Synthesis and evaluation of biological activities of 4-cyclopropyl-5-(2-fluorophenyl) arylhydrazono-2,3-dihydrothiazoles as potent antioxidant agents

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ABSTRACT
A new series of 4-cyclopropyl-5-(2-fluorophenyl)arylhydrazono-2,3-dihydrothiazole derivatives was synthesized via the reaction of prepared thiosemicarbazones with 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone in the presence of Et₃N as a catalyst through a semi Hantzsch cyclization. The optimized reaction conditions for this one-pot reaction were achieved. The products were obtained in short reaction times, high yields and high purities. Antioxidant activity of products was evaluated using DPPH (2,2-diphenyl-2-picrylhydrazyl) and ABTS 2,2-azinobis(3-ethylbenzothiazoline-sulfonate) assays. Products showed higher antioxidant activity using the ABTS method. Compounds 5c and 5g showed lower IC₅₀ values compared with ascorbic acid as a standard. Compounds 5a–5h possessed moderate to high antioxidant activity by both methods. Also, antibacterial activity of 5a–5h was evaluated against gram-positive and gram-negative bacterial strains. None of the compounds inhibited A. hydrophila, while they had moderate to low inhibitory activity against other tested bacterial strains.

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Introduction

Sulfur-containing heterocycles have been under investigation for a long time because of their interesting and diverse biological properties. Also, sulfur is an important element that is incorporated into proteins and some biomolecules.[1] Thiazoles as sulfur-containing compounds possessed various applications in medicine. Thiazole moiety is found in thiamine (vitamin B1). Also, some important drugs such as penicillin, sulfathiazole, ritonavir, abafungin, bleomycin, and tiazofurin have thiazole moiety as their integral part. Thiazoles have attracted considerable attention due to their wide biological activities such as antitrypanosomal,[2] anti-inflammatory,[3,4] anti-hypertensive,[5] anti-allergic,[6] antibacterial,[7] anti-schizophrenia,[8] anti-HIV,[9] analgesic,[10] and anti-viral.[11] Moreover, hydrazinyl thiazole derivatives exhibited interesting biological activities including antioxidant,[12,13] antibacterial,[14] antimalarial,[15] antitumor,[16] and antifungal.[17] Also, hydrazinyl thiazole derivative of isatin has been used as a naked eye chemosensor for fluoride anion detection.[18] Recently, various biological activities, e.g. antibacterial, antifungal, antioxidant, cytotoxicity and DNA cleavage of complexes derived from thiazoles have been studied.[19,20]

Compounds bearing fluorine atom have shown interesting biological activities.[21,22] According to the literature, the presence of fluorine atom can increase the potential of biological activities, e.g. fluorinated Schiff bases derived from 1,2,4-triazoles showed potent antiproliferative activity,[23] and fluorine atom enhanced insecticidal activity of 2-(3-(2,6-dichloro-4-(3,3-dichloroallyloxy)phenoxy)propoxy)-5-(trifluoromethyl)benzo[d]oxazole.[24]

Sunlight, ultraviolet light, ionizing radiation, chemical reactions, and metabolic processes can produce free radical species. Free radical species can react and oxidize DNA, lipids, and proteins, and nucleic acids in living systems and result in degenerative disease and health problems. Antioxidants are a class of compounds which trap free radicals, so can reduce the risk for chronic diseases such as cancer and heart disease.[25]

As a part of our current studies in synthesis of thiazolyl-pyrazoline derivatives,[26] bis-thiazoles,[27] thiazolyl-pyridazinones,[28] and 1,4-dihydropyridines bearing thiazole moiety and possessing high antioxidant activity[29] here, we reported synthesis of new hydrazinyl thiazoles and evaluation of their antioxidant and antibacterial activities.

Results and discussion

Chemistry

α-Halocarbonyl compounds are convenient and widely used building blocks for various types of heterocyclization and for the Hantzsch's thiazole synthesis.[30] 2-Bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (α-halocarbonyl compound) is a key moiety in prasugrel as a platelet inhibitor drug, which is more efficient than ticlopidine and clopidogrel [31] (Figure 1). Recently, it has been used in the synthesis of thiazol-2-imine derivatives [32] so this prompted us to investigate synthesis of new thiazoles using 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone. Even though, the carbonyl group 4 is condensed to a carbon of thiazole ring, but the cyclopropyl and 2-fluorophenyl moieties still presented in the skeleton of target products 5 (Scheme 1).
In this study, new 4-cyclopropyl-5-(2-fluorophenyl)arylhydrazono-2,3-dihydrothiazole derivatives 5a–5h were synthesized through the reaction of carbonyl compounds 1a–1h, thiosemicarbazide 2, and 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone 4. Thiosemicarbazone derivatives 3a–3h were prepared through the condensation of carbonyl compounds 1a–1h and thiosemicarbazide 2 in the presence of a few drops of AcOH in EtOH. Then, cyclization of thiosemicarbazones 3a–3h with 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone 4 under optimized reaction conditions led to the corresponding products 5a–5h (Scheme 1).

With the aim to obtain the optimal reaction conditions, preparation of 5a was selected as a model reaction. Results are summarized in Table 1. The effects of different basic catalysts, amounts of catalyst, and solvents were studied. According to the results, the reaction proceeded slowly in low yield in the absence of catalyst (entry 1), while product 5a was obtained in a higher yield and shorter reaction time in the presence of Et3N (entry 2). Increasing the amount of catalyst showed no substantial improvement in the yield (entry 5), while the yield was decreased by reducing the amount of catalyst to 10% (entry 4). Moreover, the effects of other polar solvents such as DMF, methanol (MeOH) and CH3CN on the yield and time of the reaction were studied (entry 6–11). Thus, compound 5a was obtained from the reaction of thiosemicarbazone 3a and 4 in the presence of Et3N (20 mol%) in high yield (95%) and short reaction time (30 min) in EtOH as the solvent under
Table 1. Optimization of reaction condition for synthesis of 5a.

