Synthesis, Antibacterial Activity and Quantum-Chemical Studies of Novel 2-Arylidenehydrazinyl-4-arylthiazole Analogues

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A new series of 2-arylidenehydrazinyl-4-arylthiazole derivatives (2a—k) was designed and synthesized through a rapid, simple, and efficient methodology in excellent isolated yield. These compounds were screened for *in vitro* antimicrobial activities against eight bacteria, *e.g. Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, and three fungi *e.g. Aspergillus oryzae*, *Candida albicans*, and *Saccharomyces cerevis*. The results indicate that some of the compounds exhibit strong antibacterial activity, depending on the bacterial strain, but show virtually no antifungal activity. The structure–antibacterial activity relationships were studied using some physicochemical and quantum-chemical parameters with the *ab initio* Hartree–Fock model at the RHF/6-31G level of theory. A good qualitative correlation between predicted lipophilic parameters and antibacterial activity has been found.

Key words thiazole; antibacterial activity; quantum-chemical calculation; 2-arylidenehydrazinyl-4-arylthiazole

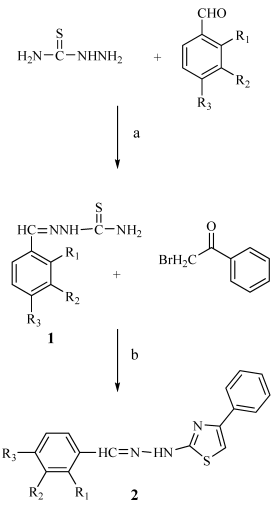
During the last few decades, increased incidence of bacterial resistance to existing drugs has become a major concern throughout the world and necessitates continuing research into new classes of antibiotics.[1)] Extensive use of antibacterial drugs and their resistance against bacterial infections has led to severe health problems. Of particular concern are severe infections caused by multidrug-resistant Gram-positive pathogens, such as *Staphylococcus* species,[2,3)] which has become a serious problem in hospitals and in the community. Resistance of wide spectrum antibacterial agents has prompted discovery and modification toward new antibiotics with a potent, wide therapeutic window, broad spectrum activity, and new mode of action.

The thiazole scaffold is an interesting building block in a variety of natural products and bioactive compounds that are useful as pharmaceuticals or agrochemical agents. Synthesis of thiazole analogues has attracted continuing interest over the years due to their wide range of pharmaceutical and biological properties, including antibacterial,[4—7)] anti-human immunodeficiency virus (HIV),[8)] hypertension,[9)] anti-inflammatory,[10)] anti-viral[11,12)] and anticancer[13,14)] activity. A survey of the literature also revealed that some new thiazole candidates showed adenosine receptor antagonists[15)] and Src family kinase inhibitor activity.[16)] More recently, Zhang *et al.*[17)] reported that 2-aminothiazole analogues acted as potential neuroprotective agents for treatment of neurological diseases and Leone *et al.*[18)] reported on modulators of transcriptional repression for treatment of Huntington's disease. The antimicrobial activities of different arylidenehydrazinyl-4-arylthiazoles were also reported.[19,20)]

Due to the observed wide range of biological activities of the thiazole derivatives and in continuation of our ongoing studies on novel biologically active molecules, we were prompted to design, synthesize, and perform antimicrobial evaluation of 2-arylidenehydrazinyl-4-arylthiazole analogues. Syntheses of desired analogues of 2-arylidenehydrazinyl-4-arylthiazole were carried out according to Hantzsch's method[21)] and the structures of the new compounds were elucidated by IR, ^{1}H-NMR, mass spectrometry, and elemental analysis. *In vitro* antibacterial activities were screened against eight bacterial strains, *e.g Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi*, and *Escherichia coli*. Antifungal activity was determined against three different fungal strains, including *Aspergillus oryzae*, *Candida albicans*, and *Saccharomyces cerevis*. Finally, quantum-chemical and physicochemical calculations were carried out with the *ab initio* Hartree–Fock model to study the relationship between the electronic properties and antibacterial activity of 2-arylidenehydrazinyl-4-arylthiazole analogues.

Results and Discussion

Synthesis The synthetic routes of arylidenethiosemicarbazones (**1a**—**k**) and 2-arylidenehydrazinyl-4-arylthiazoles (**2a**—**k**) are outlined in Chart 1. Arylidenethiosemicarbazones (**1a**—**k**) were prepared by condensation of thiosemicarbazide and substituted benzaldehyde in ethanol in excellent yield (86—98%). Structures of compounds **1a**—**k** were determined using IR and ^{1}H-NMR spectral data. IR spectrum



	R_1	R_2	R_3
1a, 2a	H	H	H
1b, 2b	H	H	Me
1c, 2c	H	H	Br
1d, 2d	H	H	OH
1e, 2e	OH	H	H
1f, 2f	H	H	NO_2
1g, 2g	H	NO_2	H
1h, 2h	H	H	Cl
1i, 2i	Cl	H	H
1j, 2j	H	H	OMe
1k, 2k	H	H	NMe_2

