Study on Condensation of *N*-Aryl Thioureas with 3-Bromo-acetylacetone: Synthesis of Aminothiazoles and Iminodihydrothiazoles, and Their *In Vitro* Antiproliferative Activity on Human Cervical Cancer Cells

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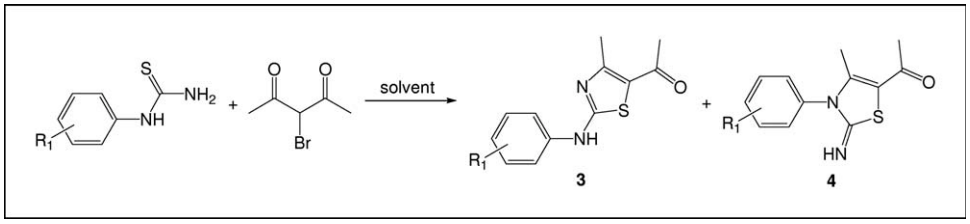

The condensation of *N*-aryl thioureas with 3-bromo-acetylacetone in neutral solvent acetone not only led to 5-acetyl-4-methyl-2-(substituted anilino) thiazoles **3** but also 2-imino-3-(substituted phenyl)-4-methyl-5-acetyl-2,3-dihydrothiazoles **4**. Further study found that different reaction solvents displayed an important role toward the ratio of aminothiazoles **3** and iminodihydrothiazoles **4**, and the reaction scope was extended. A plausible mechanism involving solvent effect and *in situ* hydrobromic acid catalyzation was proposed. Some selected isomers exhibited moderate *in vitro* antiproliferative activity on human cervical cancer cell lines (Hela, Siha).

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INTRODUCTON

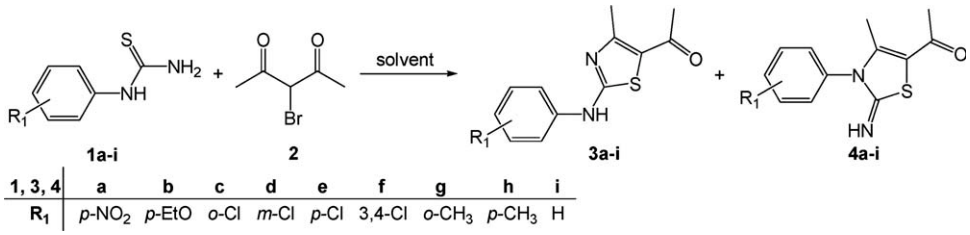
Thiazoles are an important class of heterocyclic compounds with biological activities and as versatile synthetic building blocks [1]. Thiazole derivatives exhibit a wide range of pharmacological activities such as antibacteria and fungi [2,3], anti-inflammation [4], antihypertension [5], anticonvulsion [6], anti-Parkinson's disease [7], anti-Alzheimer's disease [8], anti-HIV [9], and anticancer [10–14]. The synthesis of thiazole derivatives has drawn considerable attention. The most straight forward procedure reported by Hantzsch and Weber in 1887 involved a one-pot condensation of α-haloketones and thiourea derivatives in refluxing alcohol with long reaction time [15]. Modified procedures or novel approaches leading to thiazoles have long been explored [16–21]. As far as we known, Traumann in 1888 first observed an isomer formed by condensation of chloroacetone with *N*-methylthiourea, which was 2-imino-3,4-dimethyl-2,3-dihydrothiazole [22]. Not until a century later did Meakins and coworkers give an explanation that the acidic reaction conditions in the Hantzsch thiazole synthesis promoted the formation of iminodihydrothiazoles, while in neutral solvents, without exception, only aminothiazoles were obtained [23]. Reaction under DMSO with *in situ* formed α-haloketones in the presence of hydrochloric acid or hydrobromic acid also afforded both aminothiazoles and iminodihydrothiazoles [24].

We have carefully reexamined our work on a condensation of substituted arylthioureas with 3-bromo-acetylacetone in refluxing acetone [25] and found some desired 5-acetyl-4-methyl-2-(substituted anilino) thiazoles were not pure in that trace of 2-imino-3-(substituted phenyl)-4-methyl-5-acetyl-2,3-dihydrothiazoles were existed as indicated from ^{1}H-NMR spectra. This result encouraged us to undertake a study on the synthesis of aminothiazoles **3** and iminodihydrothiazoles **4** in neutral solvents (Scheme 1).