| Entry | Catalyst          | Solvent | Condition | Time (min) | Yield% |
|-------|-------------------|---------|-----------|------------|--------|
| 1     | –                 | EtOH    | r.t.      | 240        | 53     |
| 2     | Et₃N (20 mol%)    | EtOH    | r.t.      | 120        | 85     |
| 3     | Et₃N (20 mol%)    | EtOH    | Reflux    | 30         | 95     |
| 4     | Et₃N (10 mol%)    | EtOH    | Reflux    | 45         | 88     |
| 5     | Et₃N (40 mol%)    | EtOH    | Reflux    | 30         | 94     |
| 6     | Et₃N (20 mol%)    | DMF     | Reflux    | 30         | 91     |
| 7     | Et₃N (20 mol%)    | MeOH    | Reflux    | 40         | 82     |
| 8     | Et₃N (20 mol%)    | CH₃CN   | Reflux    | 50         | 85     |
| 9     | DBU (20 mol%)     | EtOH    | Reflux    | 40         | 80     |
| 10    | piperidine (20 mol%) | EtOH   | Reflux    | 45         | 82     |
| 11    | NaOAc (20 mol%)   | EtOH    | Reflux    | 90         | 76     |

reflux conditions. The formation of product 5a was indicated by a reaction color change from yellow to dark orange within 5 min, however completion of the reaction took place within 30 min.

The scope of this method was explored for the synthesis of new 4-cyclopropyl-5-(2-fluorophenyl)arylhydrazono-2,3-dihydrothiazoles 5a–5h with different carbonyl compounds 1a–1h under the optimized reaction conditions. The results showed that this procedure is reliable, simple setup, reproducible, high yield, economic and products purified without chromatographic methods. All products were fully characterized by IR, ¹H NMR and ¹³C NMR spectra. The physicochemical properties of synthesized compounds are presented and summarized in Table 2.

IR spectra of products 5a–5h showed characteristic absorption bands at 3320–3200, 1620–1600 and 1070–1050 cm⁻¹ due to N–H, C=Na and C–F bonds, respectively. ¹H NMR spectra of products were in accordance with expected number, chemical shifts and coupling constants. Aromatic protons appeared at 8.28–6.66 ppm. Also, aliphatic proton of cyclopropyl moiety (Hₐ) and methylene protons appeared as multiples at 1.99–1.86 and 1.20–0.88 ppm, respectively. In addition, N–H can be endo- or exo-cyclic. However, according to the literature [33] a signal at 14.50–11.45 ppm is related to exocyclic N–H. So, we can propose an exo-cyclic N–H for compound 5g according to a down field signal at 12 ppm and an endo-cyclic N–H for other compounds.

¹³C NMR spectra of compounds 5a–5h corresponded to the expected number and types of carbons. Aromatic and olefinic carbons appeared at 168.6–108.4 ppm and aliphatic carbons appeared at their expected chemical shifts at 55.9–7.8 ppm. ¹³C NMR spectra of products represented C–F couplings clearly. Fluorine atom has split all carbons of the phenyl ring. All chemical shifts and coupling constants of important carbon atoms are summarized in Table 3.

**Biological evaluation**

**Antioxidant activity**

All products were screened for their in vitro antioxidant activity. DPPH (2,2-diphenyl-2-picrylhydrazyl) radical and ABTS 2,2-azinobis(3-ethylbenzothiazoline-sulfonate) radical cation are widely used, rapid, simple, and inexpensive methods to evaluate antioxidant ability of compounds. In this research, the antioxidant activity of compounds was evaluated using colorimetric DPPH and ABTS methods. When a compound acts as an antioxidant
Table 2. Data related to synthesized 4-cyclopropyl-5-(2-fluorophenyl)arylhydrazono-2,3-dihydrothiazoles.

| Compound | Time (min) | Yield % | m.p. (°C)       |
|----------|------------|---------|-----------------|
| 5a       | 30         | 95      | 114–118         |
| 5b       | 30         | 94      | 120–123         |
| 5c       | 20         | 96      | 195–198         |
| 5d       | 35         | 89      | 89–92           |

| Compound | Time (min) | Yield % | m.p. (°C)       |
|----------|------------|---------|-----------------|
| 5e       | 20         | 93      | 182–186         |
| 5f       | 40         | 90      | 153–157         |
| 5g       | 35         | 91      | 85–87           |
| 5h       | 30         | 88      | 166–170         |

It can scavenge free radicals and lead to a decrease in absorption band at 517 and 734 nm for DPPH and ABTS solutions, respectively. Moreover, potential antioxidant activity leads to a rapid decrease in absorbance. ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. ABTS is converted to its radical cation (ABTS$^{+}$) by the addition of sodium persulfate or potassium persulfate. ABTS$^{+}$ scavenging is considered as an electron transfer reaction.\[34] ABTS radical cation is blue in color, when it reacts with an antioxidant compound the blue color changes to yellow or colorless. Also, the DPPH radical changes from purple to colorless in reaction with an antioxidant compound. DPPH$^\cdot$ scavenging may be through donation of a radical hydrogen atom (H$^\cdot$) to form a stable DPPH-H molecule.\[35]

The antioxidant activities of compounds were screened at concentrations of 125–4000 μg/mL at 517 and 734 nm for DPPH and ABTS assays, respectively. Also, IC$^{50}$
Table 3. $^{13}\text{C}$ NMR spectroscopy data of significant carbon atoms of 5a–5h.

