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Synthesis and evaluation of in vitro antioxidant, anticancer, and antibacterial properties of new benzylideneiminophenylthiazole analogues

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Abstract

A series of new benzylideneiminophenylthiazole analogues were designed and synthesized. Common spectroscopic methods, such as FT-IR, ^{1}H-, ^{13}C-NMR, and MASS spectra, and elemental analysis, were used to confirm the molecular structures. Then, the antioxidant, cytotoxicity, and anti-bacterial effects of synthesized analogues were assessed against 2,2-diphenyl-1-picrylhydrazyl (DPPH), three cancer cell lines, and two bacterial strains, respectively. Among the analogues, **7f** was detected as the most potent compound for antioxidant activity. Moreover, the compounds **7b**, **7f**, and **7 g** exhibited the maximum cytotoxicity activity against MCF-7, HepG-2, and A549 cell lines, respectively. Finally, **7e** showed the highest anti-bacterial activity against both *S. aureus* and *E. coli* strains. It was concluded from the antioxidant, cytotoxicity, and anti-bacterial effects that the benzylideneiminophenylthiazoles might serve as candidate molecules for the development of small molecules with medicinal potential.

Keywords Neoplasm, Antimicrobial, Antioxidant, Cytotoxicity

Introduction

Oxidation is a physiological procedure that occurs in all living organisms. An inconsonance between the production and accumulation of reactive nitrogen and oxygen types (ROS and RNS) and the capacity of a biological system to neutralize these reactive radicals leads to augmented oxidative stress. It can be the fundamental reason for the pathogenesis of various chronic diseases in humans [1], such as cardiovascular disease, aging, cataracts, autoimmune disorders, rheumatoid arthritis, cancer, and neurodegenerative diseases [2]. This may also change the target cell function, leading to cell death [3, 4]. Antioxidant complements or medicinal plant-based foods play a significant role in lowering oxidative harm produced through active oxygen types and free radicals [5]. Protection against radicals can be obtained by raising the amount of protection factors obtained from numerous pharmaceutical or dietary bases, such as vitamin A or C, and from synthetic or natural sources [6, 7]. Commercially available and widely used synthetic antioxidants include butylated hydroxy anisole and butylated hydroxy toluene [8].

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Cancer is well-known for an anomaly in the control mechanism that governs suppression or stimulation in normal cells [9]. According to the WHO statement issued in September 2022, cancer is the second most important cause of death worldwide, accounting for 10 million deaths in 2020. Cancer mortality is expected to rise further, with an estimated 13 million deaths by 2030 [10]. Chemotherapy is the most fundamental technique in cancer therapy because it employs a wide range of synthetic and natural compounds to destroy cancer cells [11].

Patients with cancer who are receiving chemotherapeutic treatment are more vulnerable to infectious bacteria due to a weakened immune system that affects their capability to fight infections and diseases [12]. Furthermore, ongoing infection exposure causes inflammation, which contributes to the appearance of cancer. Infectious agents such as viruses and fungi were responsible for close to two million new cancer cases [10].

Thiazole and thiazoline scaffolds have shown efficiency in antimicrobial, antifungal, antiparasitic, anticancer, and anti-proliferative activities, as well as antioxidant in preclinical reports [13]. Furthermore, among the various aromatic heterocycles, these compounds play an important role in drug design, and also these ring scaffolds can be recognized in several approved drugs [14]. Thiazole-including drugs have been shown in clinical use for more than 30 years; for instance, cefdinir, cephalosporin [15], abafungin [16], tiazofurin [17], dasatinib [18], and dabrafenib [19–23] (Fig. 1). The molecules having the thiazole scaffold with anticancer, antimicrobial, and antioxidant activities were chosen and shown in Fig. 1 too [8, 10, 24–28].

According to the mentioned results and the structural similarity of thiazole scaffold with thiazolidine, we were encouraged to synthesize derivatives that contain an imino-thiazoline moiety. Then, the synthesized derivatives were tested for antioxidant, cytotoxicity, and antibacterial properties on DPPH, three A549, HepG-2, and MCF-7 cell lines, and two *S. aureus* and *E. coli* strains, respectively. The rational design of derivatives based on previous findings is revealed in Fig. 2. In the design of current derivatives, there is an *N*-methylenethiazol-3(2*H*)-amine moiety, which was in previously reported compounds (red and blue colors in Fig. 2). Moreover, reported compounds had aromatic or heteroaromatic moiety linked to this moiety, and designed derivatives have substituted-phenyl rings too (red and green colors in Fig. 2). The substituted-imino moiety was placed in designed derivatives so that there is a methyl group in some of the previous compounds (black part in Fig. 2).