Chart 1. Reagents and Conditions: (a) EtOH–H_2O, Reflux, Yield 86—98%; (b) EtOH, Reflux, Yield 70—87%

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of arylidenethiosemicarbazones (**1a**—**k**) showed an absorption band around the 3467—3373 cm^{-1} and 3363—3239 cm^{-1} regions resulting from the $–NH_2$ and –NH– groups. ^{1}H-NMR spectra of compounds **1a**—**k** showed a singlet proton at 7.99—8.41 ppm for the –CH=N– proton and the =N–NH– proton, which appeared at 11.17—11.71 ppm. Two amino ($–NH_2$) protons appeared as two singlets at 7.67—8.26 and 7.81—8.38 ppm due to en-thiol tautomerism, while these protons disappeared in compounds **2a**—**k**.

Arylidenethiosemicarbazones (**1a**—**k**) underwent Hantzsch thioazole synthesis[21] with 2-bromo acetophenone to give 2-arylidenehydrazinyl-4-arylthiazoles (**2a**—**k**) in good yield (70—87%). Structures of compounds **2a**—**k** were elucidated using IR, ^{1}H-NMR, and electron ionization-mass spectra (EI-MS) spectral data together with elemental analysis. In IR spectrum of compounds **2a**—**k**, the characteristic N–H stretching absorption band appeared around the 3299—3092 cm^{-1} regions. ^{1}H-NMR spectra of compounds **2a**—**k** revealed the presence of the –CH=N– proton and the =N–NH– proton as two singlets at 7.66—8.62 and 11.30—11.81 ppm, respectively. The thiazole proton appeared as a multiplet at 6.45—8.14 ppm together with phenyl protons. In addition, EI-MS spectra of **2a**—**k** showed a molecular ion peak with intensities of 95—100%.

Biological Activities The newly synthesized 2-arylidenehydrazinyl-4-arylthiazoles (**2a**—**k**) together with their thiosemicarbazone precursors (**1a**—**k**) were evaluated for their *in vitro* antibacterial activities against four Gram-positive bacteria *e.g. Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, and four Gram-negative bacteria, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi*, and *E. coli* by disc diffusion methods. As presented in Table 1, among 11 thiazole derivatives (**2a**—**k**) tested, 7 compounds inhibited some Gram-positive bacteria and three compounds resisted the growth of some Gram-negative bacteria. However, their precursors (**1a**—**k**) did not show activity. Inhibition zones of synthesized compounds were measured at doses of 25 and 50 $\mu g\,disc^{-1}$ and kanamycin, a positive control, were evaluated at a dose of 25 $\mu g\,disc^{-1}$. In detail, compounds **2c** and **2j** showed strong activity against *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus megaterium*, *Salmonella typhi*, and *E. coli*. Compound **2h** showed moderate activity against *Bacillus subtilis*, *Bacillus megaterium*, and *Salmonella typhi*, while compound **2i** also exhibited moderate activity against *Bacillus cereus* and *Bacillus megaterium*. Compounds **2e**, **2f**, and **2k** demonstrated good activity against one bacterial strain only. Compounds **2f**, **2h**, and **2k** showed good activity against *Bacillus subtilis*, while the positive control, kanamycin, showed no activity at a concentration of 25 $\mu g\,disc^{-1}$. From this study, 2-arylidenehydrazinyl-4-arylthiazoles appear to have greater activity against Gram-positive bacteria compared to Gram-negative bacteria. However, more study is needed in order to clarify this point.

Structure–activity relationships (SAR) may be explained briefly as follows: introduction of electron donating groups (halogen, OMe) at the R_3 position resulted in better activity (*cf.*, **2c**, **2j**, **2h**), while the presence of more polar groups (OH, NO_2) caused no or reduced activity (*cf.*, **2d**—**f**). Activity was not dependent on the position of substituents at the phenyl ring.

Compounds **2a**—**k** were screened for their *in vitro* antifungal activity according to the disc diffusion method against *Aspergillus oryzae*, *Candida albicans*, and *Saccharomyces cerevis*. None of the compounds were significantly active at a concentration of 125 $\mu g\,disc^{-1}$.

