Mol Divers (2016) 20:497–506
DOI 10.1007/s11030-015-9654-7

ORIGINAL ARTICLE

Facile synthesis and biological assays of novel 2,4-disubstituted hydrazinyl-thiazoles analogs

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Received: 23 July 2015 / Accepted: 29 December 2015 / Published online: 11 January 2016

Abstract A convenient, one-pot multi-component synthesis of new 2,4-disubstituted hydrazinyl-thiazoles was accomplished using different aldehydes/ketones, thiosemicarbazide, and 4-methoxy phenacyl bromide in the presence of a catalytic amount of AcOH in EtOH. Products were obtained in reasonable yields and high purity. The in vitro antioxidant activity of hydrazinyl-thiazoles was evaluated by DPPH radical scavenging activity in comparison to ascorbic acid. Synthesized thiazoles **14c** and **14g** possessed the lowest IC_{50} values. Also, hydrazinyl-thiazoles were screened for their in vitro antibacterial activity against six strains of bacteria including *S. aureus, M. luteus, E. coli, Ps. aeruginosa, B. subtilis, and A. hydrophila* where some products showed good antibacterial activity. Moreover, compound **14a** showed anticancer activity against melanoma cancerous cell lines A375 with $LC_{50} = 0.55$ mg/mL, slightly selective versus normal cell lines (Hu-2) with $LC_{50} = 1.19$ mg/mL.

Keywords One-pot three-component reaction · Disubstituted hydrazinyl-thiazoles · Antioxidant · DPPH · Antibacterial · Anticancer · MCR

Introduction

Thiazoles have attracted a great deal of attention because of their diverse biological activities. Thiamine (vitamin B_1, **1**) is one of the B-complex vitamins that contains a thiazole ring, which is vitally important to keep a living organism operating properly. Commercial drugs, such as sulfathiazole **2** (antimicrobial drug), ritonavir **3** (antiretroviral drug), and abafungin **4** (antifungal drug) contain a thiazole moiety (Fig. 1).

Synthesized compounds containing a thiazole moiety have been used for the treatment of allergies [1], hypertension [2], inflammation [3], human immunodeficiency virus (HIV) infections [4], and schizophrenia [5]. Thiazoles also exhibit other biological activities, such as antibacterial [6,7], antitumor [8], analgesic [9], anticonvulsant [10], antimelanogenesis [11], antipsychotic [12], and antioxidant [13]. Also, thiazoles can act as cyclin-dependent kinase (CDK) inhibitors [14] and β-glucuronidase inhibitors [15].

Analogs such as 2-substituted-6-fluorobenzo[d]thiazoles [16], (thiazol-2-yl)hydrazine derivatives [17]. 3-Allyl-2-(substituted imino)-4-phenyl-3*H*-thiazoles, and 2,2′-(1,3-phenylene)bis(3-substituted-2-imino-4-phenyl-3*H*-thiazole) derivatives [18] showed anti-*candida*, antibacterial, and acetylcholinesterase activities. Moreover, thiazoles have been used as semiconducting materials [19], thiazole-iridium complexes as phosphorescent organic light-emitting diodes [20], and naphthol-substituted thiazoles as ratiometric fluorescent chemosensors [21].

Recently, our research group reported the one-pot synthesis of new thiazolyl-pyrazoline derivatives **5**–**6** [22], bis-thiazoles **7**–**8** [23], and thiazolylpyridazinones **9** [24] exhibiting antibacterial activity, and new 1,4-dihydropyridines **10** bearing a thiazole moiety exhibiting high antioxidant activity [25] (Fig. 2). In continuation of our previous investigation [26], we report herein the one-pot MCR synthesis of new hydrazinyl-thiazole analogs and their biological activities.

Electronic supplementary material The online version of this article (doi:10.1007/s11030-015-9654-7) contains supplementary material, which is available to authorized users.

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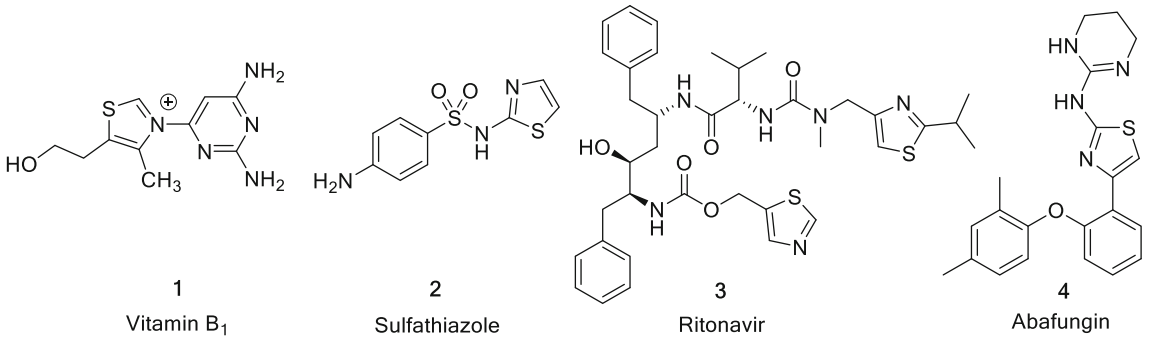