Results and discussion

Chemistry

Herein, we reported the synthesis of alkyl *N*-benzylidene-2-(substituted-imino)-5-phenylthiazol-3(2*H*)-amine

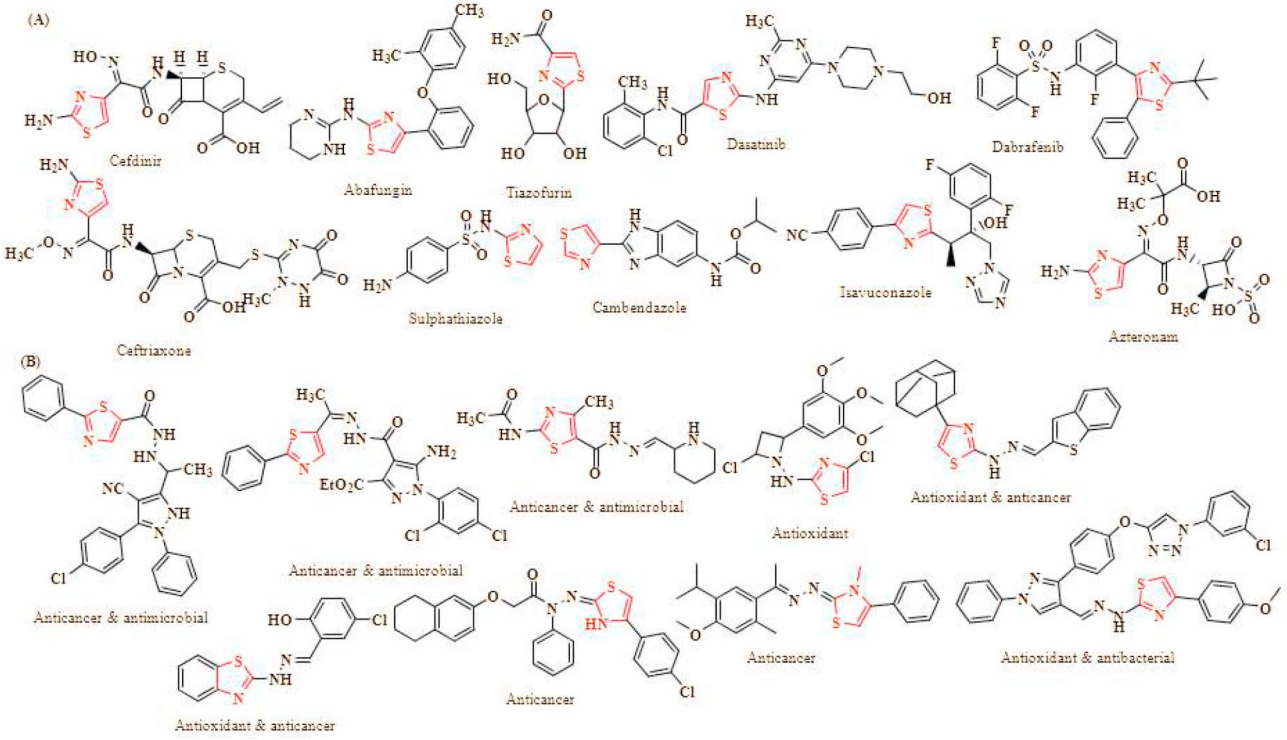


Fig. 1 Some drugs having thiazole scaffold (**A**) and antimicrobial, anticancer, and antioxidant aiming based on thiazole moiety (**B**)

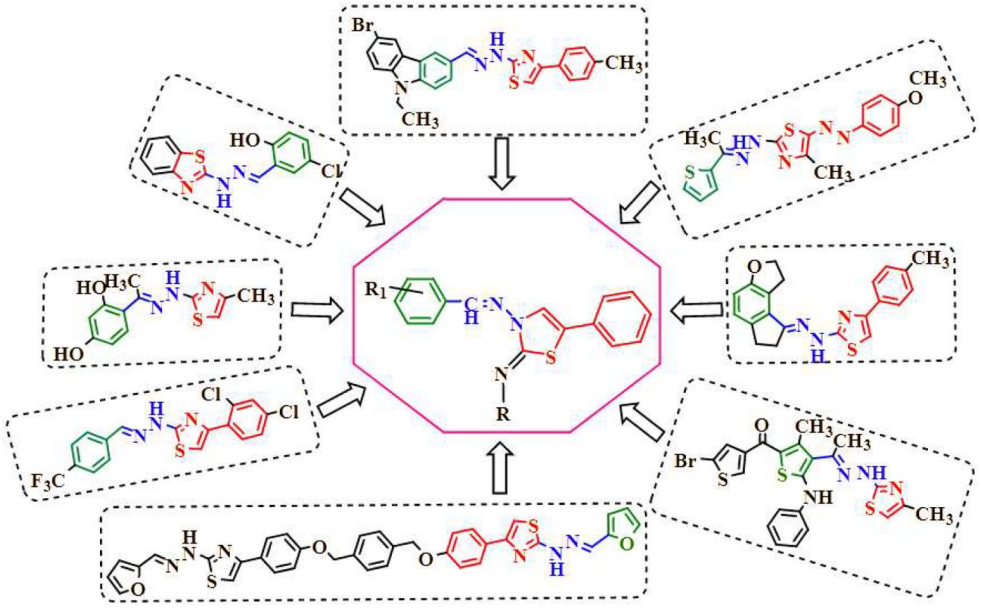
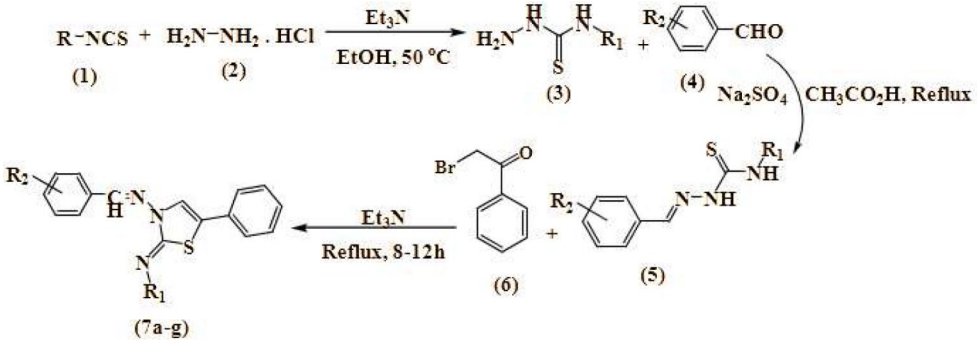