Computational Studies The frontier molecular orbitals (FMOs) and physicochemical properties of a molecule play

Table 1. Antibacterial Profile of 2-Arylidenehydrazinyl-4-arylthiazole Derivatives in Terms of the Inhibition Zone

Comp.	Conc. ($\mu g\,disc^{-1}$)	Gram-positive				Gram-negative			
		B. c.	*S. a.*	*B. s.*	*B. m.*	*P. a.*	*S. d.*	*S. t.*	*E. c.*
2a	25	—	—	—	—	—	—	—	—
	50	—	—	—	—	—	—	—	—
2b	25	—	—	—	—	—	—	—	—
	50	—	—	—	—	—	—	—	—
2c	25	10	11		12			10	12
	50	16	18	—	18	—	—	15	19
2d	25	—	—	—	—	—	—	—	—
	50	—	—	—	—	—	—	—	—
2e	25	NC	—	—	—	—	—	—	—
	50	10	—	—	—	—	—	—	—
2f	25	—	—	NC		—	—	—	—
	50	—	—	10	—	—	—	—	—
2g	25	—	—	—	—	—	—	—	—
	50	—	—	—	—	—	—	—	—
2h	25	—	—	NC	NC	—	—	NC	—
	50	—	—	11	10	—	—	12	—
2i	25	NC	—	—	NC	—	—	—	—
	50	11	—	—	11	—	—	—	—
2j	25	11	13	—	13	—	—	11	10
	50	17	19	—	18	—	—	16	15
2k	25	—	—	NC	—	—	—	—	—
	50	—	—	10	—	—	—	—	—
Kanamycin	25	10	16	—	17	—	—	15	12

Inhibitory activity is expressed as the diameter (in mm) of the observed inhibition zone. NC: not clear (opaque halos were observed). *B. c.*, *Bacillus cereus*; *S. a.*, *Staphylococcus aureus*; *B. s.*, *Bacillus subtilis*; *B. m.*, *Bacillus megaterium*; *P. a.*, *Pseudomonas aeruginosa*; *S. d.*, *Shigella dysenteriae*; *S. t.*, *Salmonella typhi*; *E. c.*, *Escherichia coli*.

Table 2. Comparison of Quantum-Chemical and Physicochemical Properties of Active (**2c**, **j**) and Inactive (**2a**) 2-Arylidenehydrazinyl-4-arylthiazole Analogues

Comp.	E_{HF} (kJ/mol)	Volume (Å^3)	E_{LUMO} (eV)	E_{HOMO} (eV)	H_F (kcal/mol)	Dipole (Db)	PSA	Log *P*
2a	−3066406.4	249.08	−1.336	−9.094	135.38	3.04	37.283	3.96
2c	−9786303.8	266.96	−1.907	−8.766	133.20	2.20	39.283	5.68
2j	−6363747.5	274.62	−1.953	−8.814	96.96	1.60	46.517	5.03

E_{HF}, total energy; E_{LUMO}, energy of lowest unoccupied molecular orbital; E_{HOMO}, energy of highest occupied molecular orbital; H_F, heat of formation; PSA, molecular polar surface area; Log *P*, calculated octanol–water partition coefficient.

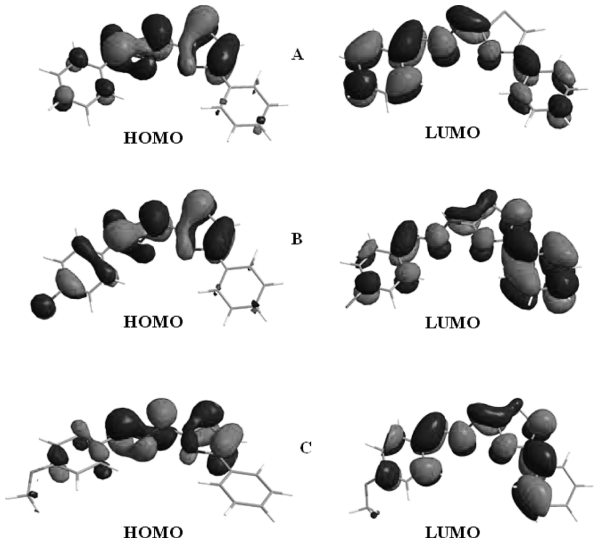

Fig. 1. HOMO and LUMO Isosurfaces for **2a** (A), **2c** (B) and **2j** (C)
Different surface colors represent opposite signs of the wave function.

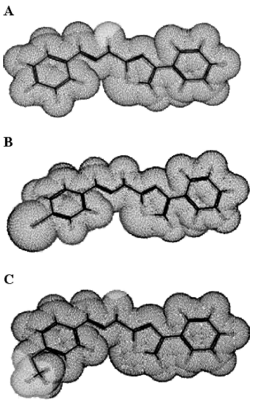

Fig. 2. Molecular Lipophilicity Potential (MLP) Maps of **2a** (A), **2c** (B) and **2j** (C)

an important role in determining molecular reactivity in biological response.[22] Biological systems are composed of a number of heterogeneous phases and drug must be transported through the different liquid phase barriers such as water, serum protein, lipid particles *etc.* to reach the site of action. Therefore, drug transport processes and drug–receptor interactions are essentially physicochemical and more complex than the homogeneous equilibria. Lipophilicity is recognized as a meaningful parameter in structure–activity relationship studies and become the single most informative and successful physicochemical property in medicinal chemistry.[23] It has become a major experimental and theoretical tool in drug design.