Fig. 1 Vitamin B_1 and drugs containing a thiazole moiety

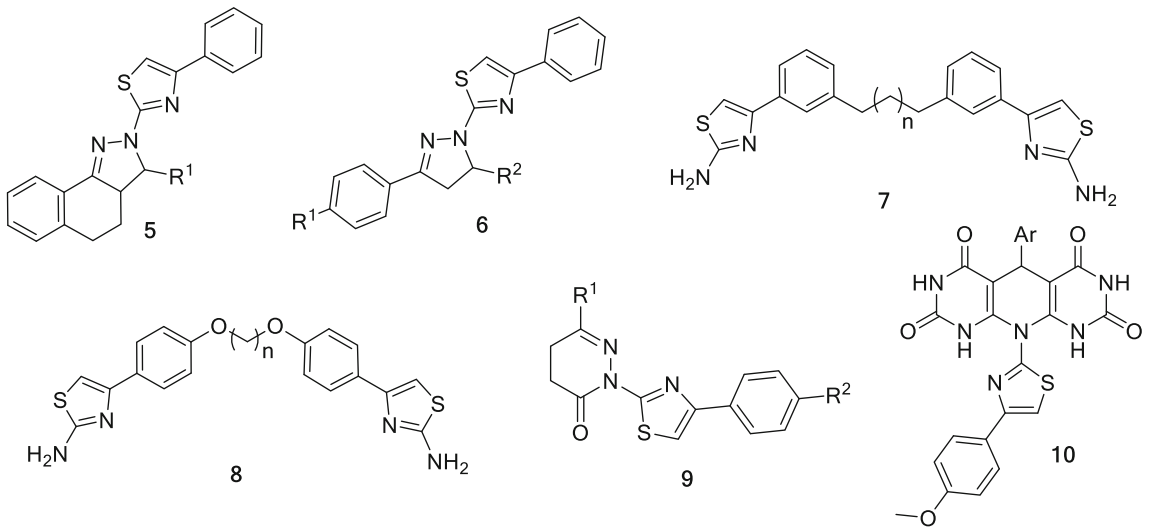


Fig. 2 Thiazoles accessed via one-pot synthesis

Results and discussion

Chemistry

New 2,4-disubstituted hydrazinyl-thiazoles **14a**–**g** were synthesized via the one-pot reaction of different aldehydes/ketones **11a**–**g**, thiosemicarbazide **12**, and 4-methoxy phenacyl bromide **13** in the presence of AcOH in EtOH under reflux conditions (Scheme 1). A literature survey indicated that hydrazone-thiazoles are generally synthesized in a two-step procedure. To the best of our knowledge, this is the first one-pot synthesis report for **14** analogs [27–30].

At the onset of our research, we explored the one-pot, multi-component preparation of **14a** in EtOH at room temperature in the absence of a catalyst (entry 1) which after 6h afforded the desired product in 56 % yield. In the presence of AcOH, the yield of **14a** increased from 56 to 60 % (entry 2). Then, the scope of the reaction was investigated under reflux conditions. As shown in Table 1, **14a** was obtained after 2h in EtOH or DMF in high yield (entries 4 & 8). Since DMF is a toxic solvent, EtOH was chosen as it is an eco-friendly and green solvent.

Our results show that the products are obtained in high yields and are easily purified using non-chromatographic methods. The advantages of this method are simple set-up and product isolation, reproducibility, using cheap and eco-friendly solvent.

In most cases, upon addition of **13** to a solution of **12** in EtOH after 2 h under reflux condition with aldehydes/ketones **11a**–**g** in the presence of few drop of AcOH, the desired product precipitated. This is a good sign for the start of the reaction. Hydrazinyl-thiazoles **14a**, **14b**, and **14c** were obtained as pure solids without the need for further purification.

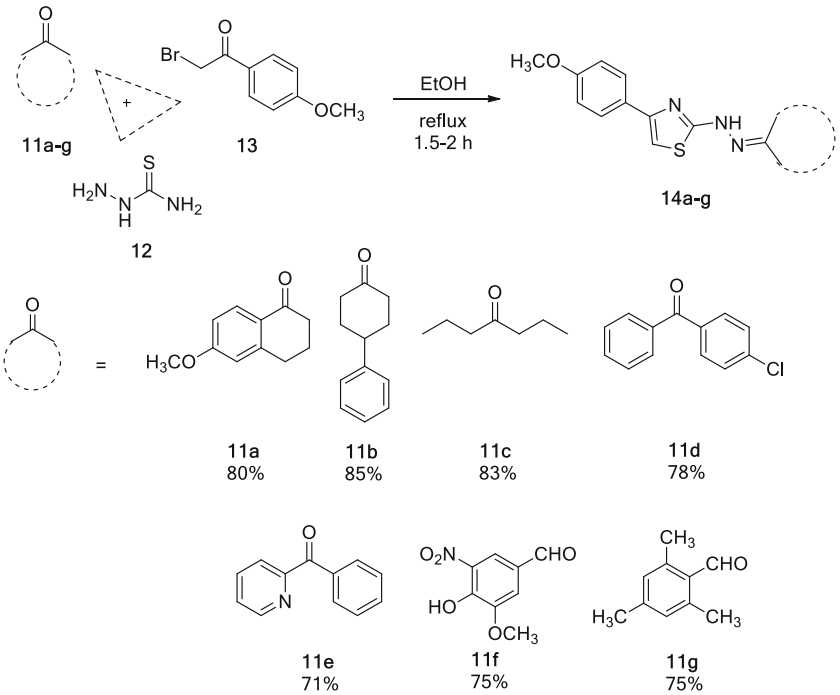
The structures of hydrazinyl-thiazoles **14a**–**g** were determined by IR, ^{1}H NMR, and ^{13}C NMR spectra. In the IR spectra, the disappearance of the carbonyl and thiosemicarbazone N–H stretching vibration bands indicates the participation of thioamide moiety in the cyclization reaction formation of desired products. In ^{1}H NMR spectra, the presence of a singlet at 7.09–6.06 ppm confirms the thiazole ring closure. Also, the OCH_3 signal appeared as a