| Compound | 5a   | 5b   | 5c   | 5d   | 5e   | 5f   | 5g   | 5h   |
|----------|------|------|------|------|------|------|------|------|
| $\text{C}_1$ | 140.8 | 139.1| 139.9| 139.9| 139.4| 146.3| 140.0| 143.3|
| $\text{C}_2$ | 159.9 | 159.9| 159.9| 159.9| 159.9| 159.9| 159.9| 159.9|
| $1_{\text{C,F}}$ | 247  | 247  | 249  | 248  | 247  | 247  | 247  | 247  |
| $2_{\text{C,F}}$ | 16   | 16   | 15   | 15   | 14   | 15   | 15   | 15   |
| $3_{\text{C,F}}$ | 7.8  | 7.8  | 8.3  | 7.9  | 8.3  | 7.8  | 8.3  | 7.9  |
| $4_{\text{C,F}}$ | 124.1| 124.4| 124.8| 124.4| 123.3| 124.1| 124.7| 124.2|
| $5_{\text{C,F}}$ | 129.1| 129.1| 131.5| 130.2| 131.4| 129.3| 131.3| 129.7|
| $6_{\text{C,F}}$ | 116.0| 116.0| 116.5| 116.2| 116.5| 116.0| 116.4| 116.1|
| $2_{\text{C,F}}$ | 122  | 122  | 122  | 122  | 122  | 122  | 122  | 122  |

Figure 2. DPPH radical-scavenging activity.

values (the concentration of compounds to scavenge 50% of DPPH or ABTS) were calculated by plotting radical scavenging activity against concentration and obtaining a line equation. Ascorbic acid was used as a standard. The investigation of antioxidant activity revealed that all the newly synthesized compounds showed potent to moderate radical scavenging activity when compared with ascorbic acid as a standard. As it is depicted in Figures 2 and 3 radical scavenging activity of products 5a–5h was dose dependent. In the DPPH assay, compounds 5a, 5b, 5e and 5g showed higher antioxidant activity at lower concentration (125–500 μg/mL) in comparison with others. While, at higher concentrations the antioxidant activity of all products was approximately equal. Moreover, compounds 5a and 5e showed a chigher antioxidant activity at low concentration (120 μg/mL) in the ABTS assay, while higher concentrations represented potent antioxidant activity.

In addition, IC$_{50}$ values of products 5a–5h were calculated (Figure 4). The IC$_{50}$ values were in the range of 1.92–0.17 and 0.96–0.08 μM for DPPH and ABTS assays, respectively.
As it is evident, the ABTS assay showed more potent antioxidant activity. It shows that compounds 5a–5h can donate radical electron better than the hydrogen radical. Product 5g was more active than ascorbic acid according to the DPPH assay, while compound 5c represented better antioxidant activity when compared with ascorbic acid in the ABTS assay. However, compounds 5a, 5c, 5e and 5g showed potent antioxidant activity. Other compounds showed moderate activity.

Thiazole moiety has an important role in antioxidant activity.[25,12] As it is depicted in Scheme 2 the endo- or exo- N–H readily can donate a hydrogen radical to the DPPH radical and generate a new radical species which can resonate through the thiazole ring and =C–N–N=C moieties. So, the new radical can be stable by resonance through this structure. Although the thiazole and =C–N–N=C moieties have an important role in antioxidant activity, however other parts of products can affect this activity as well. Probably, the high antioxidant activity of 5c can be due to the fused aromatic rings which can stabilize the free radical by resonance through a longer system. Low antioxidant activity of 5h is due to the presence of an electron-withdrawing group NO2, which resulted in destabilization of radicals (Scheme 2).[36]
Scheme 2. Proposed mechanism for antioxidant activity of compounds 5a–5h.

**Antibacterial activity**

The new synthesized compounds 5a–5h were screened for their *in vitro* antibacterial activity against Gram-positive and Gram-negative bacterial strains including: *Staphylococcus aureus* (*S. aureus*), *Micrococcus luteus* (*M. luteus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*Ps. aeruginosa*), *Bacillus subtilis* (*B. subtilis*), and *Aeromonas hydrophila* (*A. hydrophila*) using the well-diffusion method. Penicillin G and Gentamicin were used as positive controls. DMSO was used as a negative control and showed no activity against mentioned bacterial strains. The antibacterial activity of products 5a–5h was screened at a concentration of 1000 μg/mL in DMSO. The experiments were performed in triplicate. The results are presented as mean ± standard deviation in millimeter.

According to Table 4 all of the compounds were inactive against *A. hydrophila*, while all of the compounds showed antibacterial activity against *Ps. aeruginosa*. Moreover, 5a and 5f showed low antibacterial activity against *S. aureus*; however, most of the compounds possessed antibacterial activity against *E. coli* and *B. subtilis*. Furthermore, 5e showed the highest antibacterial activity against *B. subtilis* (14.3 ± 0.57) among all of the compounds. Also, 5f and 5b possessed higher antibacterial activity against *M. luteus* (10.6 ± 0.57) and *E. coli* (11.6 ± 1.15), respectively. Moreover, compounds 5a and 5c showed similar activity (10.3 ± 0.57) against *Ps. aeruginosa*. In addition, the antibacterial activity of compounds 5a–5h was not comparable to standard drugs.
Table 4. Antibacterial assay of 5a–5h. Results are presented as mean ± SD in mm.

| Compound | E. coli | A. hydrophila | Ps. aeruginosa | M. luteus | S. aureus | B. subtilis |
|----------|---------|---------------|---------------|-----------|-----------|-------------|
| 5a       | 9.3 ± 0.57 | –             | 10.3 ± 0.57   | –         | 8.6 ± 0.57 | 9.3 ± 0.57  |
| 5b       | 11.6 ± 1.15 | –             | 8.3 ± 0.57    | 8.6 ± 0.57| –         | 11.3 ± 0.57 |
| 5c       | 7.6 ± 0.57  | –             | 10.3 ± 0.57   | –         | 8.6 ± 0.57 | –           |
| 5d       | 9.3 ± 0.57  | –             | 9.3 ± 0.57    | 7.3 ± 0.57| –         | 10.3 ± 0.57 |
| 5e       | –         | –             | 9.3 ± 0.57    | 7.6 ± 0.57| –         | 14.3 ± 0.57 |
| 5f       | –         | –             | 9.3 ± 0.57    | 10.6 ± 0.57| 9.6 ± 0.57| 8.6 ± 0.57  |
| 5g       | 9.3 ± 0.57  | –             | 9 ± 1.0       | 8.3 ± 0.57| –         | 10.3 ± 0.57 |
| 5h       | 8.6 ± 0.57  | –             | 8.6 ± 0.57    | 7.6 ± 0.57| –         | –           |
| Penicillin G | 45.0 ± 1.0 | 46.6 ± 1.52   | 24.0 ± 1.0    | 54.0 ± 1.0| 23.0 ± 1.0| 32.0 ± 1.0  |
| Gentamicin | 32.0 ± 1.0 | 41.3 ± 1.57   | 29.6 ± 0.57   | 46.0 ± 1.0| 30.0 ± 1.0| 35.3 ± 0.57 |