To explain the antibacterial activity of 2-arylidenehydrazinyl-4-arylthiazoles (**2a**—**k**), the quantum-chemical and physicochemical calculations were carried out with the *ab initio* Hartree–Fock (HF) model at the 6-31G basis set. Quantum-chemical and physiochemical parameters of some selected inactive (**2a**) and active (**2c**, **j**) thiazole analogues are listed in Table 2. The determined values of HF energy (E_{HF}) of compound **2c** and **2j** are lower than that of compound **2a**, indicating the more thermodynamic stability of **2c** and **2j**. The highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO-LUMO) energy differences of compound **2c** (6.85 eV) and **2j** (6.86 eV) are less than that of compound **2a** (7.76 eV). Figure 1 shows HOMO and LUMO isosurfaces of inactive (**2a**) and active (**2c**, **2j**) thiazole analogues, in which sulfur atom does not involve in LUMO of **2a**, but involves in **2c** and **2j**, which imply the region reacting with potential biological nucleophiles. Thiazole peptide class of antibiotics exerts their antibacterial effects through binding of sulfur atom of thiazole ring and amine group to the target site and cause membrane disruption, cell lysis and inhibition of bacterial protein biosynthesis.[24]

Lipophilic character of a molecule is the most important factor for their antibacterial activity which helps to cross the cellular membrane or irreversibly damages the cellular membrane.[25] Figure 2 shows the molecular lipophilicity potential (MLP) map of inactive (**2a**) and active (**2c**, **2j**) thiazole analogues, suggesting that the latter two compounds are more lipophilic than the former compound. In the present study, the log *P* of the most active compounds **2c** (5.68) and **2j** (5.03) are much higher than those of lower active compounds **2e** (3.45), **2f** (3.92), **2h** (4.63), **2i** (4.61) and inactive compounds **2a** (3.96), **2b** (4.40) and **2d** (3.48). Although a small number of compounds are used in the present study, a trend was observed in which the antibacterial activity decreases with decreasing the log *P*. This is expected since it is known the log *P* is usually correlated with a biological activity[26] which is also consistent with our results. Molecular volume also plays an important role in quantitative structure–activity relationship (QSAR) studies to model molecular properties and biological activity. It was also observed that the active compounds such as compound **2c** (266.96 Å^3), **2h** (262.61 Å^3), **2i** (262.61 Å^3) and **2j** (274.61 Å^3) has higher molecular volume than inactive compounds, **2a** (249.08 Å^3), **2b** (265.64 Å^3), **2d** (257.09 Å^3) except compound **2f** (272.41 Å^3). But, log *P* of compound **2f** is lower than the active compounds. The correlation coefficient (r^2) between the log *P* and inhibition zone of active compounds against *B. cereus* and *B. megaterium* were found 0.70 and 0.71, respectively. The above correlations should be treated with caution, because the number of active compounds is low as well as in-

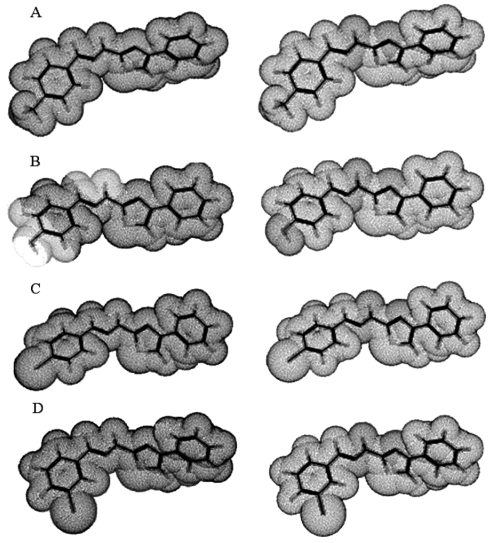

Fig. 3. Map of Lipophilicity Potential (Left) and Polar Surface Area (Right) of **2b** (A), **2d** (B), **2h** (C), and **2i** (D)

active and lower active compounds have small differences in log *P* and molecular volume, which do not fully explain the different activity. Therefore map of polar surface area and lipophilicity potential were compared for compound **2b**, **2d**, **2h** and **2i** (Fig. 3). It is noticeable that the polarity and lipophilicity are different for all molecules. The higher polarity of the surface and lower lipophilicity may explain the inactivity and lower antibacterial activity of the molecules. The design and synthesis of more 2-arylidenehydrazinyl-4-arylthiazole analogues with more lipophilic and hydrophilic substituents are in progress to elucidate this point.