Table 1 Optimization of **14a**

Entry	Catalyst	Solvent	Temp	Time (h)	Yield %[a]
1	–	EtOH	r.t.	6	56
2	AcOH (20 mol%)	EtOH	r.t.	4	60
3	AcOH (20 mol%)	EtOH	Reflux (60 °C)	2	75
4	AcOH (20 mol%)	EtOH	Reflux (80 °C)	2	80
5	AcOH (20 mol%)	H_2O	Reflux	4	65
6	AcOH (20 mol%)	EtOH:H_2O (1:1)	Reflux	3	70
7	AcOH (20 mol%)	MeOH	Reflux	3	70
8	AcOH (20 mol%)	DMF	Reflux	2	80

[a] After purification

Scheme 1 Synthesis of new thiazoles **14a–g**



singlet at 3.87–3.67 ppm. Other aromatic protons appeared in the expected region around 8.94–6.68 ppm. The N–H signal appeared as a broad singlet at 14.50–11.45 ppm. In addition, the N–H signal can be endo- or exocyclic. However, according to literature [31], the signal at 14.50–11.45 ppm is related to an exocyclic N-H. The ^{13}C NMR spectra of **14a**–**g** provided the expected number and types of carbons. The C–H of the thiazole ring appeared at 100.4–98.7 ppm. The signal at 55.9–55.3 is attributed to OCH_3 and other aromatic carbons appeared at 112.6–171.5 ppm.

In order to apply this procedure to carbonyl compounds with steric hindrance, we investigated the 3MCRs of camphor and 2-adamantanone **15** with thiosemicarbazide **12**, and 4-methoxy phenacyl bromide **13**. The reaction of camphor was unsuccessful and produced several by-products. However, **15** led to mixture of products **16A–16D** in moderate yield (Scheme 2).

The ^1H NMR spectra did not show a single product; rather, it showed a mixture of two sets of stereoisomer **16A/B** and **C/D**. The protons of the adamantane moiety appeared at 2.20–1.25 ppm, OCH_3 of isomers **16C/D** appeared at expected region at 3.96–3.72 ppm, and the proton of the thiazole moiety as well as other aromatic protons appeared at 7.80–6.71 ppm. Several signals at 3.25–3.01 and 2.74–2.58 ppm corresponded to CH–N=N isomers **C** and **D**.

Biology

Antioxidant assay

Using DPPH (diphenylpicrylhydrazyl) is one of the simplest methods to evaluate antioxidant activity. DPPH is a stable, free radical of violet color. When a compound donates a radical hydrogen atom to a DPPH molecule, it reduces

Scheme 2 Synthesis of thiazoles **16A–D** from adamantanone and proposed structures

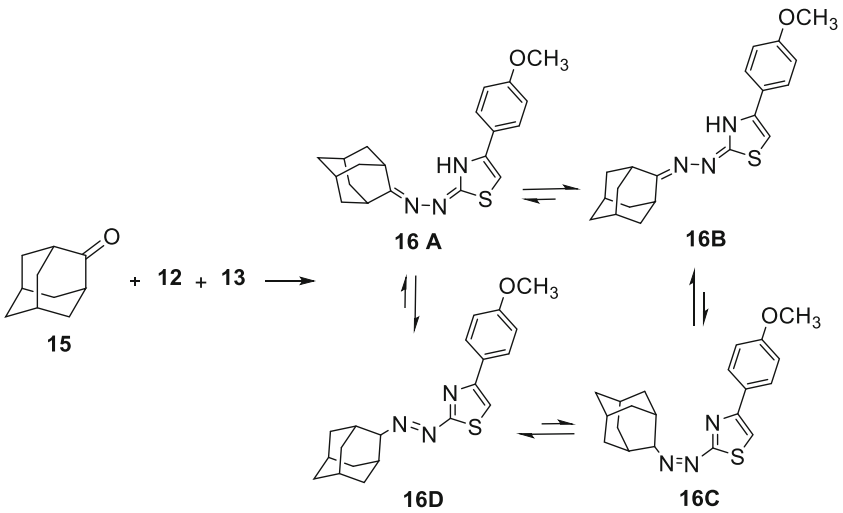
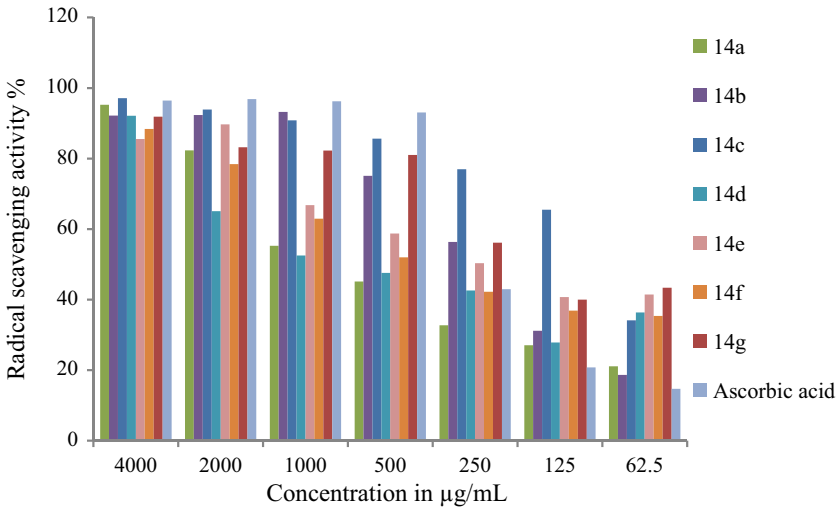