RESULTS AND DISCUSSION

In continuation of our study on the synthesis of thiazoles, we found that the reaction of *p*-nitrophenyl thiourea **1a** with 3-bromo-acetylacetone **2** under refluxing acetone was largely converted to 5-acetyl-4-methyl-2-(4-nitrophenylamino)-thiazole **3a** along with a very small amount of a second product that was identified as 2-imino-3-(4-nitrophenyl)-4-methyl-5-acetyl-2,3-dihydrothiazole **4a** and

Scheme 1. Synthetic route to thiazoles **3** and **4**.



confirmed by X-ray diffraction [26]. The molecular structure is shown in Figure 1. In planar thiazole ring, the S(1)–C(2) [1.756 (2) Å], S(1)–C(5) [1.762 (2) Å], C(2)–N(3) [1.404 (2) Å], and N(3)–C(4) [1.383 (2) Å] bond lengths corresponded to single bonds, and C(4)–C(5) was typical for double bond. Also, the C(2)–N(6) [1.265 (2) Å] indicated a double bond.

As we had confirmed the crystal structure of **4a**, the reaction conditions were studied to amplify the ratio of **4a** as a major product as shown in Table 1. It could be found that different solvents significantly affected the ratio of **3a** and **4a** (entries 3–8) and seemed to have the trend that the amount of **4a** increased in nonpolar solvents and **3a** was predominant in polar solvents. Besides, the reaction temperature did not fundamentally change the ratio between **3a** and **4a** neither in acetone (entries 1–3) nor in carbon tetrachloride (entries 8–10); however, reflux temperature led to higher isolated yields and greatly shortened the reaction time. In addition, we tried to use 3-chloro-acetylacetone in refluxing acetone, the ratio (**3a**:**4a** = 99:1) was almost the same as with 3-bromo-acetylacetone (entry 3).

Considering the reaction time and isolated yields, we extended the reaction scope in refluxing solvents with different substituted arylthioureas as shown in Table 2. It could be found that acetone was not an efficient regioselective solvent except for *p*-nitrophenyl thiourea **1a**. For other substituted arylthioureas, it was not obvious that compounds **3** were favorable in refluxing acetone; however, compounds **4** seemed to have overwhelming yields in refluxing carbon tetrachloride.

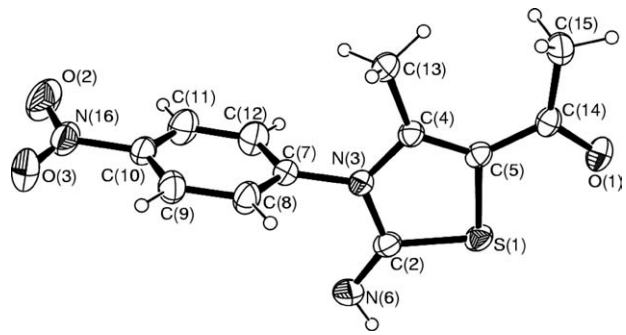


Figure 1. ORTEP view of iminodihydrothiazole **4a** (thermal ellipsoids at 30% probability).

In addition, we found that the salts of iminodihydrothiazoles **4** were generally less soluble in acetone than those of aminothiazoles **3**, so the salts of **4** could be obtained conveniently by filtration after cooling the reaction mixture before neutralization. This method avoided chromatographic separation to get pure **4**, but the yields still on the lower level than that resulted from carbon tetrachloride.

The UV absorption spectra of some aminothiazoles **3**, iminodihydrothiazoles **4**, and their hydrochlorides in absolute ethanol at $10^{-4}M$ concentration are profiled in Figure 2. In general, aminothiazoles **3** had longer wavelengths of their absorption maxima than that of iminodihydrothiazoles **4**, showing compounds **3** possessed stronger aromatic conjugated systems. Moreover, from the similar wavelengths of absorption maxima between **3** and their hydrochlorides, it could be concluded that the conjugated systems were not destroyed after forming hydrochlorides. However, the hydrochlorides of iminodihydrothiazoles **4** strikingly led to blue shifts, indicating protonation of heterocyclic nitrogen atom with a lone pair of electrons impaired conjugation and gave rise to shorter wavelengths of the absorption maxima.