Conclusion

The present study reports on the synthesis of novel 2-arylidenehydrazinyl-4-arylthiazole derivatives and their antibacterial activities. Using Hantzsch's method, 11 compounds could be prepared from arylidenethiosemicarbazones and their inhibition activities against eight bacteria and three fungi were evaluated. Compounds **2c** and **2j** containing bromo or methoxy substituent on the *para*-position of the arylidene phenyl ring exhibited strong activity against *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus megaterium*, *Salmonella typhi*, and *E. coli*. Polar group substitution resulted in a loss of activity (**2d**—**f**). Quantum-chemical and physicochemical calculations indicate that antibacterial activity correlates well with calculated log *P* and HOMO-LUMO energy difference of molecules. The promising antibacterial activity of compounds **2c** and **2j** and the results of computational studies would be helpful in synthesis of a large library of 2-arylidenehydrazinyl-4-arylthiazole analogues for extensive antimicrobial studies, which would be used to develop a more appropriate drug candidate.

Experimental

General All the reagents used were of commercial grade. Reactions were monitored by TLC using silica gel F_{254} plates (Merck, Germany) and the compounds were visualized either by exposure to UV light or dipping in iodine chamber.

Physical Measurements Melting points were determined on an X-5 melting point apparatus (Yuxiagyiqi, Gongyi City Yuxiang Instruments Co., Ltd., China) and are uncorrected. IR spectra were obtained on an FTIR-8430S (Shimadzu, Japan) in KBr discs. NMR spectra were recorded on an AM-400MH (Bruker, U.S.A.) in DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. Mass spectra were measured on an HP-5988 spectrometer (EI, 70 eV). Elemental analyses (C, H, N) were performed by means of a Perkin-Elmer 2400 II CHN elemental analyzer.

General Procedures for Preparation of Thiosemicarbazone Analogues (1a—k) To a stirred solution of thiosemicarbazide (1 mmol) in an ethanol–water mixture, an ethanolic solution of substituted benzaldehydes (1 mmol) was added slowly and refluxed for 10—20 min. After cooling the reaction mixture to an ambient temperature, the mixture was filtered to give a solid crude product, which was crystallized from ethanol to furnish pure compounds **1a**—**k** with a yield of 86—98%.

2-Benzylidenehydrazinecarbothioamide (**1a**): Yield 98%, mp 164—165 °C. ^{1}H-NMR (ppm) δ: 7.35—7.52 (m, 3H, Ar-H), 7.87—8.00 (m, 2H, Ar-H), 7.74 and 7.81 (2×s, 2H, –NH_2), 8.17 (s, 1H, CH), 11.40 (s, 1H, –NH–). IR (cm^{-1}): 3422 (NH_2), 3251 (NH), 1590 (C=N), 1298 (C=S).

2-(4-Methylbenzylidene)hydrazinecarbothioamide (**1b**): Yield 80%, mp 175—176 °C. ^{1}H-NMR (ppm) δ: 2.42 (s, 3H, –CH_3), 6.96 (d, 2H, J=8.2 Hz, Ar-H), 7.76 (d, 2H, J=8.2 Hz, Ar-H), 7.92 and 8.12 (2×s, 2H, –NH_2), 8.03 (s, 1H, CH), 11.33 (s, 1H, –NH–). IR (cm^{-1}): 3390 (NH_2), 3270 (NH), 1610 (C=N), 1260 (C=S).

2-(4-Bromobenzylidene)hydrazinecarbothioamide (**1c**): Yield 91%, mp 219—220 °C. ^{1}H-NMR (ppm) δ: 7.43 (d, 2H, J=8.1 Hz, Ar-H), 7.84 (d, 2H, J=8.1 Hz, Ar-H), 8.01 and 8.07 (2×s, 2H, –NH_2), 8.23 (s, 1H, CH), 11.47 (s, 1H, –NH–). IR (cm^{-1}): 3431 (NH_2), 3278 (NH), 1609 (C=N), 1279 (C=S).

2-(4-Hydroxybenzylidene)hydrazinecarbothioamide (**1d**): Yield 92%, mp 217—218 °C. ^{1}H-NMR (ppm) δ: 6.73 (d, 2H, J=8.2 Hz, Ar-H), 7.60 (d, 2H, J=8.2 Hz, Ar-H), 7.81 and 7.91 (2×s, 2H, –NH_2), 8.04 (s, 1H, CH), 9.84 (s, 1H, OH), 11.22 (s, 1H, –NH–). IR (cm^{-1}): 3467 (NH_2), 3359 (NH), 1608 (C=N), 1232 (C=S).

2-(2-Hydroxybenzylidene)hydrazinecarbothioamide (**1e**): Yield 93%, mp 222—223 °C. ^{1}H-NMR (ppm) δ: 6.77—6.91 (m, 2H, Ar-H), 7.21—7.35 (m, 2H, Ar-H), 7.89 and 7.95 (2×s, 2H, –NH_2), 8.35 (s, 1H, CH), 9.86 (s, 1H, OH), 11.36 (s, 1H, –NH–). IR (cm^{-1}): 3442 (NH_2), 3317 (NH), 1614 (C=N), 1265(C=S).