Fig. 3 DPPH radical scavenging activity of hydrazinyl-thiazoles **14a–g**

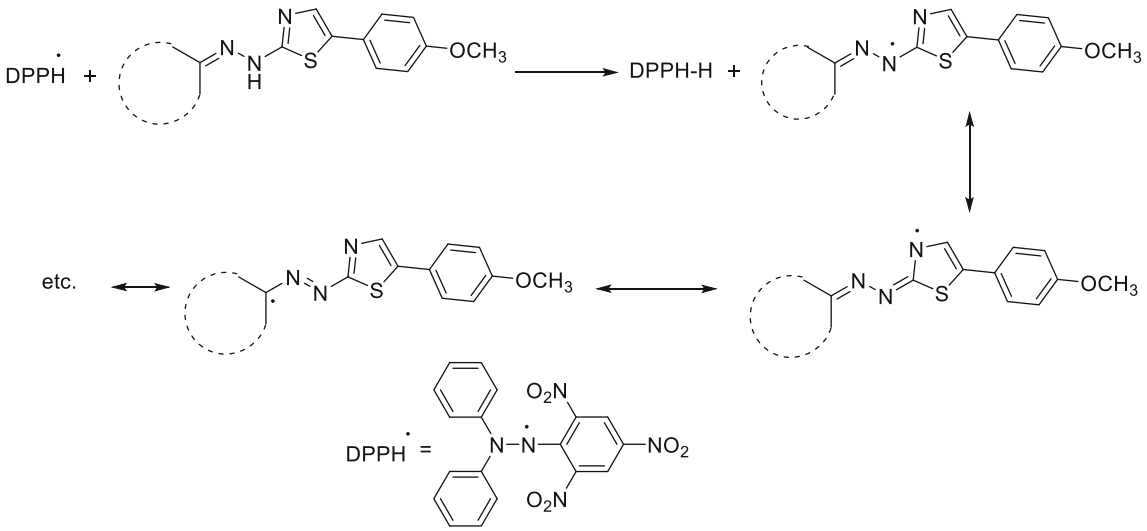


DPPH and so the absorbance of DPPH decreases. In brief, the higher the antioxidant activity, the lighter the violet hue. The antioxidant activity of hydrazinyl-thiazoles **14a–g** was evaluated by the DPPH radical scavenging activity method. In this method, a decrease in absorption band at 517 nm indicates that the test compound possesses antioxidant activity. The radical scavenging activity of **14a–g** was screened at a concentration of 4000–62.5 µg/mL and monitored at 517 nm. Also, IC_{50} values (the concentration of **14a–g** to scavenge 50 % of DPPH radical concentration) were calculated from line equation which can be obtained from Fig. 3. Ascorbic acid was used as standard. All hydrazinyl-thiazoles **14a–g** showed dose-dependent antioxidant activity (Fig. 3).

The IC_{50} values of **14a–g** are in the range 2.90–0.23 µM (Table 2). It is worth noting that hydrazinyl-thiazole **14c** has the lowest IC_{50} value (0.23 µM) while compound **14a** has the highest IC_{50} value (2.90 µM) compared to the IC_{50} value of ascorbic acid (antioxidant compound) (1.30 µM). The higher antioxidant activity is reflected in a lower IC_{50}. Compounds **14a–g** were determined to exhibit potent radical scavenging activity in a DPPH assay with IC_{50} values in the following order**14c**, **14g**, **14b**, **14e**, ascorbic acid, **14f**, **14d**, and **14a** respectively.

According to the literature [32] and our recent report [25], the high antioxidant activity of **14a–g** is attributed to the presence of the thiazole ring. The N–H at the C_2 position of the thiazole ring can readily donate a hydrogen radical to DPPH radical and form a radical of the test compound. Therefore, the radical is highly resonance-stabilized through the thiazole ring and =C–N–NH–C moiety (Scheme 3).

In addition to the presence of the thiazole moiety, the high antioxidant activity of **14g** can be due to the presence of benzylic hydrogens. These benzylic hydrogens are easily converted to the stable benzyl radical by DPPH. Furthermore, the antioxidant activity of **14b** and **14c** can be due to the presence of aliphatic hydrogens which can form tertiary, secondary, and primary carbon radicals. As it is mentioned in Table 2, these compounds exhibit better activity than ascorbic acid. Therefore, the presence of benzylic and



Scheme 3 Proposed mechanism of radical scavenging activity

Table 2 IC_{50} values for DPPH radical scavenging activity of hydrazinyl-thiazoles **14a–g**

Compound	DPPH assay IC_{50}(μM)
14a	2.90
14b	0.87
14c	0.23
14d	2.10
14e	0.88
14f	1.69
14g	0.28
Ascorbic acid	1.30

aliphatic radicals as well thiazole and =C–N–NH–C moieties possibly play an effective role in antioxidant activity (Scheme 3).

Antibacterial assay

Products **14a–g** were screened for their in vitro antibacterial activity against Gram-positive and Gram-negative bacteria strains including *Staphylococcus aureus (S. aureus), Micrococcus luteus (M. luteus), Escherichia coli (E. coli), Pseudomonas aeruginosa (Ps. aeruginosa), Bacillus subtilis (B. subtilis), Aeromonas hydrophila (A. hydrophila)* using a well-diffusion method. DMSO was used as negative control and showed no activity against the above bacterial strains. Penicillin G and Cefixime were used as positive controls (Table 3).

Hydrazinyl-thiazoles **14a–g** were evaluated for their antibacterial activity at a concentration of 4000 μg/mL in DMSO. The experiments were performed in triplicate. The results are presented as mean ± standard deviation in millimeter. According to the results, only **14d** was active against all six bacterial strains, and **14f** had the highest antibacterial activity against *S. aureus* and *M. luteus.* Compound **14d** was more active against *E. coli* and also showed significant inhibition activity against *B. subtilis.* In addition, **14e** showed significant inhibition activity against *Ps. aeruginosa*. Only **14b** showed good antibacterial activity against *A. hydrophila*.