A plausible mechanism was proposed as illustrated in Scheme 2. Similar to the Hantzsch thiazole synthesis, the reactions were proposed to involve a sequence of three stages from nucleophilic displacement of halogen

Table 1

The effects of solvents and temperature in the synthesis of **3a** and **4a**.

Entry	Solvent	Temperature (°C)	Reaction time (h)	Ratio[a] **3a**	Ratio[a] **4a**
1	Acetone	0	22	85	15
2	Acetone	25	4	84	16
3	Acetone	Reflux	1	99	1
4	THF	Reflux	1	98	2
5	EtOH	Reflux	1	85	15
6	Benzene	Reflux	1	47	53
7	$CHCl_3$	Reflux	1	18	82
8	CCl_4	Reflux	1	12	88
9	CCl_4	0	24	7	93
10	CCl_4	25	12	7	93

[a] The ratio was determined by HPLC.

Table 2

Synthesis of thiazoles **3** and **4**.

Entry	R_1	Solvent[a]	**3**	Yield[b] (%)	t_R[c] (min)	**4**	Yield[b] (%)	t_R[c] (min)
1	*p*-NO_2	Acetone	**3a**	90.3	7.35	**4a**	2.0	1.91
2	*p*-EtO	Acetone	**3b**	37.7	6.50	**4b**	37.7	1.90
3	*o*-Cl	Acetone	**3c**	31.4	5.13	**4c**	52.6	1.64
4	*m*-Cl	Acetone	**3d**	44.3	9.32	**4d**	35.3	2.24
5	*p*-Cl	Acetone	**3e**	31.5	9.05	**4e**	45.6	1.94
6	3,4-Cl	Acetone	**3f**	45.8	16.44	**4f**	19.2	2.04
7	*o*-CH_3	Acetone	**3g**	45.5	5.41	**4g**	40.7	1.79
8	*p*-CH_3	Acetone	**3h**	46.3	9.20	**4h**	23.9	2.45
9	H	Acetone	**3i**	41.6	5.75	**4i**	17.1	1.99
10	*p*-NO_2	CCl_4	**3a**	9.5	7.35	**4a**	80.3	1.91
11	*o*-Cl	CCl_4	**3c**	4.1	5.13	**4c**	78.6	1.64
12	*p*-Cl	CCl_4	**3e**	15.5	9.05	**4e**	76.7	1.94
13	H	CCl_4	**3i**	14.5	5.75	**4i**	72.3	1.99

[a] Under reflux.
[b] Isolated yield.
[c] Retention time determined by HPLC.

by sulfur together with nitrogen attacking carbonyl group, cyclization with proton transfer, and dehydration [23]. The final ratio of aminothiazoles **3** and iminodihydrothiazoles **4** was depended on the equilibrium of **6** and **9**, which might probably affected by the nature of solvents, and *in situ* released hydrobromic acid might accelerate the formation of compounds **4** as it was reported that in neutral medium entirely aminothiazoles **3** were exclusively obtained and only under acidic conditions could compounds **4** be obtained [23]. It was observed from HPLC detection that the formation of **4** could be greatly inhibited when we added equivalent pyridine or triethylamine into the reaction in advance as hydrobromic acid capturers. Our study with *o*-chlorophenyl thiourea **1c** as an example showed that the final ratio of **3c**:**4c** in acetone reached 99.4:0.6 with pyridine,

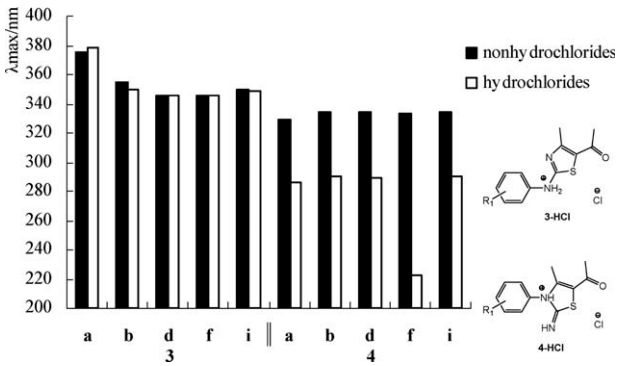


Figure 2. UV comparison of compounds **3**, **4**, and their hydrochlorides.

and no **4c** was detected by HPLC with triethylamine. In addition, from our results discussed above, it could also be speculated that nonpolar solvents might enhance the yields of iminodihydrothiazoles **4**.