2-(4-Nitrobenzylidene)hydrazinecarbothioamide (**1f**): Yield 98%, mp 230—231 °C. ^{1}H-NMR (ppm) δ: 8.07 (d, 2H, J=8.2 Hz, Ar-H), 8.22 (d, 2H, J=8.2 Hz, Ar-H), 8.26 and 8.38 (2×s, 2H, –NH_2), 8.41 (s, 1H, CH), 11.71 (s, 1H, –NH–). IR (cm^{-1}): 3490 (NH_2), 3363 (NH), 1589 (C=N), 1247 (C=S).

2-(3-Nitrobenzylidene)hydrazinecarbothioamide (**1g**): Yield 98%, mp 215—216 °C. ^{1}H-NMR (ppm) δ: 7.64—781 (m, 2H, Ar-H), 8.42—8.63 (m, 2H, Ar-H), 7.67 and 8.18 (2×s, 2H, –NH_2), 8.19 (s, 1H, CH), 11.60 (s, 1H, –NH–). IR (cm^{-1}): 3393 (NH_2), 3239 (NH), 1603 (C=N), 1299 (C=S).

2-(4-Chlorobenzylidene)hydrazinecarbothioamide (**1h**): Yield 89%, mp 209—211 °C. ^{1}H-NMR (ppm) δ: 7.43 (d, 2H, J=8.2 Hz, Ar-H), 7.84 (d, 2H, J=8.2 Hz, Ar-H), 8.00 and 8.02 (2×s, 2H, –NH_2),, 8.23 (s, 1H, CH), 11.47 (s, 1H, –NH–). IR (cm^{-1}): 3436 (NH_2), 3278 (NH), 3105 (C–H), 1600 (C=N), 1282 (C=S).

2-(2-Chlorobenzylidene)hydrazinecarbothioamide (**1i**): Yield 91%, mp 220—221 °C. ^{1}H-NMR (ppm) δ: 6.74—6.95 (m, 2H, Ar-H), 7.16—7.27 (m, 2H, Ar-H), 7.94 and 7.99 (2×s, 2H, –NH_2), 8.40 (s, 1H, CH), 11.39 (s, 1H, –NH–). IR (cm^{-1}): 3438 (NH_2), 3321 (NH), 1619 (C=N), 1275 (C=S).

2-(4-Methoxybenzylidene)hydrazinecarbothioamide (**1j**): Yield 88%, mp 172—173 °C. ^{1}H-NMR (ppm) δ: 3.80 (s, 3H, CH_3), 6.96 (d, 2H, J=8.3 Hz, Ar-H), 7.76 (d, 2H, J=8.3 Hz, Ar-H), 7.92 and 8.12 (2×s, 2H, –NH_2), 8.03 (s, 1H, CH), 11.33 (s, 1H, –NH–). IR (cm^{-1}): 3390 (NH_2), 3270 (NH), 1610 (C=N), 1260 (C=S).

2-(4-(Dimethylamino)benzylidene)hydrazinecarbothioamide (**1k**): Yield 86%, mp 193—194 °C. ^{1}H-NMR (ppm) δ: 2.94 (s, 6H, CH_3), 6.67 (d, 2H, J=8.2 Hz, Ar-H), 7.55 (d, 2H, J=8.2 Hz, Ar-H), 7.75 and 7.91 (2×s, 2H, –NH_2), 7.99 (s, 1H, CH), 11.17 (s, 1H, NH). IR (cm^{-1}): 3373 (NH_2), 3330 (NH), 1600 (C=N), 1369 (C=S).

General Procedure for Preparation of 1,3-Thiazole Analogues (2a—k) A mixture of thiosemicarbazone (1 mmol) and 2-bromoacetophenone (198 mg, 1 mmol) in ethanol was refluxed for 30—60 min and then cooled to ambient temperature. The resulting precipitate was filtered and washed with water to give a crude product, which was purified by crystallization in a *N*,*N*-dimethylformamide (DMF)–EtOH mixture, affording pure 1,3-thiazole derivatives with a yield of 70—87%.

2-(2-Benzylidenehydrazinyl)-4-phenylthiazole (**2a**): Yield 80%, mp 202—203 °C. ^{1}H-NMR (ppm) δ: 6.77—7.75 (m, 11H, Ar-H+thiazole-H), 8.22 (s, H, CH=N), 11.30 (s, 1H, NH). IR (cm^{-1}): 3196 (NH), 1621, 1558,

1489 (C=C, C=N). EI-MS *m/z* (%): 279 (M^+, 100), 105 (20). *Anal.* Calcd for $C_{16}H_{13}N_3S$: C 68.79; H 4.69; N 15.04. Found: C 68.82; H 4.66; N 15.10.