Some reports showed moderate antibacterial activity of hydrazinyl-thiazoles. For example, Bharti and co-workers [33] studied the antibacterial activity of several hydrazinyl-thiazole derivatives, finding that their thiazoles showed no antibacterial activity against *E. coli* and only a few were active against *Ps. aeruginosa.* However, in our study, **14c–e** had moderate antibacterial activity against *E. coli*, while **14e** good antibacterial activity against *Ps. aeruginosa.* Moreover, Lee et al. [34] studied the antibacterial activity of hydrazinyl-thiazoles on several bacterial strains. According to their report, none of their synthesized compounds had antibacterial activity against *Ps. aeruginosa,* and only a few of them had good activity against *S. aureus, B. subtilis, and E. coli.* Also, the hydrazinyl-thiazoles showed no antibacterial activity against *A. hydrophila*.

Anticancer assay

Melanoma is a cancer that develops in melanocytes (the pigment cells present in the skin). It is known that melanoma can spread to other parts of the body causing serious illness and death. Melanoma can be more serious than two other forms of skin cancers, e.g., basal-cell cancer (BCC) and squamous-cell cancer (SCC). The primary treatment for melanoma is surgery. There are a few drugs used in chemotherapy of melanoma cancer; however, none of them

Table 3 Antibacterial activity of hydrazinyl-thiazoles **14a–g** (4000 μg/mL) as zone of inhibition in millimeter

Antibacterial activity (mean ± SD) (mm)						
Compound	*S. aureus*	*M. luteus*	*E. coli*	*Ps. aeruginosa*	*B. subtilis*	*A. hydrophila*
14a	–	8.33 ± 0.57	–	7.00 ± 0.73	6.66 ± 0.57	–
14b	7.33 ± 1.15	–	9.66 ± 1.15	7.33 ± 1.52	7.66 ± 0.57	11.00 ± 1.0
14c	6.66 ± 0.57	7.66 ± 0.57	11.00 ± 1.0	9.66 ± 0.57	11.00 ± 1.0	–
14d	7.66 ± 0.57	8.66 ± 0.57	13.00 ± 1.0	9.33 ± 0.57	21.33 ± 0.57	7.66 ± 0.57
14e	8.66 ± 0.57	–	11.33 ± 0.57	17.33 ± 0.57	12.33 ± 1.15	–
14f	11.33 ± 1.52	10.33 ± 0.57	–	7.66 ± 0.57	–	–
14g	7.66 ± 1.15	–	–	–	7.66 ± 0.57	6.66 ± 0.57
DMSO[a]	–	–	–	–	–	–
Penicillin G[b]	23	55	45	24	33	48
Cefixime[b]	38	35	39	30	32	41

[a] Negative control
[b] Positive control

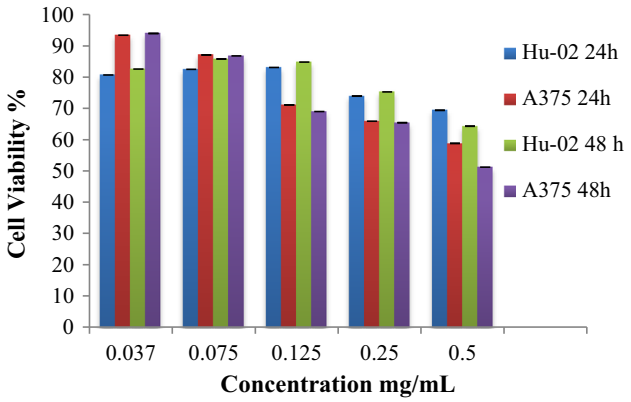


Fig. 4 In vitro cytotoxic activity of **14a** on human melanoma cancer cell lines A375. Results are presented as the percentage of cell viability ± SD using the MTT protocol

contain hydrazinyl-thiazole moiety, e.g., dacarbazine and temozolomide contain imidazole and carboxamide moieties, respectively. An in vitro cytotoxicity study of **14a** was carried out on human melanoma cancer cell lines A375 and Hu-02 (normal human skin cell lines) obtained from the National Centre for Cell Science, Tehran, Iran. Cell viability of **14a** was evaluated using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay protocol at concentrations of 0.5, 0.25, 0.125, 0.075, 0.037 mg/mL in DMSO. DMSO was used as negative control. The MTT method is based on the reduction of soluble MTT by mitochondrial reductase of the viable cells to insoluble purple crystals of formazan ((*E*, *Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan). The time of exposure was 24 h and 48 h (Fig. 4). Results are reported as the percent of cell viability ± standard deviation. Statistical analysis demonstrated that **14a** had cytotoxic activity on Hu-02 (69.4 %) and A375 (58.8 %) cell lines at 24 h time of exposure and on Hu-02 (64.3 %) and A375 (51.2 %) at 48 h time of exposure at a concentration of 0.5 mg/mL ($P \leq 0.05$).

Table 4 The LC_{50} values of cytotoxic effects of **14a** on melanoma cancer cell lines (A375) (mean ± SD)

Entry	LC_{50} (mg/mL)			
	Hu-02 (24 h)	A375 (24 h)	Hu-02 (48 h)	A375 (48 h)
14a	1.19 ± 0.12	0.55 ± 0.01	0.81 ± 0.06	0.47 ± 0.02

The LC_{50} values of **14a** are shown in Table 4. The LC_{50} values are between 1.19 and 0.55 mg/mL. As it is shown, the LC_{50} values on melanoma cancer cells (A375) are lower than on skin cells (Hu-02). However, increasing the time of exposure from 24 h to 48 h did not have a significant effect on the LC_{50} values.

Conclusions

Several new hydrazinyl-thiazoles derivatives **14a–g** were synthesized via one-pot reaction of aldehydes/ketones **11a–g**, thiosemicarbazide **12**, and 4-methoxy phenacyl bromide **13** through a reliable procedure for high yields and high purity. Hydrazinyl-thiazoles **14a**, **14b**, and **14c** were obtained as pure solids without further purification. All products showed high antioxidant activity in comparison to ascorbic acid. Compounds **14b** and **14e** had the highest antioxidant activity; their IC_{50} was less than that of ascorbic acid. Compounds **14a–g** showed low to moderate antibacterial activity. Compound **14d** possessed the highest antibacterial activity against *B. subtilis*. Compound **14a** had cytotoxic activity at a concentration of 0.5 mg/mL upon 24 h and 48 h time of exposure on melanoma cancer cell lines.