The *in vitro* antiproliferative activity of some selected isomers on human cervical cancer cell lines (Hela and Siha) was evaluated as shown in Table 3. The antiproliferative activity of all compounds was weaker than that of cisplatin (DDP), but some still exhibited moderate anticervical activity. It seemed that isomers with *p*-substituted phenyl moieties were less effective against cervical cancer cells. The selectivity between aminothiazoles **3** and iminodihydrothiazoles **4** on cell lines (Hela or Siha) was not obvious. Further work is being done to illustrate the detailed structure–activity relationships with more aminothiazoles **3** and iminodihydrothiazoles **4**.

EXPERIMENTAL

Chemistry. Melting points were taken on an XRC-1 apparatus and are uncorrected. Infrared spectra were obtained on a

Scheme 2. Plausible mechanism for thiazoles **3** and **4**.

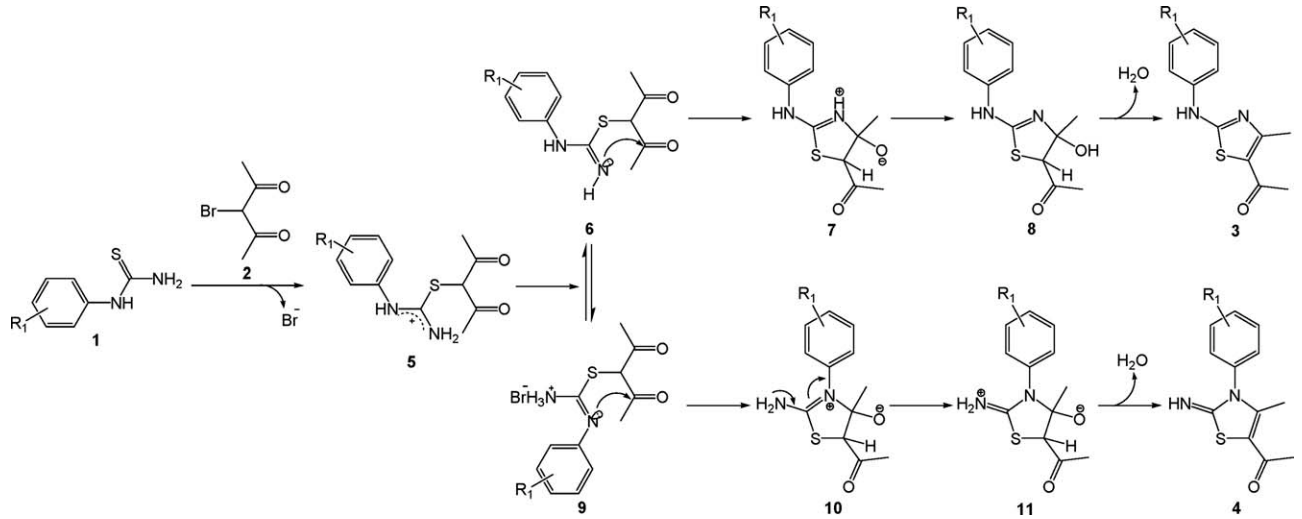


Table 3

Antiproliferative activity of some selected isomers on human cervical cancer cell lines.

Compd.	Human cervical cancer cell lines IC_{50} (μM)	
	Hela	Siha
3a	>100	>100
4a	>100	>100
3b	>100	>100
4b	>100	>100
3d	>100	>100
4d	76.1	97.3
3f	56.5	84.4
4f	49.5	29.8
3g	37.1	13.3
4g	42.0	52.3
3i	31.3	21.3
4i	66.1	33.4
DDP	5.53	5.57