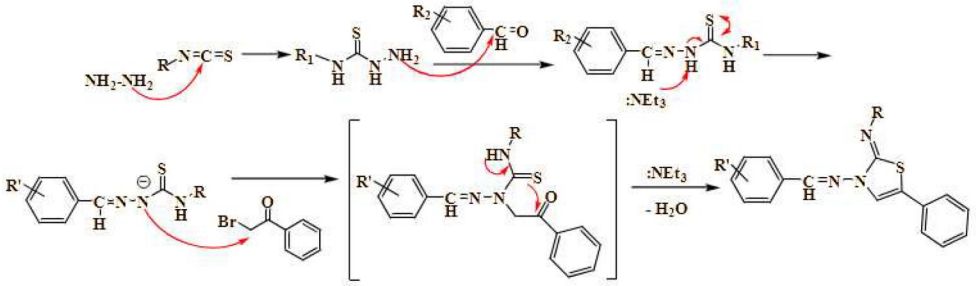
Fig. 2 The design strategy for synthesized compounds as antioxidant, antimicrobial, and anticancer agents



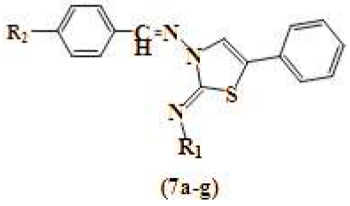
Scheme 1 Synthesis of alkyl *N*-benzylidene-2-(substituted-imino)-5-phenylthiazol-3(2*H*)-amine derivatives (**7a-g**)

derivatives (**7a-g**) by using a one-pot, three-step, four-component procedure. The synthesis procedure for the titled derivatives (**7a-g**) is shown in Scheme 1. The reaction of hydrazine, various benzaldehydes, and appropriate isothiocyanate with phenacyl bromide afforded substituted alkyl *N*-benzylidene-2-(substituted-imino)-5-phenylthiazol-3(2*H*)-amine (**7a-g**). All the derivatives were cleanly isolated by straightway filtration and purified by ethanol/water (1:1 v/v). During this process, the derivatives obtained had good yields. The compounds were confirmed using spectral data, including FT-IR, ^{1}H-NMR, ^{13}C-NMR, and MASS spectra, and elemental analysis. The ^{1}H-NMR spectra of synthesized derivatives **7a-g** exhibited a peak at 6.99–7.76 ppm for CH of the thiazole ring proton, and a singlet peak at 8.34–8.76 ppm belonged to the CH proton of the benzylidene moiety. The FT-IR spectra of the derivatives established characteristic absorption peaks for the C=N bond at 1606–1625 cm^{-1} and a strong band related to C-S at 1212–1271 cm^{-1} (ring closure).

The probable reaction mechanism of the one-pot, three-step, and four-component procedure for the synthesis of alkyl *N*-benzylidene-2-(substituted-imino)-5-phenylthiazol-3(2*H*)-amine derivatives (**7a-g**) is depicted in Scheme 2. Hydrazine reacted with substituted isothiocyanate to give hydrazinecarbothioamide intermediate **3**. Then, the condensation reaction of intermediate **3** with benzaldehyde derivatives afforded intermediate **5**. Subsequently, Et_3N, as a basic catalyst, deprotonated the hydrazone NH of intermediate **5**, and this transformation was due to the higher acidity of the NH proton connected to the hydrazone moiety. After that, the nitrogen atom attacked the bromide atom of phenacyl bromide, and then, in the presence of excess Et_3N, the condensation cyclization reaction gave the target compounds (**7a-g**) (Scheme 2).