Experimental section

Materials and instruments

Starting materials, reagents, biological cultures, and solvents were obtained from commercial suppliers Fluka, Merck, Sd-fine, Quelab and were used without further purification. All reactions were monitored by silica gel-coated TLC plates (60 F_{254} Merck). IR spectra were recorded on a Shimadzu IR-470 spectrophotometer in anhydrous KBr. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz and 500 MHz Bruker spectrometers using DMSO-d_6 and $CDCl_3$ as solvents. Chemical shifts are expressed relative to TMS as singlet (s), doublet (d), triplet (t), multiple (m), doublet of doublet (dd), doublet of triplet (dt), triplet of triplet (tt), doublet of doublet of doublet (ddd), and quintet (quin). Coupling constants are expressed in Hertz (Hz). Elemental analyses were carried out on a Carlo–Erba EA1110 CNNO-S analyzer. Melting points were determined with a Mettler Fp5 apparatus, and were uncorrected. Absorbance in the antioxidant assay was recorded on an Unico 2100 spectrophotometer.

General procedure for the synthesis of hydrazinyl-thiazoles 14a–g

To a solution of thiosemicarbazide **12** (1 mmol) in EtOH, aldehydes/ketones **11a–g** (1 mmol) and a few drops of AcOH were added and this mixture was refluxed and stirred. After a few minutes (1–15 min), 4-methoxy phenacyl bromide **13** (1 mmol) was added and the reaction was refluxed and stirred until completion (1.5–2 h). Thin layer chromatography (TLC) was used to monitor the progress of the reaction (EtOAc:*n*-hexane 3:6). After completion of the reaction, the reaction mixture was cooled down to room temperature. The resulting solid was filtered and washed with or recrystallized in EtOH.

*2-(2-(6-Methoxy-3,4-dihydronaphthalen-1(2**H**)-ylidene)hydrazinyl)-4-(4-methoxyphenyl)thiazole* **(14a)***:*

Cream solid (0.3 g, 80 %); mp 233–236 °C (from EtOH); IR (KBr) ν_{max} 3210 (stretch N–H), 3100 (stretch C–H aromatic), 2920, 2830 (stretch C–H aliphatic), 1610 (stretch C=N), 1570, 1500 (stretch C=C), 1250, 1240, 1045 (stretch C–O), 825, 760, 700 (OOP. C–H) cm^{-1}; R_f (83 %, hexane: EtOAc 6:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H, NH), 8.02 (*d*, J = 8.8Hz, 1H), 7.67 (*d*, J = 8.0 Hz, 2H), 6.99 (*d*, J = 8.8 Hz, 2H), 6.82 (dd, J = 8.8, 2.8 Hz, 1H), 6.68 (*d*, J = 2.4 Hz, 1H), 6.06 (*s*, 1H), 3.84 (*s*, 3H), 3.83 (*s*, 3H), 2.90 (*t*, J = 6.4 Hz, 2H), 2.79 (*t*, J = 6.0 Hz, 2H), 2.02 (quin, J = 6.3 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 161.2, 160.7, 154.0, 142.5, 127.1, 126.9, 123.7, 121.5, 114.7, 113.5, 112.6, 99.1, 55.4, 55.3, 29.7, 27.2, 21.6 ppm; Anal. calcd. for $C_{21}H_{21}N_3O_2S$: C, 66.49, H, 5.56, N, 11.10. Found: C, 66.51; H, 5.59; N, 11.06 %.

4-(4-Methoxyphenyl)-2-(2-(4-phenylcyclohexylidene) hydrazinyl)thiazole **(14b)**

Orange solid (0.3 g, 85 %); mp 186–189 °C (from EtOH); IR (KBr) ν_{max} 3210 (stretch N–H), 3020 (stretch C–H aromatic), 2920 (stretch C–H aliphatic), 1610 (stretch C=N), 1560, 1540, 1510 (stretch C=C), 1250, 1040 (stretch C–O), 820, 740, 690 (OOP. C–H) cm^{-1}; R_f (80 %, hexane: EtOAc 6:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (*s*, 1H, NH), 7.67 (*d*, J = 8.8 Hz, 2H), 7.33 (*t*, J = 7.2 Hz, 2H), 7.26–7.22 (*m*, 3H), 7.01 (*d*, J = 8.8 Hz, 2H), 6.56 (*s*, 1H), 3.86 (*s*, 3H), 3.32 (*d*, J = 14.8 Hz, 1H), 2.88 (tt, J = 12, 3.3 Hz, 1H), 2.72 (*d*, J = 14.4 Hz, 1H), 2.47 (dt, J = 14, 4.9 Hz, 1H), 2.32 (dt, J = 14.1, 5.3 Hz, 1H), 2.27–2.17 (m, 2H), 1.79 (ddd, J = 25.5, 13.1, 4.3 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 164.2, 161.5, 145.1, 140.8, 129.0, 127.5, 127.1, 127.0, 120.3, 115.4, 98.8, 55.8, 43.5, 35.2, 34.6, 33.3, 29.4 ppm; Anal. calcd. for $C_{22}H_{23}N_3OS$: C, 70.03; H, 6.11; N, 11.09. Found: C, 69.98; H, 6.15; N, 11.12 %.