2-(2-(4-Methylbenzylidene)hydrazinyl)-4-phenylthiazole (**2b**): Yield 83%, mp 205—206 °C. ^{1}H-NMR (ppm) δ: 2.44 (s, 3H, CH_3), 6.84—7.43 (m, 10H, Ar-H+thiazole-H), 7.87 (s, H, CH=N), 11.47 (s, 1H, NH). IR (cm^{-1}): 3172 (NH), 1622, 1562, 1512 (C=C, C=N). EI-MS *m/z* (%): 294 (M^+, 100), 159 (25), 120 (19), 104 (20). *Anal.* Calcd for $C_{17}H_{15}N_3S$: C 69.59; H 5.15; N 14.32. Found: C 69.67; H 5.11; N 14.41.

2-(2-(4-Bromobenzylidene)hydrazinyl)-4-phenylthiazole (**2c**): Yield 87%, mp 237—238 °C. ^{1}H-NMR (ppm) δ: 6.86 (d, 2H, J=8.1 Hz, Ar-H), 7.83 (d, 2H, J=8.1 Hz, Ar-H), 7.01—7.43 (m, 6H, Ar-H+thiazole-H), 7.92 (s, H, CH=N), 11.50 (s, 1H, NH). IR (cm^{-1}): 3299 (NH), 1599, 1570, 1481 (C=C, C=N), EI-MS *m/z* (%): 360 (M+3, 35), 359 (M+2, 100), 358 (M+1, 32), 357 (M^+, 95). *Anal.* Calcd for $C_{16}H_{12}BrN_3S$: C 53.64; H 3.38; N 11.73. Found: C 53.69; H 3.41; N 11.69.

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-4-phenylthiazole (**2d**): Yield 83%, mp 241—243 °C. ^{1}H-NMR (ppm) δ: 6.94—7.70 (m, 10H, Ar-H+thiazole-H), 7.97 (s, H, CH=N), 8.96 (s, 1H, –OH), 11.51 (s, 1H, NH). IR (cm^{-1}): 3092 (NH), 1602, 1574, 1493 (C=C, C=N). EI-MS *m/z* (%): 295 (M^+, 100), 175 (20), 120 (20). *Anal.* Calcd for $C_{16}H_{13}N_3OS$: C 65.06; H 4.44; N 14.23. Found: C 65.11; H 4.41; N 14.19.

2-(2-(2-Hydroxybenzylidene)hydrazinyl)-4-phenylthiazole (**2e**): Yield 85%, mp 211—213 °C. ^{1}H-NMR (ppm) δ: 6.90—7.84 (m, 10H, Ar-H+thiazole-H), 8.61 (s, H, CH=N), 9.91 (s, H, OH), 11.46 (s, 1H, NH). IR (cm^{-1}): 3275 (NH), 2866, 1623, 1558, 1494 (C=C, C=N). EI-MS *m/z* (%): 295 (M^+, 100), 176 (30), 107 (20). *Anal.* Calcd for $C_{16}H_{13}N_3OS$: C 65.06; H 4.44; N 14.23. Found: C 65.03; H 4.47; N 14.27.

2-(2-(4-Nitrobenzylidene)hydrazinyl)-4-phenylthiazole (**2f**): Yield 70%, mp 250—251 °C. ^{1}H-NMR (ppm) δ: 6.45—7.62 (m, 10H, Ar-H+thiazole-H), 7.66 (s, H, CH=N), 11.81 (s, 1H, NH). IR (cm^{-1}): 3145 (NH), 1622, 1557, 1506 (C=C, C=N). EI-MS *m/z* (%): 325 (M^+, 100), 107 (20). *Anal.* Calcd for $C_{16}H_{12}N_4O_2S$: C 59.25; H 3.73; N17.27. Found: C 59.27; H 3.79; N 17.30.

2-(2-(3-Nitrobenzylidene)hydrazinyl)-4-phenylthiazole (**2g**): Yield 75%, mp 228—230 °C. ^{1}H-NMR (ppm) δ: 6.92—8.14 (m, 10H, Ar-H+thiazole-H), 8.42 (s, H, CH=N), 11.61 (s, 1H, NH). IR (cm^{-1}): 3166 (NH), 2922, 1602, 1525, 1483 (C=C, C=N). EI-MS *m/z* (%): 325 (M^+, 100), 107 (20). *Anal.* Calcd for $C_{16}H_{12}N_4O_2S$: C 59.25; H 3.73; N 17.27. Found: C 59.23; H 3.80; N 17.32.

2-(2-(4-Chlorobenzylidene)hydrazinyl)-4-phenylthiazole (**2h**): Yield 87%, mp 199—200 °C. ^{1}H-NMR (ppm) δ: 6.86 (d, 2H, J=7.9 Hz, Ar-H), 7.83 (d, 2H, J=7.9 Hz, Ar-H), 7.08—7.43 (m, 6H, Ar-H+thiazole-H), 11.50 (s, 1H, NH). IR (cm^{-1}): 3299 (NH), 1599, 1570, 1481 (C=C, C=N). EI-MS *m/z* (%): 315 (M+2, 40), 313 (M^+, 100). *Anal.* Calcd for $C_{16}H_{12}ClN_3S$: C 61.24; H 3.85; N 13.39. Found: C 61.28; H 3.82; N 13.34.