Scheme 2 The reaction probable mechanism for the one-pot, three-step, and four-component synthesis of alkyl *N*-benzylidene-2-(substituted-imino)-5-phenylthiazol-3(2*H*)-amine (**7a-g**)



(7a-g)

Compounds	R_1	R_2	IC_{50} (μM)	DPPH Scavenging Activity (%) (200 μM)
7a	Pyridine	4-F	356.09 ± 0.059	78.51
7b	Pyridine	3,4-di-OCH_3	328.61 ± 0.049	81.95
7c	NO_2	4-F	847.48 ± 0.16	57.12
7d	NO_2	4-Br	-	5.29
7e	CH_3	4-CH_3	-	4.78
7f	CH_3	4-OCH_3	311.76 ± 0.042	79.32
7 g	CH_3	4-Br	-	7.46
BHT	-	-	207.45	90.45

Table 1 Antioxidant activity of compounds (**7a-g**)

Biological activities

Antioxidant activity

The antioxidant activity of the new analogues **7a-g** was investigated by the radical scavenging method 2,2-diphenyl-1 picrylhydrazyl (DPPH). Butylated hydroxyl toluene (BHT) was used as the positive control. This technique is modest, accurate, and inexpensive. In this method, antioxidant activity is also evaluated at room temperature, which removes the degradation of the screened derivatives [24]. DPPH is a stable free radical that is extensively used to estimate antioxidants' free radical scavenging properties. When DPPH reacts with a hydrogen donor, the purple color diminishes or disappears due to its conversion to 2,2-diphenyl-1-picryl hydrazine, resulting in a reduction in absorbance. In the presence of scavengers in the fractions, the more the decrease in absorption, the more effective its antioxidant activity is [29]. All of the screened derivatives (**7a-g**) demonstrated weak to moderate antioxidant activity (Table 1).

Compound **7f** exhibited the highest antioxidant activity, while compounds **7d**, **7e**, and **7 g** showed no antioxidant activity among the compounds. All compounds exhibited less activity than BHT as a standard. The obtained results allowed the conclusion that the type and electronic effects of substituents affected radical scavenging. Antioxidant activity was increased by the electron-donating groups (to stabilize the free radicals) while decreased by the electron-withdrawing groups (to destabilize free radicals) [30]. Compounds **7b** and **7f** with electron-donating substituents, such as methoxy and the methyl substituents at the C4 position of the phenyl ring showed the highest antioxidant properties. In addition, it was detected that the methoxy group showed a higher level of activity than methyl group. Furthermore, the methoxy derivatives had higher antioxidant activity than the other derivatives. The methoxy group could act as a good electron-donating group and raise the electronic cloud alongside the molecule.

Generally, halogens show diverse electronic effects related to their positions on the phenyl ring. Although halogens have both electron-donating inductive and electron-withdrawing resonance effects, their electron-withdrawing inductive effects are superior [31]. It was documented that chlorine and fluorine are electron-donating groups at the C4 position of phenyl ring, while they exhibit an electron-withdrawing effect at the C3

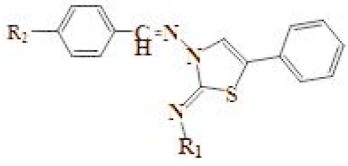
position of phenyl ring [32]. According to this result, compounds **7a**, **7c**, **7d**, and **7 g** having the fluorine and the bromine at the C4 position of phenyl ring showed less antioxidant activity than **7b** and **7f** with methoxy group at the same position. Interestingly, compounds bearing the fluorine atom were more potent than compounds with the bromine atom. As mentioned, increasing the electron-donating properties of the groups at the C4 position of the phenyl ring increased the antioxidant activity of the compounds. On the other hand, destabilized free radicals were also observed by the strong electron-withdrawing group, and this effect was possibly removed using the aromatic effect of the phenyl rings. Besides, replacing the nitro group in **7c** with a pyridyl ring in **7a** at the imine thiazole moiety improved antioxidant activity [32].

Cytotoxicity activity

In vitro cytotoxicity activity of all derivatives **7a-g** was tested against lung carcinoma (A549), hepatocellular carcinoma (HepG-2), and human breast carcinoma cells (MCF-7). Doxorubicin was used as a standard. The cytotoxicity activity was determined using the methylthiazolyl tetrazolium (MTT) assay. The concentration of the compounds needed to inhibit 50% of the cells (IC_{50}) was evaluated and revealed in Table 2.

Results revealed that compound **7b** was more selective against the MCF-7 cell line than the HepG-2 and the A549, while compound **7f** was more selective against the HepG-2 cell line. Most of the screened derivatives were found to be the most effective against the MCF-7 cell line. Compound **7b**, having two methoxy groups at the C3 and C4 positions on the phenyl ring and a pyridyl ring on the imine thiazole moiety, showed the highest activity against MCF-7 cell line. Compound **7f** is placed in the second rank after **7b**. Removing a methoxy group from the C3 position on the phenyl ring and replacing the pyridyl ring with methyl reduced cytotoxicity activity in this cell line. Thus, the presence of an electron-donating group on the phenyl ring had a positive effect on activity. Changing the methoxy group at the C4 position on the phenyl ring in **7f** with the methyl group at the same position in **7e** slightly decreased activity against the MCF-7 cell line. Lipophilic and small size groups at the C4 position on the phenyl ring decreased cytotoxicity activity. Similarly, replacing the methyl or the methoxy with a bromine atom showed lower activity against MCF-7. Based on the results, the electron-withdrawing group and lipophilic at the C4 position on the phenyl ring had a negative effect on the MCF-7 cell line. Switching the methyl on the imine thiazole moiety in **7 g** with the nitro group in **7d** dramatically reduced activity. On the other hand, compound **7c** having a fluorine atom at the C4 position on the phenyl ring and the nitro on imine thiazole moiety, intensely decreased activity compared to **7d**. Based on the findings, the presence of a strong electron-withdrawing group (NO_2) on imine thiazole moiety significantly reduced activity against MCF-7 cell line.