Thermo Nicolet Avatar 370 FTIR spectrophotometer. ^{1}H-NMR spectra were recorded on a Brucker AC 400 spectrometer operating at 400 MHz or a Bruker AVANCE III spectrometer at 500 MHz using TMS as the internal standard. MS spectra were run on an HP5989B instrument or a Waters GCT Premier with EI source. HRMS spectra were recorded on Agilent 6210 TOF LC/MS. HPLC were performed on a Shimadzu LC-10AT Liquid Chromatograph (Diamonsil C_{18}, 250 mm × 4.6 mm) with MeOH/H_2O (8:2) as an eluent, T_f = 1.0 mL/min, λ = 254 nm. UV spectra were recorded on a Shimadzu UV-2400PC spectrophotometer. All the chemicals and solvents were of analytical reagent and used as received. Arylthioureas **1** and 3-bromo-acetylacetone **2** were prepared according to literature methods [25].

General procedure for aminothiazoles 3 and iminodihydrothiazoles 4 in acetone. To a stirred mixture of arylthiourea **1** (10 mmol) in acetone (40 mL) was added a solution of 3-bromo-acetylacetone **2** (1.8 g, 10 mmol) in acetone (5 mL), keeping the reaction temperature below 25°C throughout the addition. Then, the reaction was kept to reflux temperature for about 1 h (completion by TLC). After cooling, the mixture was neutralized with 5% aqueous K_2CO_3 to pH 9, and the resulting precipitate was filtered and purified by preparative layer chromatography, eluting with petroleum ether/ethyl acetate (2:1) to afford **3** and **4**, respectively. The isolated yields are presented in Table 2.

5-Acetyl-4-methyl-2-(*p*-nitrophenylamino)-thiazole (3a). Yellow crystal, mp: 208–209°C (lit. [27]: 205°C); IR ν_{max} (KBr)/cm^{-1}: 3282, 3104, 1611, 1597, 1577, 1478, 1320, 1253; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.32 (d, J = 9.2 Hz, 2H, Ar-H), 7.57 (d, J = 9.2 Hz, 2H, Ar-H), 2.71 (s, 3H, CH_3), 2.55 (s, 3H, CH_3CO); EIMS *m/z* (%): 277 [M^+](90), 262(100), 247(5), 234(7), 216(20), 86(13), 71(16), 50(7).

5-Acetyl-4-methyl-2-imino-3-(*p*-nitrophenyl)-2,3-dihydrothiazole (4a). Yelow crystal, mp: 168–169°C; IR ν_{max} (KBr)/cm^{-1}: 3287, 3075, 1607, 1568, 1523, 1350, 1324, 1103; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.42 (d, J = 8.8 Hz, 2H, Ar-H), 7.51 (d, J = 9.2 Hz, 2H, Ar-H), 2.36 (s, 3H, CH_3), 2.25 (s, 3H, CH_3CO); EIMS *m/z* (%): 277 [M^+](100), 276(100), 262(15), 230(20), 163(76), 117(55), 90(7), 76(30); HRMS (APCI): calcd for $C_{12}H_{11}N_3O_3S$+H 278.0599. Found 278.0600. Crystal data: Monoclinic, $P2_1/c$, a = 11.446 (2) Å, b = 11.1475 (19) Å, c = 10.5155 (18) Å, β = 103.108 (2)°, V = 1306.8 (4) Å^3, Z = 4, D_x = 1.409 mg m^{-3}, Mo Kα radiation, μ = 0.26 mm^{-1}, T = 296 (2) K, 8104 measured reflections, 2957 independent reflections, R_{int} = 0.020, fine R_1 = 0.038, $wR(F^2)$ = 0.110.

CCDC 653947 contains the supplementary crystallographic data for **4a**. They can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request.cif.

5-Acetyl-4-methyl-2-(*p*-ethoxyphenylamino)-thiazole (3b). Pale yellow crystal, mp: 172–174°C (lit. [27]: 176°C); IR ν_{max} (KBr)/cm^{-1}: 3273, 3132, 2977, 1609, 1554, 1510, 1484, 1372, 1326; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.25 (d, J = 8.5 Hz, 2H, Ar-H), 6.93 (d, J = 8.5 Hz, 2H, Ar-H), 4.04 (q, J = 7.0 Hz, 2H, CH_3CH_2), 2.55 (s, 3H, CH_3), 2.41 (s, 3H, CH_3CO), 1.43 (t, J = 7.0 Hz, 3H, CH_2CH_3); EIMS *m/z* (%): 276 [M^+](83), 261(13), 247(100), 233(14), 205(7), 178(4), 150(3), 134(7).

