C–S–C), 1248, 1028 (C–O–C). ^{1}H-NMR ($CHCl_3$, δ ppm): 2.34 (s, 3H, C_6H_4–CH_3); 3.82 (s, 3H, OCH_3); 6.65 (s, 1H, thiazoline-C_5-H); 6.44 (d, 1H, *J:* 2.3 Hz, benzoquinonyl-C_6-H); 6.62-6.69 (m, 2H, benzoquinonyl-$C_{3,4}$-H); 6.88 (d, 2H, *J:* 8.4 Hz, methoxyphenyl-$C_{3,5}$-H); 7.17 (d, 2H, *J:* 8.4 Hz, *p*-tolyl-$C_{3,5}$-H); 7.20 (d, 2H, *J:* 8.4 Hz, *p*-tolyl-$C_{2,6}$-H); 7.63 (d, 2H, *J:* 8.4 Hz, methoxyphenyl-$C_{2,6}$-H); 8.18 (s, 1H, CH=N). Anal. calcd. (%) for $C_{24}H_{19}N_3O_3S$: C 67.12, H 4.46, N 9.78. Found: C 66.85, H 4.32, N 9.54.

N^1-4-methylbenzylidene-N^2-[3-(2,5-dimethoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5f**

Crystallized from methanol. Yield 95 %. M.p. 188–190 °C (with decomposition). IR (KBr, υ cm^{-1}): 1654 (C=O), 1598 (C=N), 1262, 1163 (C–S–C), 1217, 1042 (C–O–C). ^{1}H-NMR ($CHCl_3$, δ ppm): 2.35 (s, 3H, C_6H_4–CH_3); 3.75, 3.82 (two s, each 3H, OCH_3); 6.46 (d, 1H, *J:* 2.3 Hz, benzoquinonyl-C_6-H); 6.62 (s, 1H, thiazoline-C_5-H); 6.63-6.69 (m, 2H, benzoquinonyl-$C_{3,4}$-H); 6.80 (d, 1H, *J:* 9.2 Hz, dimethoxyphenyl-C_3-H); 6.87 (dd, 1H, *J:* 9.2, 3.05 Hz, dimethoxyphenyl-C_4-H); 7.18 (d, 2H, *J:* 8.4 Hz, *p*-tolyl-$C_{3,5}$-H); 7.21 (d, 2H, *J:* 8.4 Hz, *p*-tolyl-$C_{2,6}$-H); 7.56 (d, 1H, *J:* 3.05 Hz, dimethoxyphenyl-C_6-H); 8.64 (s, 1H, CH=N). Anal. calcd. (%) for $C_{25}H_{21}N_3O_4S{\cdot}½$ H_2O: C 64.09, H 4.73, N 8.97. Found: C 64.22, H 4.48, N 9.54.

N^1-4-methylbenzylidene-N^2-[3-(4-hydroxy-3-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5g**

Crystallized from benzene. Yield 90 %. M.p. 88–90 °C (with decomposition). IR (KBr, υ cm^{-1}): 3409 (OH), 1657 (C=O), 1589 (C=N), 1280, 1159 (C–S–C), 1230, 1058 (C–O–C). Anal. calcd. (%) for $C_{24}H_{19}N_3O_4S$: C 64.71, H 4.30, N 9.43. Found: C 64.70, H 4.38, N 8.90.

N^1-4-methylbenzylidene-N^2-[3-(3-hydroxy-4-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5h**

Crystallized from methanol. Yield 84 %. M.p. 200–202 °C (with decomposition). IR (KBr, υ cm^{-1}): 3225 (OH), 1661 (C=O), 1606 (C=N), 1278, 1175 (C–S–C), 1246, 1071 (C–O–C). Anal. calcd. (%) for $C_{24}H_{19}N_3O_4S$: C 64.71, H 4.30, N 9.43. Found: C 64.50, H 4.20, N 9.15.

N^1-4-chlorobenzylidene-N^2-[3-(4-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5i**

Crystallized from ethanol. Yield 98 %. M.p. 188–190 °C (with decomposition). IR (KBr, υ cm^{-1}): 1652 (C=O), 1608 (C=N), 1285, 1168 (C–S–C), 1244, 1030 (C–O–C). ^{1}H-NMR ($CHCl_3$, δ ppm): 3.83 (s, 3H, OCH_3); 6.54 (d, 1H, *J:* 2.3 Hz, benzoquinonyl-C_6-H); 6.63 (s, 1H, thiazoline-C_5-H); 6.63-6.73 (m, 2H, benzoquinonyl-$C_{3,4}$-H); 6.89 (d, 2H, *J:* 8.4 Hz, methoxyphenyl-$C_{3,5}$-H); 7.26 (d, 2H, *J:* 8.4 Hz, *p*-chlorophenyl-$C_{2,6}$-H); 7.38 (d, 2H, *J:* 8.4 Hz, *p*-chlorophenyl-$C_{3,5}$-H); 7.64 (d, 2H, *J:* 8.4 Hz, methoxyphenyl-$C_{2,6}$-H); 8.22 (s, 1H, CH=N). ^{13}C-NMR (DMSO-d_6, δ ppm): 55.3, 111.6, 114, 127.8, 129.1, 129.5, 129.6, 132.3, 132.6, 134, 135.9, 136.4, 136.5, 136.6, 153.76, 161.11, 167.76, 184.07, 186.40. Anal. calcd. (%) for $C_{23}H_{16}ClN_3O_3S \cdot ½ H_2O$: C 60.20, H 3.73, N 9.16. Found: C 60.24, H 3.56, N 9.05. EI MS, *m/z* (%)**:** 449 (79.5) ($M^{+\cdot}$), 118 (100).