Conclusion

In conclusion we have reported an efficient, convenient and easy setup procedure for synthesis of new 4-cyclopentyl-5-(2-fluorophenyl)arylhydrazono-2,3-dihydrothiazole derivatives. The optimized procedure led to high yields and high purities products in short reaction time. The obtained data revealed that products namely 5a, 5c, 5e and 5g exhibited promising antioxidant activity. The important role of hydrazinyl-thiazole moiety of synthesized compounds in antioxidant activity was discussed. The antioxidant activity by the ABTS method was higher than the DPPH method; this presents potential electron donation capacity of products besides their hydrogen atom transfer capacity. Products showed moderate to low antibacterial activity.

Experimental

Materials and instruments

Starting materials containing ketones, thiosemicarbazide, and 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone were obtained from Fluka company (Switzerland) and Merck company (Germany), antioxidant reagents were supplied from Sigma company (Germany), and biological cultures were obtained from Merck company (Germany) and Quelab company (Canada). All chemicals were used without further purification. All reactions were monitored by TLC performed on silica gel plates (60 F254 Merck). IR spectra were recorded on a Shimadzu IR-470 spectrophotometer in anhydrous potassium bromide (KBr). 1H NMR and 13C NMR spectra were recorded on a 400 MHz Bruker spectrometers using CDCl3 as the solvent and chemical shifts are expressed relative to TMS. Coupling constants were expressed in hertz (Hz). Melting points were determined using a Mettler Fp5 apparatus and are uncorrected. Absorbance of antioxidant assays was recorded on the Unico 2100 spectrophotometer. Elemental analyses were made by a Carlo–Erba EA1110 CNNO-S analyzer.

General procedure for synthesis of 5a–5h

Thiosemicarbazide 2 (2 mmol, 0.18 g) was added to a solution of carbonyl compound 1a–1h (2 mmol) in 10 mL EtOH as the solvent in the presence of a few drops of AcOH and refluxed for 2 h. The mixture was cooled down and the resulting precipitate was filtered off and dried at room temperature. Resulted thiosemicarbazone 3a-h (1 mmol) was dissolved in EtOH (5 mL) and 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone 4 (1 mmol) was
added to the mixture. The solution was refluxed in the presence of Et₃N (20% mole) for appropriate time. The progress of the reaction was monitored by TLC (n-hexane:EtOAc 7:2). After completion of the reaction, pH was controlled and the mixture was neutralized with saturated Na₂CO₃ solution. The solids was filtered off and dried at room temperature. The products obtained from EtOH:H₂O recrystallization.

4-Cyclopropy-5-(2-fluorophenyl)-2-((6-methoxy-3,4-dihydronaphthalen-1(2H)-ylidene)hydrazono)-2,3-dihydrothiazole 5a

Brown solid, yield: 95%, m.p. 114–118°C, IR (KBr, cm⁻¹) ν: 3320 (stretch N–H), 2930, 2820 (stretch C–H ali.), 1620 (stretch C=O), 1540, 1490 (stretch C=C), 1320 (stretch C–N), 1240, 1010 (stretch C–O), 1060 (stretch C–F), 890, 840, 810, 750 (OOP. C–H). ¹H NMR (CDCl₃, 400 MHz) δ: 8.07 (d, J = 8.8 Hz, 1H, Hₙ), 7.55 (dt, J = 7.1, 1.6 Hz, 1H, H₉), 7.38–7.32 (m, 1H, H₇), 6.82 (dd, J = 8.8, 2.4 Hz, 1H, H₆), 6.66 (d, J = 2.0 Hz, 1H, H₅), 3.84 (s, 3H, H₃), 2.78 (t, J = 6.0 Hz, 2H, H₂), 2.60 (t, J = 6.4 Hz, 2H, H₁). Anal. calcd. for C₂₃H₂₂FN₃OS: C, 67.75; H, 5.49; N, 10.36. Found: C, 67.80; H, 5.45; N, 10.30%.

4-Cyclopropy-5-(2-fluorophenyl)-2-((3,4-dihydronaphthalen-1(2H)-ylidene)hydrazono)-2,3-dihydrothiazole 5b

Light orange solid, yield: 94%, m.p. 120–123°C, IR (KBr, cm⁻¹) ν: 3310 (stretch N–H), 2930 (stretch C–H ali.), 1610 (stretch C=O), 1540, 1480 (stretch C=C), 1280 (stretch C–N), 1060 (stretch C–F), 760, 750, 730 (OOP. C–H). ¹H NMR (CDCl₃, 400 MHz) δ: 8.15–8.12 (m, 1H, Hₙ), 7.99 (br, s, 1H, NH), 7.57 (t, J = 7.6 Hz, 1H, H₉), 7.37–7.09 (m, 6H, H₆, H₇, H₈, H₉, H₄, H₅). ¹³C NMR (CDCl₃, 100 MHz) δ: 168.3 (C₅), 160.1, 159.9 (J_C,F = 247 Hz, C₂), 150.2 (C₆), 146.4, 140.8 (C₇), 132.3 (J_C,F = 3 Hz, C₉), 129.1 (J_C,F = 8 Hz, C₉), 126.4, 125.0, 121.5 ppm. Anal. calcd. for C₂₂H₂₀FN₃S: C, 70.02; H, 5.36; N, 11.10. Found: C, 69.98; H, 5.33; N, 11.14%.