5-Acetyl-4-methyl-2-imino-3-(*p*-ethoxyphenyl)-2,3-dihydrothiazole (4b). Gray white crystal, mp: 113–115°C; IR ν_{max} (KBr)/cm^{-1}: 3295, 2973, 1583, 1562, 1511, 1359, 1322, 1251; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.15 (d, J = 9.0 Hz, 2H, Ar-H), 7.03 (d, J = 9.0 Hz, 2H, Ar-H), 4.07 (q, J = 7.0 Hz, 2H, CH_3CH_2), 2.34 (s, 3H, CH_3), 2.21 (s, 3H, CH_3CO), 1.45 (t, J = 7.0 Hz, 3H, CH_2CH_3); EIMS *m/z* (%): 276 [M^+](100), 275(57), 261(7), 247(48), 233(7), 205(5), 162(52), 134(19); HRMS (APCI): calcd for $C_{14}H_{16}N_2O_2S$+H 277.1011. Found 277.1013.

5-Acetyl-4-methyl-2-(*o*-chlorophenylamino)-thiazole (3c). White crystal, mp: 163–165°C; IR ν_{max} (KBr)/cm^{-1}: 3235, 3000, 1633, 1543, 1480, 1439, 1384, 1314; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.94 (d, J = 8.0 Hz, 1H, Ar-H), 7.44 (d, J = 8.0 Hz, 1H, Ar-H), 7.34 (t, J = 7.8 Hz, 1H, Ar-H), 7.07 (t, J = 7.8 Hz, 1H, Ar-H), 2.64 (s, 3H, CH_3), 2.48 (s, 3H, CH_3CO); EIMS *m/z* (%): 266 [M^+](73), 251(82), 231(100), 223(10), 189(15), 138(20), 86(15), 71(25).

5-Acetyl-4-methyl-2-imino-3-(*o*-chlorophenyl)-2,3-dihydrothiazole (4c). White crystal, mp: 90–92°C; IR ν_{max} (KBr)/cm^{-1}: 3252, 1622, 1382, 1476, 1435, 1383, 1363, 1326; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.62 (d, J = 9.2 Hz, 1H, Ar-H), 7.51–7.46 (m, 2H, Ar-H), 7.37 (d, J = 9.2 Hz, 1H, Ar-H), 2.37 (s, 3H, CH_3), 2.20 (s, 3H, CH_3CO); EIMS *m/z* (%): 266 [M^+](32), 231(99), 189(17), 152(60), 111(17), 75(20); HRMS (APCI): calcd for $C_{12}H_{11}ClN_2OS$+H 267.0359. Found 267.0365.

5-Acetyl-4-methyl-2-(*m*-chlorophenylamino)-thiazole (3d). Pale yellow crystal, mp: 191–193°C; IR ν_{max} (KBr)/cm^{-1}: 3289, 3131, 1613, 1594, 1553, 1490, 1371, 1319; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.41 (s, 1H, Ar-H), 7.33 (t, J = 8.0 Hz, 1H, Ar-H), 7.24 (d, J = 7.8 Hz, 1H, Ar-H), 7.14 (d, J = 7.5 Hz, 1H, Ar-H), 2.61 (s, 3H, CH_3), 2.48 (s, 3H, CH_3CO); EIMS *m/z* (%): 266 [M^+](84), 251(100), 223(10), 182(10), 138(9), 111(10), 86(13), 71(22); HRMS (APCI): calcd for $C_{12}H_{11}ClN_2OS$+H 267.0359. Found 267.0359.

5-Acetyl-4-methyl-2-imino-3-(*m*-chlorophenyl)-2,3-dihydrothiazole (4d). White crystal, mp: 156–158°C; IR ν_{max} (KBr)/cm^{-1}: 3330, 3063, 1578, 1472, 1424, 1384, 1358, 1323; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.52–7.48 (m, 2H, Ar-H), 7.29 (s, 1H, Ar-H), 7.19–7.17 (m, 1H, Ar-H), 2.35 (s, 3H, CH_3), 2.23 (s, 3H, CH_3CO); EIMS *m/z* (%): 266 [M^+](72), 265(100),

251(13), 223(8), 152(64), 111(32), 85(6), 75(16); HRMS (APCI): calcd for $C_{12}H_{11}ClN_2OS+H$ 267.0359. Found 267.0341.