N^1-4-chlorobenzylidene-N^2-[3-(2,5-dimethoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5j**

Crystallized from methanol. Yield 85 %. M.p. 170–172 °C (with decomposition). IR (KBr, υ cm^{-1}): 1651 (C=O), 1580 (C=N), 1283, 1164 (C–S–C), 1260, 1041 (C–O–C). Anal. calcd. (%) for $C_{24}H_{18}ClN_3O_4S$: C 60.06, H 3.78, N 8.76. Found: C 59.80, H 3.85, N 8.90.

N^1-4-chlorobenzylidene-N^2-[3-(4-hydroxy-3-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5k**

Crystallized from benzene. Yield 97 %. M.p. 164 °C (with decomposition). IR (KBr, υ cm^{-1}): 3455 (OH), 1649 (C=O), 1596 (C=N), 1268, 1198 (C–S–C), 1250, 1034

Table 5 Minimal inhibitory concentrations (MIC) and minimal germicidal concentrations (MBC/MFC) of tested compounds in μg/ml

Comp. no.	Gram-positive bacteria				Gram-negative bacteria				Fungi	
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
4a	50	50	50	50	50	50	50	50	50	50
4b	50	50	12.5	25	12.5	25	12.5	25	25	50
4c	12.5	25	50	50	12.5	25	50	50	12.5	25
4d	100	100	12.5	25	50	50	12.5	25	100	100
4e	50	50	50	50	12.5	25	50	50	100	100
4f	12.5	25	12.5	25	25	50	25	50	50	50
4g	25	50	50	50	50	100	50	100	50	50
4h	25	50	25	50	25	50	25	50	12.5	25
4i	100	100	50	50	25	50	50	50	50	50
4j	50	100	50	50	25	50	50	50	50	100
4k	50	50	50	50	100	100	100	100	100	100
4l	25	50	50	100	100	100	100	100	25	50
5a	50	50	50	50	25	50	50	50	50	50
5b	50	50	50	50	50	50	50	50	50	50
5c	25	50	50	100	100	100	100	100	25	50
5d	50	50	100	100	50	50	100	100	100	100
5e	50	50	50	50	25	50	12.5	25	50	50
5f	12.5	25	12.5	25	25	50	25	50	25	50
5g	25	50	100	100	50	50	100	100	50	50
5h	100	100	50	50	50	50	50	50	50	100
5i	100	100	100	100	100	100	100	100	100	100
5j	25	50	100	100	100	100	100	100	50	100
5k	100	100	100	100	100	100	50	100	50	50
5l	50	50	25	50	25	50	50	50	12.5	25
A*	5	–	12.5	–	10	–	50	–	–	–
C**	–	–	–	–	–	–	–	–	5	–

* *A* Ampicillin trihydrate (Standard broad spectrum antibiotic)

** *C* Clotrimazole (Standard broad spectrum antifungal agent)

(C–O–C). Anal. calcd. (%) for $C_{23}H_{16}ClN_3O_4S$: C 59.29, H 3.46, N 9.02. Found: C 59.74, H 3.20, N 8.66.

N^1-4-chlorobenzylidene-N^2-[3-(3-hydroxy-4-methoxyphenyl)-4-(1,4-benzoquinon-2-yl)-1,3-thiazol-2-ylidene]hydrazine **5l**

Crystallized from methanol. Yield 91 %. M.p. 200 °C (with decomposition). IR (KBr, υ cm^{-1}): 3238 (OH), 1659 (C=O), 1596 (C=N), 1277, 1168 (C–S–C), 1250, 1061 (C–O–C). Anal. calcd. (%) for $C_{23}H_{16}ClN_3O_4S{\cdot}½H_2O$: C 58.17, H 3.61, N 8.85. Found: C 58.01, H 3.08, N 8.63.

Antimicrobial screening

Inhibition-zone measurements

All the synthesized compounds were evaluated by the agar cup diffusion technique (Jain and Kar, 1971) using a 1 mg/ml solution in DMSO. The test organisms were *S. aureus* (DSM 1104) and *B. subtilis* (ATCC 6633) as Gram-positive bacteria and *E. coli* (ATCC 11775) and *P. aeruginosa* (ATCC 10145) as Gram-negative bacteria. *C. albicans* (DSM 70014) was also used as a representative for fungi. Each 100 ml of sterile molten agar (at 45 °C) received 1 ml of 6 h-broth culture and then the seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 ml of the 1 mg/ml solution of the test compounds. The plates were then incubated at 37 °C for 24 h or in case of *C. albicans* for 48 h. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial while Clotrimazole was used as antifungal reference.

MIC measurement

The MIC of the most active compounds were measured by the twofold serial broth dilution method (Scott, 1989). The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at 37 °C. Twofold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 µg/ml. The tubes were then inoculated with the test organisms; each 5 ml received 0.1 ml of the above inoculum and were incubated at 37 °C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 5.

Minimal germicidal concentration (MBC or MFC) measurement

MIC tests were extended to measure the MBC or MFC as follows: A loopfull from the tube not showing visible growth (MIC) was spread over a quarter of Müller–Hinton agar plate. After incubation, 24 h for bacteria and 48 h for fungi, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC of that compound for the respective test organism. The MBC/MFC values of the tested compounds are listed in Table 5.

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