Against HepG-2 cell line, compound **7f** bearing a methoxy group in the C4 position on the phenyl ring and a methyl group on imine thiazole moiety was the most potent analogue among compounds. After this compound, compound **7b** exhibited the highest cytotoxicity activity. An extra methoxy group on phenyl ring and the replacement of the methyl with the nitro group on imine thiazole moiety reduced activity against HepG-2 cell line. Changing the methoxy group in **7f** with the methyl and the bromine in **7e** and **7 g**, respectively, at the C4 position



(7a-g)

Compounds	R_1	R_2	IC_{50}(μM)		
			MCF-7	HepG-2	A549
7a	Pyridine	4-F	88.10 ± 0.09	167.34 ± 1.11	> 200
7b	Pyridine	3,4-di-OCH_3	15.43 ± 0.32	58.32 ± 0.67	145.69 ± 1.02
7c	NO_2	4-F	> 200	> 200	119.08 ± 0.94
7d	NO_2	4-Br	127.91 ± 1.08	95.23 ± 1.12	114.03 ± 1.04
7e	CH_3	4-CH_3	48.23 ± 0.18	62.56 ± 0.78	95.14 ± 0.18
7f	CH_3	4-OCH_3	31.67 ± 1.08	20.04 ± 0.87	98.19 ± 1.09
7 g	CH_3	4-Br	75.84 ± 0.12	77.21 ± 0.05	72.94 ± 0.87
Doxorubicin	-	-	9.58	15.83	12.31

Table 2 The findings of screened derivatives against MCF-7, A549, and HepG-2 human cancer cell lines

of the phenyl ring decreased activity. Moreover, the existence of an electron-withdrawing group (F and Br or NO_2) at the C4 position of the phenyl ring and on imine thiazole moiety remarkably decreased activity against HepG-2 cell line, similar to MCF-7 cell line.

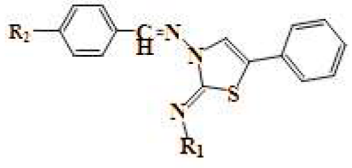
Compound **7 g** with a bromine atom at the C4 position on the phenyl ring and the methyl on imine thiazole moiety exhibited the highest activity against A549 cell line. Replacing the bromine atom with a methoxy or methyl group decreased activity against A549 cell line. It revealed that the presence of an electron-donating group on the phenyl ring had a negative effect on A549 cell line. This finding was inconsistent with HepG-2 and MCF-7 cell lines. Furthermore, placing the fluorine atom on the phenyl ring and the nitro group on imine thiazole moiety dramatically decreased cytotoxicity activity against A549 cell line. It concluded that the presence of an electron-donating group as methoxy in **7b** and **7f** and methyl group in **7e** at the C4 position of the phenyl ring increased cytotoxicity activity against HepG-2 and MCF7 cell lines, while it decreased cytotoxicity activity against A549 cell line. These results were in agreement with previous reports [10, 33].

Anti-bacterial activity

The titled derivatives were tested for in vitro anti-bacterial activity on a gram-positive strain, *S. aureus*, and a gram-negative strain, *E. coli*. The antimicrobial activity of thiazole-containing compounds against multidrug-resistant strains of *S. aureus* has been reported [34]. Table 3 shows the minimum inhibitory concentration (MIC) values for screened derivatives. The findings showed that the type and nature of the substituents on the phenyl ring and imine thiazole moiety had a significant effect on the anti-bacterial activity of the derivatives. The screened derivatives showed less anti-bacterial activity against gram-negative than gram-positive bacteria. All compounds exhibited less anti-bacterial activity compared to ciprofloxacin as standard. Whereas, compound **7e** displayed higher activity compared to ciprofloxacin against *S. aureus* strain.

The analogues having moderate electron-donating substituents (e.g., CH_3) showed higher anti-bacterial activity than strong electron-donating substituents such as methoxy [35]. The presence of a methoxy group at the C4 position on the phenyl ring in **7f** revealed less anti-bacterial effect than a methyl group at the same position in **7e**.