2-(2-(2-Chlorobenzylidene)hydrazinyl)-4-phenylthiazole (**2i**): Yield 85%, mp 234—235 °C. ^{1}H-NMR (ppm) δ: 6.90—7.84 (m, 10H, Ar-H+thiazole-H), 8.62 (s, 1H, CH=N), 11.46 (s, 1H, NH). IR (cm^{-1}): 3305 (NH), 1589, 1564, 1483 (C=C, C=N). EI-MS *m/z* (%): 315 (M+2, 39), 313 (M^+, 100). *Anal.* Calcd for $C_{16}H_{12}ClN_3S$: C 61.24; H 3.85; N 13.39. Found: C 61.27; H 3.84; N 13.33.

2-(2-(4-Methoxybenzylidene)hydrazinyl)-4-phenylthiazole (**2j**): Yield 83%, mp 204—205 °C. ^{1}H-NMR (ppm) δ: 2.94 (s, 3H, OCH_3), 6.84—7.43 (m, 10 H, Ar-H+thiazole-H), 8.01 (s, 1H, CH=N), 11.48 (s, 1H, NH). IR (cm^{-1}): 3202 (NH), 1622, 1562, 1512 (C=C, C=N). EI-MS *m/z* (%): 309 (M^+, 100), 175 (15), 120 (29), 107 (20). *Anal.* Calcd for $C_{17}H_{15}ON_3S$: C 66.00; H 4.89; N 13.58. Found: C 66.04; H 4.84; N 13.55.

2-(2-(4-*N*,*N*-Dimethylbenzylidene)hydrazinyl)-4-phenylthiazole (**2k**): Yield 84%, mp 207—208 °C. ^{1}H-NMR (ppm) δ: 2.98 (s, 6H, NMe_2), 6.62—7.83 (m, 10H, Ar-H+thiazole-H), 7.98 (s, 1H, CH=N), 11.66 (s, 1H, NH). IR (cm^{-1}): 3109 (NH), 1615, 1543, 1508 (C=C, C=N). EI-MS *m/z* (%): 323 (M^+, 100), 149 (20). *Anal.* Calcd for $C_{18}H_{18}N_4S$: C 67.05; H 5.63; N 17.38. Found: C 67.03; H 5.59; N 17.32.

Antibacterial Screening *In vitro* bactericidal activity of novel 2-arylidenehydrazinyl-4-arylthiazole analogues was determined by the Kirby–Bauer disc diffusion method.[27] Briefly, nutrient agar (NA) media (Difco) was used as basal medium for test bacteria. These agar media were inoculated with 0.2 ml of the 24 h liquid cultures containing microorganisms. Sample discs were placed gently on pre-inoculated agar plates and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, and *E. coli* were incubated aerobically at 37 °C and *Bacillus cereus*, *Shigella dysenteriae*, *Bacillus subtilis*, and *Bacillus megaterium* at 30 °C for 24 h. Discs with only dimethyl sulfoxide (DMSO) were used as a control and kanamycin was used as a positive control. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones.

Antifungal Screening Using a standard disc diffusion method, 2-arylidenehydrazinyl-4-arylthiazole analogues were tested *in vitro* for their antifungal properties toward *Candida albicans*, *Aspergillus oryzae*, and *Saccharomyces cerevis*.[27] Briefly, potato dextrose agar (Scharlau Chemi SA, U.S.A.) was used as basal medium for testing of fungi. Sterilized melted PDA medium (*ca.* 45 °C) was poured into a petridish (90 mm) and solidified. Prepared discs of samples were placed gently on solidified agar plates, and freshly seeded with the test organisms using sterile forceps. Discs with DMSO and kanamycin were used as negative and positive controls, respectively. Plates were incubated at 30±1 °C for 72 h. DMSO was used as a solvent for preparation of desired solutions of the test samples.

Computational Methods The molecular geometries of the thiazole analogues were built with a standard bond length and angles using ChemBio3D ultra Ver. 12 molecular modeling program (CambridgeSoft Corporation, Cambridge, MA 02140, U.S.A.). The energy was minimized by semi-empirical molecular orbital PM3 method[28] and then by the Hartree–Fock method at 6-31G basis set with R-Closed-Shell wave function using GAMESS Interface in the ChemBio3D ultra Ver. 12. Muliken charges and properties of frontier molecular orbitals of the compounds were analyzed using the results calculated at RHF/6-31G level. The map of molecular lipophilicity potential (MLP) and polar surface area (PSA) were viewed in Molinspiration Galaxy 3D Structure Generator (ver. 2010.02 beta) using optimized structure generated by RHF/6-31G level.

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