4-Cyclopropy-5-(2-fluorophenyl)-2-((1-(naphthalen-2-yl)ethylidene)hydrazono)-2,3-dihydrothiazole 5c

Yellow solid, yield: 96%, m.p. 195–198°C, IR (KBr, cm⁻¹) ν: 3030 (stretch C–H ar.), 1600 (stretch C≡N), 1540, 1490 (stretch C≡C), 1340 (stretch C–N), 1070 (stretch C–F), 820, 810, 750 (OOP. C–H). ¹H NMR (CDCl₃, 400 MHz) δ: 8.17 (s, 1H, Ho), 8.03 (dd, J = 8.8, 1.6 Hz, 1H, H₁), 7.94–7.92 (m, 1H, Hi), 7.89–7.85 (m, 2H, H₄, H₅), 7.59–7.47 (m, 4H, H₆, H₇, H₈, NH), 7.33–7.24 (m, 3H, H₉, H₁₀, H₁₁), 2.69 (s, 3H, H₂, CH₃), 1.99–1.93 (m, 1H, H₁₂), 1.20–1.11 (m, 4H, H₃, H₄, H₅, H₆) ppm. ¹³C NMR (CDCl₃, 100 MHz) δ: 168.1 (C₅), 159.9 (J_C,F = 247 Hz, C₂), 150.4 (C₆), 146.2, 139.1 (C₁), 132.3 (J_C,F = 3 Hz, C₉), 132.1, 129.1 (J_C,F = 8 Hz, Cₐ), 128.8, 128.4, 126.3, 124.6, 124.1 (J_C,F = 4 Hz, C₇), 120.4 (J_C,F = 16 Hz, C₈), 116.0 (J_C,F = 22 Hz, C₉), 113.2 (C₅), 55.3, 29.4 (C₆), 25.2 (C₇), 21.5 (C₈), 11.1 (C₇ or C₈), 11.1 (C₉ or C₁₀), 7.8 (C₆) ppm. Anal. calcd. for C₂₂H₂₀FN₃S: C, 67.75; H, 5.36; N, 11.10. Found: C, 69.98; H, 5.33; N, 11.14%.
123.2, 116.7 ($^2J_{C,F} = 15$ Hz, C$_3$), 116.5 ($^2J_{C,F} = 22$ Hz, C$_g$), 112.3 (C$_4$), 15.7 (CH$_3$), 9.3 (C$_b$ or C$_a$), 9.3 (C$_a$ or C$_b$), 8.3 (C$_c$) ppm. Anal. calcd. for C$_{24}$H$_{20}$FN$_3$S: C, 71.82; H, 5.04; N, 10.49. Found: C, 71.78; H, 5.01; N, 10.45%.

4-Cyclopropy-5-(2-fluorophenyl)-2-((1-(p-tolyl)ethylidene)hydrazono)-2,3-dihydrothiazole 5d

Brown solid, yield: 89%, m.p. 89–92°C, IR (KBr, cm$^{-1}$) $\nu$: 3080 (stretch C–H ar.), 2910 (stretch C–H ali.), 1600 (stretch C=O), 1560, 1540, 1510, 1480 (stretch C=C), 1300 (stretch C–N), 1060 (stretch C–F), 810, 750 (OOP. C–H). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 7.68 (d, $J = 8.4$ Hz, 1H, H$_i$), 7.53 (dt, $J = 7.6$, 1.6 Hz, 1H, H$_g$), 6.72–6.78 (m, 1H, H$_e$), 6.64–6.58 (m, 1H, H$_d$), 5.77–5.59 (s, 3H, H$_h$ or H$_k$), 3.50–3.46 (m, 2H, H$_i$, H$_j$, NH), 2.40 (s, 3H, H$_h$ or H$_k$), 1.96–1.88 (m, 1H, H$_c$), 1.07–0.94 (m, 4H, H$_a$, H$_a'$, H$_b$, H$_b'$) ppm. $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 168.6 (C$_5$), 159.9 ($^1J_{C,F} = 248$ Hz, C$_2$), 157.2 (C$_6$), 140.4, 139.9 (C$_1$), 130.2 ($^3J_{C,F} = 8$ Hz, C$_7$), 129.2, 129.2, 128.9, 126.9, 124.4 ($^4J_{C,F} = 4$ Hz, C$_8$), 118.6 ($^2J_{C,F} = 15$ Hz, C$_3$), 116.2 ($^2J_{C,F} = 21$ Hz, C$_g$), 112.5 (C$_4$), 21.3 (CH$_3$–C$_k$), 14.5 (CH$_3$–C$_h$), 10.2 (C$_a$, C$_b$), 7.9 (C$_c$) ppm. Anal. calcd. for C$_{21}$H$_{18}$FN$_3$S: C, 69.03; H, 5.50; N, 10.52%.