In substituted-halogen compounds, the anti-bacterial activity improved from the fluorine to the bromine atom [35]. Based on the findings, the bromine substituent on the phenyl ring indicated higher activity compared to the fluorine substituent when the methyl group was on imine thiazole moiety. While it showed lower activity when the nitro group was on imine thiazole moiety. Thus, it seems that the nature and electronic effect of the substituents on imine thiazole moiety affected anti-bacterial activity. These results indicated that increasing electron-withdrawing capability and hydrophilicity led to a reduction in activity. It was generally agreed that derivatives having moderate electron-donating substituents are the best candidates for attaining the best anti-bacterial activity. The electron density of the derivatives may shed light on the reason for this result. It has been reported that electron-donating substituents increased electron density, which improved the anti-bacterial activity of the analogues [36, 37]. Regardless, a very high electron density showed no anti-bacterial activity. As a result, derivatives



(7a-g)

Compounds	R_1	R_2	MIC (μM)	
			E. coli	*S. aureus*
7a	Pyridine	4-F	> 100	> 100
7b	Pyridine	3,4-di-OCH_3	55.43 ± 0.76	35.49 ± 0.81
7c	NO_2	4-F	75.04 ± 1.10	48.68 ± 0.85
7d	NO_2	4-Br	> 100	57.12 ± 1.04
7e	CH_3	4-CH_3	21.15 ± 0.95	15.31 ± 0.89
7f	CH_3	4-OCH_3	47.91 ± 1.12	26.23 ± 1.03
7 g	CH_3	4-Br	59.38 ± 1.07	38.06 ± 0.92
Ciprofloxacin	-	-	16.01	20.87

Table 3 Anti-bacterial activity of derivatives **7a-g** against *E. Coli* and *S. aureus* strains

with methoxy substituents showed weak anti-bacterial activity [38, 39].

Experimental

Chemistry

All chemicals were purchased from Sigma-Aldrich (USA) and were applied without further purification. FT-IR spectra of all the compounds were recorded using a Perkin-Elmer apparatus. ^{1}H- and ^{13}C-NMR spectra were measured (DMSO-d_6 solution) with a Bruker Ultrashield (at 400 and 100 MHz) instrument. Elemental analyses of the compounds for C, H and N were performed using a Heraeus CHN-O-Rapid analyzer.

General procedure for the synthesis of alkyl N-benzylidene-2-(substituted-imino)-5-phenylthiazol-3(2 H)-amines (7a-g)

Appropriate isothiocyanate **1** (1 mmol) and hydrazine **2** (1 mmol) were mixed in the presence of Et_3N and absolute ethanol (15 mL) at room temperature to provide intermediate *N*-arylhydrazinecarbothioamid **3**. Then, intermediate **5** was prepared by adding substituted benzaldehydes **4** (1 mmol) to the mixture in the presence of acid acetic and Na_2SO_4. In the next step, phenacyl bromide **6** and Et_3N were added to the mixture at reflux conditions for a specified time (8–12 h). TLC was used to monitor the reaction's progress. Afterward, the mixture was cooled to room temperature and poured into the ice bath. Then, the powder residue was filtered and re-crystallized from EtOH/H_2O (1:1 v/v) to give pure products (**7a-g**).

The spectral characteristics of the provided derivatives have been detailed below. Also, the ^{1}H-NMR and ^{13}C-NMR spectra are given in the Figs. **S1-S14** in the Supplementary data.

(4-Fluoro-benzylidene)-[5-phenyl-2-(pyridin-3-ylimino)-thiazol-3-yl]-amine (7a)

Yield: 63%; Yellow precipitates; mp: 163–164 °C; R_f = 0.68 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}= 3416, 3131, 2987, 2832, 1621, 1602, 1544, 1484, 1295, 1227; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.79 (1H, s, CH), 8.49 (1H, d, J=2.0 Hz, ArH), 8.32 (1H, dd, J_1=1.2 Hz, J_2=1.2 Hz, ArH), 8.28 (1H, s, CH), 8.02 (2 H, dd, J_1=5.6 Hz, J_2=5.6 Hz, ArH), 7.83 (2 H, dd, J_1=6.0 Hz, J_2=6.0 Hz, ArH), 7.71–7.63 (2 H, m, ArH), 7.43 (2 H, t, J=8.8 Hz, ArH), 7.35–7.30 (3 H, m, ArH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 161.25, 157.35, 152.74, 149.8, 147.3, 141.2, 135.6, 130.7, 129.4, 128.6, 127.2, 126.4, 126.1, 123.4, 116.6, 116.3, 115.4; Mass m/z (%): 374 (M), 296.2, 122.8. Anal. calcd for $C_{21}H_{15}FN_4S$: C 67.36, H 4.04, N 14.96, S 8.56%, found: C 67.01, H 3.86, N 15.21, S 8.37%.