2-(2-(Heptan-4-ylidene)hydrazinyl)-4-(4-methoxyphenyl) thiazole **(14c)**

Orange solid (0.26 g, 83 %); mp 127–131 °C (from EtOH); IR (KBr) ν_{max} 3210 (stretch N–H), 3100 (stretch C–H aromatic), 2950, 2850 (stretch C–H aliphatic), 1610 (stretch C=N), 1560, 1540, 1505 (stretch C=C), 1250, 1020 (stretch C–O), 830 (OOP. C–H) cm^{-1}; R_f (82 %, hexane: EtOAc 6:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.39 (*s*, 1H, NH), 7.66 (*d*, J = 8.2 Hz, 2H), 6.99 (*d*, J = 7.8 Hz, 2H), 6.53 (*s*, 1H), 3.85 (*s*, 3H), 2.49 (*t*, J = 9 Hz, J = 8 Hz, 2H), 2.34 (*t*, J = 7.6 Hz, 2H), 1.70–1.61 (*m*, 4H), 1.11 (*t*, J = 7.2 Hz, 3H), 0.98 (*t*, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO − d_6) δ 170.2, 166.1, 161.5, 140.7, 127.5, 120.4, 115.3, 98.7, 55.8, 38.9, 33.8, 19.8, 19.7, 14.6, 14.1 ppm; Anal. calcd. for $C_{17}H_{23}N_3OS$: C, 64.35; H, 7.28; N, 13.21. Found: C, 64.30; H, 7.31; N, 13.17 %.

2-(2-((4-Chlorophenyl)(phenyl)methylene)hydrazinyl)-4-(4-methoxyphenyl)thiazole **(14d)**

Orange crystal (0.32 g, 78 %); mp 239–242 °C (from EtOH); IR (KBr) ν_{max} 3120 (stretch C–H aromatic), 1610 (stretch C=N), 1580, 1560, 1540 (stretch C=C), 1250, 1030 (stretch C–O), 830, 740, 690 (OOP. C–H) cm^{-1}; R_f (73 %, hexane: EtOAc 6:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.07 (*s*, 1H, NH), 7.69 (*d*, J = 8.8 Hz, 2H), 7.66 (*d*, J = 8.4 Hz, 2H), 7.60 (dd, J = 7.8, 1.3 Hz, 2H), 7.49 (tt, J = 6.6, 1.8 Hz, 1H), 7.42 (tt, J = 8.8, 1.2, 2H), 7.35 (*d*, J = 8.8 Hz, 2H), 6.85 (*d*, J = 8.8 Hz, 2H), 6.63 (*s*, 1H) 3.85 (*s*, 3H) ppm; ^{13}C

NMR (125 MHz, $CDCl_3$/DMSO $-d_6$) δ 170.0, 161.3, 137.2, 135.7, 131.3, 130.7, 130.6, 130.4, 129.7, 129.6, 128.9, 128.5, 128.3, 127.6, 115.1, 114.6, 100.4, 55.7 ppm; Anal. calcd. for $C_{23}H_{18}ClN_3OS$: C, 65.81; H, 4.29; N, 9.98. Found: C, 65.85; H, 4.33; N, 10.03 %.

4-(4-Methoxyphenyl)-2-(phenyl(pyridin-2-yl)methylene) hydrazinyl)thiazole **(14e)**

Red solid (0.27 g, 71 %); mp 215–220 °C (from EtOH); IR (KBr) ν_{max} 1605 (stretch C=N), 1550, 1510, 1480 (stretch C=C), 1240, 1050 (stretch C–O), 830, 760, 730, 700 (OOP. C–H) cm^{-1}; R_f (74 %, hexane: EtOAc 6:3); ^1H NMR (400 MHz, $CDCl_3$) δ 14.51 (*s*, 1H, NH), 8.93 (*d*, $J = 3.6$ Hz, 1H), 7.83–7.77 (*m*, 3H), 7.64–7.60 (*m*, 2H), 7.52–7.39 (*m*, 5H), 6.97 (*d*, $J = 8.8$ Hz, 2H), 6.78 (*s*, 1H), 3.87 (*s*, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 160.6, 150.2, 148.6, 138.7, 136.0, 129.9, 129.6, 129.3, 128.7, 128.6, 127.6, 127.3, 126.0, 124.7, 114.6, 100.3, 55.3 ppm; Anal. calcd. for $C_{22}H_{18}N_4OS$: C, 68.41; H, 4.72; N, 14.53. Found: C, 68.36; H, 4.67; N, 14.48 %.

2-Methoxy-4-((2-(4-(4-methoxyphenyl)thiazol-2-yl) hydrazono)methyl)-6-nitrophenol **(14f)**

Red solid (0.3 g, 75 %); mp 207–209 °C (from EtOH); IR (KBr) ν_{max} 3250 (stretch N–H), 3100 (stretch C–H aromatic), 2980, 2830 (stretch C–H aliphatic), 1610 (stretch C=N), 1570, 1480 (stretch C=C), 1540, 1340 (stretch NO_2), 1250, 1050 (stretch C–O), 840, 760, 740, 690 (OOP. C–H) cm^{-1}; R_f (60 %, hexane: EtOAc 6:3); ^1H NMR (500 MHz, DMSO $- d_6$) δ ∼ 11.45 (*s*, 1H, NH), 7.95 (*s*, 1H), 7.74 (*d*, $J = 8.76$ Hz, 2H), 7.67 (dd, $J = 1.52$ Hz, 1H), 7.49 (dd, $J = 1.48$ Hz, 1H), 7.09 (*s*, 1H), 6.92 (*d*, $J = 8.7$ Hz, 2H), 3.90 (*s*, 3H), 3.74 (*s*, 3H) ppm; ^{13}C NMR (125 MHz, DMSO $- d_6$) δ 168.8, 159.6, 151.1, 150.6, 144.2, 140.2, 138.1, 128.3, 127.6, 126.2, 115.1, 114.8, 113.1, 102.3, 57.4, 55.9 ppm; Anal. calcd. for $C_{18}H_{16}N_4O_5S$: C, 54.03; H, 4.06; N, 13.97. Found: C, 53.97; H, 4.02; N, 14.04 %.