4-Cyclopropy-2-((2,3-dihydro-1H-inden-1-ylidene)hydrazono)-5-(2-fluorophenyl)-2,3-dihydrothiazole 5e

Light yellow solid, yield: 93%, m.p. 182–186°C, IR (KBr, cm$^{-1}$) $\nu$: 3050 (stretch C–H ar.), 2910 (stretch C–H ali.), 1610 (stretch C=O), 1340 (stretch C–N), 1055 (stretch C–F), 810, 750 (OOP. C–H). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 7.78 (d, $J = 7.6$ Hz, 1H, H$_m$), 7.54 (dt, $J = 7.4$, 1.4 Hz, 1H, H$_g$), 7.51–7.44 (m, 2H, H$_k$, H$_l$), 7.39 (d, $J = 7.6$ Hz, 1H, H$_j$), 7.36–7.23 (m, 4H, H$_d$, H$_e$, H$_f$, NH), 3.24–3.21 (m, 2H, H$_i$), 3.15–3.12 (m, 2H, H$_h$), 1.97–1.91 (m, 1H, H$_c$), 1.13–1.09 (m, 4H, H$_a$, H$_a'$, H$_b$, H$_b'$) ppm. $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 167.3 (C$_5$), 165.7, 159.9 ($^1J_{C,F} = 248$ Hz, C$_2$), 149.8 (C$_6$), 139.4, 132.0, 131.6 ($^3J_{C,F} = 2$ Hz, C$_d$), 131.4 ($^3J_{C,F} = 9$ Hz, C$_f$), 127.3, 125.8, 123.3 ($^4J_{C,F} = 4$ Hz, C$_e$), 122.3, 116.7 ($^2J_{C,F} = 14$ Hz, C$_3$), 116.5 ($^2J_{C,F} = 22$ Hz, C$_g$), 111.7 (C$_a$), 29.3 (C$_i$), 28.3 (C$_h$), 9.2 (C$_b$ or C$_a$), 9.2 (C$_a$ or C$_b$), 8.3 (C$_c$) ppm. Anal. calcd. for C$_{21}$H$_{18}$FN$_3$S: C, 69.43; H, 5.03; N, 11.58. Found: C, 69.39; H, 5.01; N, 11.54%.

4-Cyclopropy-2-((1-(3,4-dimethoxyphenyl)ethylidene)hydrazono)-5-(2-fluorophenyl)-2,3-dihydrothiazole 5f

Brown solid, yield: 90%, m.p. 153–157°C, IR (KBr, cm$^{-1}$) $\nu$: 3250 (stretch N–H), 2950, 2830 (stretch C–H ali.), 1610 (stretch C=O), 1550, 1510 (stretch C=C), 1330 (stretch C–N), 1240, 1020 (stretch C–O), 880, 860, 750 (OOP. C–H). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 7.55 (dt, $J = 7.5$, 1.8 Hz, 1H, H$_i$), 7.50 (d, $J = 2$ Hz, 1H, H$_j$), 7.41–7.31 (m, 1H, H$_k$), 7.25–7.17 (m, 3H, H$_d$, H$_e$, H$_m$), 6.87 (d, $J = 8.4$ Hz, 1H, H$_j$), 3.96 (s, 3H, H$_j$ or H$_k$), 3.93 (s, 3H, H$_k$ or H$_j$), 2.26 (s, 3H, H$_h$, CH$_3$), 1.91–1.86 (m, 1H, H$_i$), 1.00–0.89 (m, 4H, H$_a$, H$_a'$, H$_b$, H$_b'$) ppm. $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 168.1 (C$_5$), 159.9 ($^1J_{C,F} = 247$ Hz, C$_2$), 150.2, 149.9, 148.9 (C$_6$), 146.3 (C$_1$), 132.3 ($^3J_{C,F} = 3$ Hz, C$_d$), 130.4, 129.3 ($^3J_{C,F} = 8$ Hz, C$_f$), 124.1 ($^4J_{C,F} = 3$ Hz, C$_e$), 120.2 ($^2J_{C,F} = 15$ Hz, C$_3$), 119.1, 116.0 ($^2J_{C,F} = 21$ Hz, C$_g$), 112.9 (C$_4$), 110.3, 108.4, 55.9 (C$_j$ or C$_k$), 55.8 (C$_k$ or C$_j$), 12.9 (C$_h$), 11.0 (C$_b$ or C$_a$), 11.0 (C$_a$ or C$_b$), 7.8 (C$_c$) ppm. Anal. calcd. for C$_{22}$H$_{22}$FN$_3$O$_2$S: C, 69.39; H, 5.01; N, 11.54%.
4-Cyclopropy-5-(2-fluorophenyl)-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)thiazole 5g

Brown solid, yield: 91%, m.p. 85–87°C, IR (KBr, cm⁻¹) ν: 3200 (stetch N–H), 2920, 2820 (stretch C–H ali.), 1620 (stretch C=O), 1070 (stretch C–F), 850, 840, 810, 755 (OOP. C–H). ¹H NMR (CDCl₃, 400 MHz) δ: 12.0 (s, br, 1H, NH), 7.75 (d, J = 9.2 Hz, 2H, H₁), 7.49–7.44 (m, 1H, Hε), 7.31–7.21 (m, 2H, Hd, Hf), 6.93 (d, J = 8.8 Hz, Hj), 3.86 (s, 3H, Hk, OCH₃), 2.25 (s, 3H, Hh, CH₃), 1.96–1.90 (m, 1H, Hc), 1.16–1.07 (m, 4H, Ha, Ha', Hb, Hb') ppm. ¹³C NMR (CDCl₃, 100 MHz) δ: 167.9 (C₅), 161.6, 159.9 (¹JCF = 249 Hz, C₂), 155.2 (C₆), 140.0 (C₁), 131.7 (²JCF = 2 Hz, C₄), 133.3 (³JCF = 8 Hz, C₈), 128.5, 128.2 (Cᵢ), 124.7 (⁴JCF = 3 Hz, C₆), 116.9 (⁵JCF = 15 Hz, C₃), 116.4 (⁶JCF = 22 Hz, C₇), 113.9 (C₇), 112.0 (C₄), 55.4 (C₉), 15.6 (Cₖ), 9.3 (C₉ or Cₖ), 9.3 (C₉ or Cₖ), 8.3 (C₇) ppm. Anal. calcd. for C₂₁H₂₀FN₃OS: C, 66.15; H, 5.30; N, 11.05. Found: C, 66.10; H, 5.25; N, 10.98%.