(3-Methyl-4-phenyl-3 H-thiazol-2-ylidene)-pyridin-3-yl-amine (7b)

Yield: 63%; Yellow precipitates; mp: 193–194 °C; R_f = 0.74 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}= 3479, 3130, 2987, 2836, 1618, 1606, 1576, 1500, 1481, 1243, 1174; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 10.24 (1H, s, CH), 8.75 (1H, d, J=2.4 Hz, ArH), 8.53 (1H, dd, J_1=2.4 Hz, J_2=2.4 Hz, ArH), 8.25–8.22 (2 H, m, ArH), 7.71–7.72 (1H, m, ArH), 7.55 (2 H, dd, J_1=7.6 Hz, J_2=7.6 Hz, ArH), 7.49 (1H, s, CH), 7.42 (1H, t, J=6.0 Hz, ArH), 7.37–7.32 (2 H, m, ArH), 7.29–7.27 (2 H, m, ArH), 3.40 (6 H, s, 2 × OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 148.6, 145.5, 143.5, 142.6, 141.8, 139.4, 138.6, 136.8, 134.2, 132.7, 128.3, 126.5, 126.3, 126.2, 124.25, 123.3, 122.7, 95.3, 93.1, 43.1; Mass m/z (%): 416.7 (M), 339.1, 175.3. Anal. calcd for $C_{23}H_{20}N_4O_2S$: C 66.33, H 4.84, N 13.45, S 7.70%, found: C 66.68, H 4.63, N 13.6, S 7.96%.

(3-((4-Fluorobenzylidene)amino)-5-phenylthiazol-2(3 H)-ylidene)nitramide (7c)

Yield: 79%; White precipitates; mp: 115–116 °C; R_f = 0.69 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}= 3458, 3133, 3005, 2872, 1618, 1597, 1545, 1507, 1329, 1228; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.41 (1H, s, CH), 7.82 (2 H, dd, J_1=6.0 Hz, J_2=5.6 Hz, ArH), 7.56 (2 H, d, J=7.2 Hz, ArH), 7.50 (2 H, t, J=7.2 Hz, ArH), 7.45–7.41 (1H, m, ArH), 7.34 (2 H, t, J=8.8 Hz, ArH), 7.05 (1H, s, CH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 169.2, 164.2, 162.8, 151.2, 143.2, 133.3, 129.5, 129.3, 128.7, 127.36, 116.43, 94.5; Mass m/z (%): 342.3 (M), 220.1, 142.9; Anal. calcd for $C_{16}H_{11}FN_4O_2S$: C 56.13, H 3.24, N 16.37, S 9.36%, found: C 55.81, H 3.12, N 16.64, S 9.54%.

(3-((4-Bromobenzylidene)amino)-5-phenylthiazol-2(3 H)-ylidene)nitramide (7d)

Yield: 78%; yellow precipitates; mp: 183–184 °C; R_f = 0.73 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}= 3476, 3150, 3017, 2818, 1612, 1541, 1481, 1400, 1271, 1164; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.76 (1H, s, CH), 7.88 (5 H, brd, J=8.4 Hz, ArH), 7.77 (5 H, brd, J=8.4 Hz, ArH & CH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 177.8, 140.4, 134.4, 132.1, 131.5, 128.6, 128.1, 127.8, 127.4, 126.2, 122.5, 30.1; Mass m/z (%): 403.2 (M), 220.1, 142.9; Anal. calcd for $C_{16}H_{11}BrN_4O_2S$: C 47.66, H 2.75, N 13.89, S 7.95%, found: C 47.98, H 2.53, N 14.17, S 7.84%.

(4-Methylbenzylidene)-2-(methylimino)-5-phenylthiazol-3(2 H)-amine (7e)

Yield: 60%; Yellow precipitates; mp: 150–152 °C; R_f = 0.62 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}: 3483, 3144, 3050, 2982, 1614, 1572, 1547, 1446,

1303, 1212; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.36 (1H, s, CH), 7.66 (2 H, d, J=8.4 Hz, ArH), 7.56 (3 H, d, J=6.8 Hz, ArH), 7.49 (2 H, t, J=7.2 Hz, ArH), 7.43 (1H, d, J=7.2 Hz, ArH), 7.30 (1H, d, J=8.0 Hz, ArH), 7.01 (1H, s, CH), 2.72 (3 H, s, CH_3), 2.39 (3 H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 168.1, 151.9, 141.9, 139.3, 132.5, 129.7, 129.3, 128.6, 128.4, 128.1, 126.3, 125.5, 41.6, 21.6; Mass m/z (%): 307.4 (M), 189.1, 112.1; Anal. calcd for $C_{18}H_{17}N_3S$: C 70.33, H 5.57, N 13.67, S 10.43%, found: C 70.64, H 5.83, N 13.52, S 10.61%.

(4-Methoxybenzylidene)-2-(methylimino)-5-phenylthiazol-3(2 H)-amine (7f)

Yield : 59%; Yellow precipitates; mp: 144–145 °C; R_f = 0.75 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}= 3442, 3138, 3009, 2820, 1613, 1575, 1545, 1445, 1248, 1201; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.35 (1H, s, CH), 7.72 (2 H, d, J=8.8 Hz, ArH), 7.57 (2 H, t, J=8.8 Hz, ArH), 7.49 (2 H, t, J=7.6 Hz, ArH), 7.44 (1H, d, J=7.2 Hz, ArH), 7.06 (2 H, d, J=8.8 Hz, ArH), 6.99 (1H, s, CH), 3.86 (3 H, s, OCH_3), 2.71 (3 H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 167.3, 160.8, 152.3, 142.6, 128.7, 128.5, 127.8, 127.6, 126.2, 114.2, 95.7, 55.7, 41.3, 30.1; Mass m/z (%): 323.4 (M), 189.1, 112.1; Anal. calcd for $C_{18}H_{17}N_3OS$: C 66.85, H 5.30, N 12.99, S 9.91%, found: C 66.55, H 5.55, N 12.85, S 9.82%.