4-(4-Methoxyphenyl)-2-(2-(2,4,6-trimethylbenzylidene) hydrozinyl)thiazole **(14g)**

Cream solid (0.26 g, 75 %); mp 229–233 °C (from EtOH); IR (KBr) ν_{max} 3400 (stretch N–H), 3100 (stretch C–H aromatic), 2950, 2820 (stretch C–H aliphatic), 1610 (stretch C=N), 1510 (stretch C=C), 1250, 1020 (stretch C–O), 850, 750, 740, (OOP. C–H) cm^{-1}; R_f (75 %, hexane: EtOAc 6:3); ^1H NMR (500 MHz, DMSO $- d_6$) δ ∼ 13.00 (*s*, 1H, NH), 8.65 (*s*, 1H), 7.52 (*d*, $J = 8.6$ Hz, 2H), 6.81 (*d*, $J = 6.9$ Hz, 2H), 6.75 (*s*, 2H), 6.56 (*s*, 1H), 3.67 (*s*, 3H), 2.31 (*s*, 6H), 2.13 (*s*, 3H) ppm; ^{13}C NMR (125 MHz, DMS0$-d_6$) δ DMSO$-d_6$) δ ∼ 170, 161.3, 151.2, 141.1, 140.9, 139.1, 130.4, 127.9, 126.8, 120.6, 115.0, 100.2, 55.7, 22.2, 21.5 ppm. Anal. calcd. for $C_{20}H_{21}N_3OS$: C, 68.37; H, 6.05; N, 11.94. Found: C, 68.32; H, 5.98; N, 11.98 %.

2-(Adamantan-2-ylidenehydraono)-4-(4-methoxyphenyl)-2,3-dihydrothiaole **(16)**

Orange solid (0.17g, 50 %); mp 140–148 °C (from EtOH); IR (KBr) ν_{max} 2917, 2849 (stretch C-H aliphatic), 1605 (stretch C=N), 1549, 1503 (stretch C=C) cm^{-1}; R_f (35 %, hexane: EtOAc 6:3); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80–6.71 (m, Ar, NH, H-thiazole), 3.96–3.72 (OCH_3), 3.01–2.55 (CH–C=N of C and D), 2.20–1.25 (CH and CH_2 adamantan) ppm.

Biological methods

DPPH radical scavenging assay

The DPPH radical scavenging activity of **14a–d** was evaluated according to the literature [25]. A 2,2-diphenyl-2-picrylhydrazyl (DPPH) solution was prepared by dissolving an appropriate amount of DPPH in MeOH to give a concentration of 6.25×10^{-5} M. Compounds **14a–g** and DPPH with different concentrations (4000, 2000, 1000, 500, 250, 125, 62.5 μg/mL) in MeOH were prepared. Then, 0.1 mL of each hydrazinyl-thiazole solution was added to 3.9 mL of DPPH solution and was shaken vigorously. Samples were kept in darkness for 30 min and then their absorbance was measured at 517 nm. MeOH was used as 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_{control} is the absorbance of the negative control (containing all reagents except test compounds), A_{sample} the absorbance of the test compounds. IC_{50} values of the test compounds were determined by plotting the radical scavenging activity percentage against concentration of the test compound.

Antibacterial assay

The antibacterial activity of hydrazinyl-thiazoles was evaluated biologically using a well-diffusion method. First, nutrient agar and nutrient broth cultures were prepared according to manufacturer's 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 sterilized glass tube. Each well received 30 μL of the test compounds at concentration of 4000 μg/ml in DMSO. Then, plates were incubated at 37 °C for 24 h, after this

time the zone of inhibition was measured. The values are expressed in millimeters (mm). The experiments were performed in triplicate. The results are reported as mean ± SD of zone of inhibition in millimeter. Antibacterial activity of each hydrazinyl-thiazole was compared with Penicillin G and Cefixime as standards. DMSO was used as negative control.

In vitro anticancer assay

The anticancer activity of the hydrazinyl-thiazole **14a** was determined against melanoma cancerous cell lines Hu-02 and A375 using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The melanoma cancerous cell lines were provided by the Iranian Biological Resource Center, Tehran. The MTT method is based on the reductive cleavage of the tetrazolium ring of MTT into insoluble purple formazan. 100 μL of the cell suspension were added to each well of 96-well microplate and then incubated for 12–24 h at 37 °C in a CO_2 incubator. Compound **14a** was diluted in concentrations of 0.037–0.5 mg/mL in DMSO by two-fold serial dilution. DMSO was used as negative control. Different concentrations of a test compound were added to each well and then incubated for 24 and 48 h at 37 °C in a CO_2 incubator. Then, 10 μL of MTT (5 mg/mL) stock solution in PBS were added to each well and incubated for 4 h, the upper solution was removed, and 100 μL of DMSO were added to the media. The plates were shaken slowly until the insoluble crystals of the produced formazan dissolved. The plates were kept for 2–4 h in the dark. After that, the plates were read at 360 nm on an ELISA reader. The experiments were performed in triplicate.

The percentage of cell viability was calculated using the following formula:

$$\%\ \text{cell viability} = \frac{\text{Mean Absorbance of sample}}{\text{Mean Absorbance of control}} \times 100$$

The LC_{50} values (lethal concentration of the test compound required to kill 50 % of sample population) were determined by plotting cell viability percentage against concentration of the sample.

Acknowledgments The authors are greatly thankful to Dr. Zohreh Ramezanpour and Miss Somaie Rasouli Dogaheh for their helpful assistance in running biological assays. The authors appreciate the valuable and helpful comments of Professor Dr. Guillermo A. Morales, Editor-In-Chief of Molecular Diversity.

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