4-Cyclopropy-5-(2-fluorophenyl)-(2-((1-(4-nitrophenyl)ethylidene)hydrazono)-2,3-dihydrothiazole 5h

Light red solid, yield: 88%, m.p. 166–170°C, IR (KBr, cm⁻¹) ν: 3000 (stretch C–H ar.), 1615 (stretch C=O), 1590, 1480 (stretch C=C), 1510, 1340 (stretch NO₂), 1050 (stretch C–F), 860, 810, 750, 740 (OOP. C–H). ¹H NMR (CDCl₃, 400 MHz) δ: 8.28 (d, J = 6.8 Hz, 2H, Hj), 7.96 (d, J = 8.8 Hz, 2H, H₁), 7.55 (dt, J = 7.6, 1.6 Hz, 1H, Hg), 7.47–7.43 (m, 1H, Hc), 1.04–1.00 (m, 4H, Ha, Ha', Hb, Hb') ppm. ¹³C NMR (CDCl₃, 100 MHz) δ: 167.3 (C₅), 159.9 (¹JCF = 247 Hz, C₂), 149.7 (C₆), 147.7, 144.0, 143.3 (C₁), 132.2 (³JCF = 2 Hz, C₈), 129.7 (³JCF = 8 Hz, C₇), 126.5 (Cᵢ), 124.2 (⁴JCF = 4 Hz, C₆), 123.7 (C₇), 119.6 (³JCF = 15 Hz, C₃), 116.1 (³JCF = 22 Hz, C₇), 113.9 (C₄), 13.0 (Cₖ), 10.8 (C₉ or Cₖ), 7.9 (C₇) ppm. Anal. calcd. for C₂₀H₁₇FN₄O₂S: C, 60.63; H, 4.30; N, 14.16. Found: C, 60.61; H, 4.35; N, 14.12%.

Biology

DPPH radical-scavenging activity assay

DPPH radical-scavenging activity of compounds was evaluated according to the literature.[29] Appropriate amount of DPPH was dissolved in MeOH to give a concentration of 6.25 × 10⁻⁵ M. A series of sample solution at concentrations of 4000, 2000, 1000, 500, 250, 125 μg/mL in MeOH was prepared by two-fold serial dilution. To 0.1 mL of each sample solution was added 3.9 mL of fresh DPPH solution and was shaken vigorously. Samples were kept in darkness for 30 min then their absorbance was measured at 517 nm. MeOH was used as a blank. Radical-scavenging activity was calculated as follows:

\[
\text{Radical scavenging activity} \% = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100,
\]

where \(A_{\text{control}}\) is the absorbance of negative control (3.9 mL DPPH + 0.1 mL MeOH) and \(A_{\text{sample}}\) the absorbance of the test compounds.
**ABTS assay**

The ABTS assay of compounds was evaluated according to the literature.[37] A solution of ABTS (7.4 mM) in MeOH and a solution of potassium persulfate (K₂S₂O₈) (2.6 mM) as an oxidizing agent in MeOH were mixed in equal volumes and allowed to react for 12 h in the dark at room temperature to produce the ABTS radical cation (ABTS⁺) stock solution. Then, the resulted stock solution was diluted with MeOH to give an absorbance of 1.1 ± 0.02 at 734 nm. A series of sample solution at concentrations of 4000, 2000, 1000, 500, 250, 125 μg/mL in MeOH was prepared by two-fold serial dilution. Then, 150 μL of the sample solution was added to 3.0 mL of the ABTS⁺ solution, this mixture was shaken and incubated in the dark for 2 h. Then, the absorbance of each solution was recorded at 734 nm. MeOH was used as a blank. Radical-scavenging activity was calculated by a similar formula of the DPPH radical-scavenging assay.

The IC₅₀ values of each compound for DPPH and ABTS assays were calculated by plotting the inhibition percentage against concentration of the samples and the results were expressed in μM.

**Antibacterial assay**

The antibacterial activity of hydrazinyl-thiazoles 5a–5h was evaluated biologically using the well-diffusion method against *S. aureus* (ATCC 29213), *M. luteus* (ATCC 4698), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *B. subtilis* (DSM 6887), and *A. hydrophila* (ATCC 7966) supplied from the Iranian biological resource center, Tehran, Iran. First, nutrient agar and nutrient broth cultures were prepared according to manufacturers’ instructions and were incubated at 37°C. After incubation for the appropriate time, a suspension of 30 μL of each bacterium was added to the nutrient agar plates. Cups (5 mm in diameter) were cut in the agar using a sterilized glass tube. Each well received 30 μL of the test compounds at a concentration of 1000 μg/ml in DMSO. Then, plates were incubated at 37°C for 24 h, after this time the zone of inhibition was measured and values are expressed in millimeters (mm). The experiments were performed in triplicate. The results are reported as mean ± standard deviation of zone of inhibition in millimeter. Antibacterial activity of each hydrazinyl-thiazole was compared with penicillin G and gentamicin as standard drugs. DMSO was used as a negative control.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

[1] Atmaca G. Antioxidant effects of sulfur-containing amino acids. Yonsei Med J. 2004;45:776–788.

[2] Zelisko N, Atamanyuk D, Vasylenko O, Grellier P, Lesyk R. Synthesis and antitrypanosomal activity of new 6,6,7-trisubstituted thiopyrano[2,3-d][1,3]thiazoles. Bioorg Med Chem Lett. 2012;22:7071–7074.
[3] Aggarwal R, Kumar S, Kaushik P, Kaushik D, Gupta GK. Synthesis and pharmacological evaluation of some novel 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles. Eur J Med Chem. 2013;62:508–514.

[4] Sharma RN, Xavier FP, Vasu KK, Chaturvedi SC, Pancholi SS. Synthesis of 4-benzyl-1,3-thiazole derivatives as potential anti-inflammatory agents: an analogous-based drug design approach. J Enzyme Inhib Med Chem. 2009;24:890–897.

[5] Patt WC, Hamilton HW, Taylor MD, et al. Structure–activity relationship of a series of 2-amino-4-thiazole containing rennin inhibitors. J Med Chem. 1992;35:2562–2572.