(4-Bromobenzylidene)-2-(methylimino)-5-phenylthiazol-3(2 H)-amine (7 g)

Yield: 70%; White precipitates; mp: 224–225 °C; R_f = 0.71 (n-hexane/ethyl acetate 1:2); FT-IR (KBr, cm^{-1}) ν_{max}= 3410, 3132, 3008, 2989, 1625, 1570, 1481, 1190, 1155; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.39 (1H, s, CH), 8.06 (2 H, s, ArH), 7.83 (2 H, d, J=8.4 Hz, ArH), 7.72–7.67 (3 H, m, ArH), 7.56 (1H, d, J=7.2 Hz, ArH), 7.50 (1H, t, J=7.2 Hz, ArH), 7.43 (1H, t, J=7.2 Hz, ArH), 7.08 (1H, s, CH), 2.73 (3 H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ/ppm: 161.2, 155.6, 137.6, 135.4, 134.2, 133.5, 132.4, 131.2, 128.4, 127.6, 127.3, 125.4, 42.5; Mass m/z (%): 372.3 (M), 189.1, 112.1; Anal. calcd for $C_{17}H_{14}BrN_3S$: C 54.85, H 3.79, N 11.29, S 8.61%, found: C 55.14, H 3.68, N 11.58, S 8.44%.

Biological activity

Antioxidant activity

The antioxidant activity of derivatives was assessed according to Sembiring et al. [40] method in a 96-well plate with slight modification. 20 µL of a sample solution of compounds in different concentrations (100–2000 ppm) was added to 180 20 µL of DPPH solution (0.147 mM) in each wall. After 1 h incubation in the dark room, absorbance was read at 520 nm by an ELIZA Reader. The scavenging ability (%) was considered as follows:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of standard} - \text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

BHT and DMSO were used as positive and negative controls, respectively. All examinations were carried out in triplicate.

Cytotoxicity activity

In vitro cytotoxicity activity of all derivatives was tested on HepG-2, A549, and MCF-7 cell lines by MTT assay. MCF-7, A549, and HepG2 cancer cell lines were obtained from Iranian Biological Resource Center (IBRC), Tehran, Iran. The positive control was doxorubicin, while DMSO (1%; Merck, Germany) and cell suspension were used as the negative controls. The cytotoxicity assay procedure for compounds was performed according to the process described in the previous report [41].

Anti-bacterial activity

S. aureus PTCC 1337 and *E. coli* PTCC 1338 were used to test the compounds' anti-bacterial activity, and the MIC was considered by the microplate Alamar blue assay method. Mueller-Hinton agar was used to culture the bacterial strains. Ciprofloxacin was used as a standard anti-bacterial drug. The anti-bacterial assay procedure for compounds was performed according to the process in the previous research [42].

Conclusion

In summary, some of the new benzylideneiminophenylthiazole analogues were synthesized, and their structures were confirmed using FT-IR, NMR, and MASS spectra. Then, the in vitro antioxidant, cytotoxicity, and anti-bacterial properties of analogues were evaluated. The screened derivatives showed weak to moderate antioxidant activity. Compound **7f** was the most potent compound against DPPH. According to findings, compounds having the nitro group as strong an electron-withdrawing group destabilized free radicals, however, this result might be removed using the aromatic effect of the phenyl rings. Besides, the presence of a pyridine ring instead of a nitro group and a fluorine atom on phenyl ring improved the antioxidant properties. However, the presence of a methoxy on the phenyl ring showed the highest antioxidant activity. In addition, derivatives exhibited moderate to good cytotoxicity activity against A549, HepG-2, and MCF-7 cell lines. Compounds **7b**, **7f**, and **7 g** showed the highest cytotoxicity activity against MCF-7, HepG-2, and A549 cell lines, respectively. The existence of methoxy as an electron-donating group in **7b** and **7f** and a methyl group **7e** at the C4 position of the phenyl ring increased cytotoxicity activity against HePG-2 and MCF7 while

decreased cytotoxicity activity against A549. On the other hand, anti-bacterial activity was tested against two *E. coli* and *S. aureus* strains. Compound **7e** showed the highest anti-bacterial activity against the two strains. It is generally agreed that compounds having electron-donating groups are good candidates for achieving the best anti-bacterial activity. All of the screened compounds showed moderate to good activity against both strains.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-024-01273-5.

Supplementary Material 1

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Author contributions
A.K. and J.B.S synthesized and purified the compounds, and analyzed 1 H-NMR, 13 C-NMR and FT-IR. A.K. and M.M. performed the biological tests. S.S. designed and supervised the current study, analyzed the obtained data and wrote the manuscript. All authors reviewed the manuscript.

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Data availability
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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