[6] Hargrave KD, Hess FK, Oliver JT. N-(4-Substituted-thiazolyl)oxamic acid derivatives, new series of potent, orally active anti-allergy agents. J Med Chem. 1983;26:1158–1163.

[7] Lobo PL, Poojary B, Manjunatha K, et al. Synthesis and antimicrobial evaluation of some new 2-((6-oxo-5,6-dihydro[1,3]thiazolo[3,2-b]-2-aryloxymethyl-1,2,4-triazol-5-yl)-N-arylacetamides. Z Naturforsch. 2010;65:617–624.

[8] Vicini P, Geronikakib A, Incertia M, et al. Synthesis and biological evaluation of benzo[d]isothiazole, benzothiazole and thiazole Schiff bases. Bioorg Med Chem. 2003;11: 4785–4789.

[9] Osman H, Arshad A, Lam CK, Lam C, Bagley, MC. Microwave-assisted synthesis and antioxidant properties of hydrazinyl thiazolyl coumarin derivatives. Chem Cent J. 2012;6:32–41.

[10] An TNM, Kumar MA, Chang SH, Kim MY, Kim J-A, Lee KD. Synthesis, anticancer and antioxidant activity of novel 2,4-disubstituted thiazoles. Bull Korean Chem Soc. 2014;35:1619–1624.

[11] Alam MS, Liu L, Lee YE, Lee D-U. Synthesis, antibacterial activity and quantum-chemical studies of novel 2-arylidenehydrazinyl-4-arylthiazole analogous. Chem Pharm Bull. 2011;59:568–573.

[12] Ignat A, Lovasz T, Vâsilescu M, et al. Heterocycles 27. Microwave assisted synthesis and antitumour activity of novel phenothiazinyl-thiazolyl-hydrazine derivatives. Arch Pharm Chem Life Sci. 2012;345:574–83.

[13] Karegowdar P, Karthikeyan MS, Prasad DJ, Mahalinga M, Holla BS, Kumari NS. Eur J Med Chem. 2008;44:261–267.

[14] Nagesh GY, Mruthyunjayswamy BHM. Synthesis, characterization and biological relevance of some metal (II) complexes with oxygen, nitrogen and oxygen (ONO) donor Schiff base ligand derived from thiazole and 2-hydroxy-1-naphthaldehyde. J Mol Struct. 2015;1085:198–206.

[15] Ismail FMD. Important fluorinated drugs in experimental and clinical use. J Fluorine Chem. 2002;118:27–33.

[16] Kirk KL. The use of selective fluorination in drug design and development. Curr Top Med Chem. 2006;6:1445–1445.
[23] Kumar BNP, Mohana KN, Mallesha L. Synthesis and antiproliferative activity of some new fluorinated Schiff bases derived from 1,2,4-triazoles. J Fluorine Chem. 2013;156:15–20.
[24] Guan A, Qin Y, Wang J, Li B. Synthesis and insecticidal activity of novel dihalopropene derivatives containing benzoazole moiety: a structure–activity relationship study. J Fluorine Chem. 2103;156:120–123.
[25] Jaitak V, Sharma K, Kalia K, et al. Antioxidant activity of Potentilla fulgens: an alpine plant of western Himalaya. J Food Compost Anal. 2010;23:142–147.
[26] Sharifzadeh B, Mahmoodi NO, Mamaghani M, Tabatabaeian K, Chirani AS, Nikokar I. Facile regioselective synthesis of novel bioactive thiazolyl-pyrazoline derivatives via a three-component reaction and their antimicrobial activity. Bioorg Med Chem Lett. 2013;23:548–551.
[27] Mahmoodi NO, Parvizi J, Sharifzadeh B, et al. Facile regioselective synthesis of novel bis-thiazole derivatives and their antimicrobial activity. Arch Pharm Chem Life Sci. 2013;346:860–864.
[28] Mahmoodi NO, Safari N, Sharifzadeh B. One-pot synthesis of novel 2-(thiazol-2-yl)-4,5-dihydropyridazin-3(2H)-one derivatives catalyzed by activated KSF. Synth Commun. 2014;44:245–250.
[29] Mahmoodi NO, Ramzanpour S, Ghanbari Pirbasti F. One-pot multi-component synthesis of 1,4-dihydropyridines using Zn$^{2+}$@KSF and evaluation their antibacterial and antioxidant activities. Arch Pharm. 2015;348:275–282.
[30] Metzger JV. Thiazole and its derivatives, part 1. New York: John Wiley & Sons; 1979. p. 166–310.
[31] Pan X, Huang R, Zhang J, et al. Efficient synthesis of prasugrel, a novel P2Y$_{12}$ receptor inhibitor. Tetrahedron Lett. 2012;53:5364–5366.
[32] Abbasi Shiran J, Yahyazadeh A, Yamin BM, et al. Basic ionic liquid as catalyst and reaction media for the one-pot three-component regioselective synthesis of various thiazol-2-imine derivatives. J Heterocyclic Chem. Article in press. doi:10.1002/jhet.2406
[33] Hassan AA, Ibrahim YR, El-Sheref EM, Abdel-Aziz M, Bräse S, Nieger M. Synthesis and antibacterial activity of 4aryl-2-(1-substituted ethylidene)thiazoles. Arch. Pharm. Chem. Life Sci. 2013;346:562–570. doi:10.1002/ardp.201300099
[34] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med. 1999;9:1231–1237.
[35] Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant using the DPPH$^*$ free radical method. Food Sci Technol. 1997;30:609–615.
[36] Jaishree V, Ramdas N, Sachin J, Ramesh B. In vitro antioxidant properties of new thiazole derivatives. J Saudi Chem Soc. 2012;16:371–376.
[37] Jin L, Zhang Y, Yan L, Guo Y, Niu L. Phenolic compounds and antioxidant activity of bulb extracts of six Lilium species native to China. Molecules. 2012;17:9361–9378.