5-Acetyl-4-methyl-2-(*p*-chlorophenylamino)-thiazole (3e). Gray white crystal, mp: 195–197°C (lit. [27]: 185°C); IR ν_{max} (KBr)/cm^{-1}: 3276, 3127, 1608, 1554, 1492, 1373, 1319, 1237; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.37 (d, J = 8.8 Hz, 2H, Ar-H), 7.32 (d, J = 8.8 Hz, 2H, Ar-H), 2.60 (s, 3H, CH_3), 2.47 (s, 3H, CH_3CO); EIMS *m/z* (%): 266 [M^+](90), 251(100), 223(10), 182(10), 152(20), 138(15), 111(12), 86(17).

5-Acetyl-4-methyl-2-imino-3-(*p*-chlorophenyl)-2,3-dihydrothiazole (4e). Gray white crystal, mp: 121–123°C; IR ν_{max} (KBr)/cm^{-1}: 3207, 3014, 1652, 1633, 1587, 1489, 1383, 1315; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.53 (d, J = 8.4 Hz, 2H, Ar-H), 7.21 (d, J = 8.4 Hz, 2H, Ar-H), 2.35 (s, 3H, CH_3), 2.22 (s, 3H, CH_3CO); EIMS *m/z* (%): 266 [M^+](90), 251(20), 152(100), 129(15), 111(57), 97(15), 83(20), 75(30); HRMS (APCI): calcd for $C_{12}H_{11}ClN_2OS+H$ 267.0359. Found 247.0347.

5-Acetyl-4-methyl-2-(3,4-dichlorophenylamino)-thiazole (3f). Pale yellow crystal, mp: 220–221°C; IR ν_{max} (KBr)/cm^{-1}: 3270, 3070, 1604, 1587, 1546, 1472, 1362, 1318; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.56 (d, J = 2.0 Hz, 1H, Ar-H), 7.47 (d, J = 8.5 Hz, 1H, Ar-H), 7.24 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H, Ar-H), 2.65 (s, 3H, CH_3), 2.50 (s, 3H, CH_3CO); EIMS *m/z* (%): 300 [M^+](80), 285(100), 257(12), 218(7), 186(6), 172(7), 86(13), 71(9).

5-Acetyl-4-methyl-2-imino-3-(3,4-dichlorophenyl)-2,3-dihydrothiazole (4f). Pale yellow crystal, mp: 195–196°C; IR ν_{max} (KBr)/cm^{-1}: 3306, 3057, 1647, 1604, 1549, 1472, 1305, 1091; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.63 (d, J = 8.5 Hz, 1H, Ar-H), 7.41 (d, J = 2.0 Hz, 1H, Ar-H), 7.15 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H, Ar-H), 2.35 (s, 3H, CH_3), 2.24 (s, 3H, CH_3CO); EIMS *m/z* (%): 300 [M^+](88), 299(100), 285(20), 257(10), 186(73), 145(30), 109(15), 75(7); HRMS (APCI): calcd for $C_{12}H_{10}Cl_2N_2OS+H$ 300.9969. Found 300.9945.

5-Acetyl-4-methyl-2-(*o*-methylphenylamino)-thiazole (3g). White crystal, mp: 153–154°C (lit. [28]: 169–170°C); IR ν_{max} (KBr)/cm^{-1}: 3165, 3062, 2930, 1598, 1541, 1500, 1431, 1328; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.49 (d, J = 8.0 Hz, 1H, Ar-H), 7.31–7.26 (m, 2H, Ar-H), 7.21 (d, J = 7.5 Hz, 1H, Ar-H), 2.51 (s, 3H, CH_3), 2.40 (s, 3H, CH_3CO), 2.33 (s, 3H, Ar-CH_3); EIMS *m/z* (%): 246 [M^+](100), 231(57), 213(16), 203(26), 170(27), 118(27), 91(19), 71(22).

5-Acetyl-4-methyl-2-imino-3-(*o*-methylphenyl)-2,3-dihydrothiazole (4g). Yellow crystal, mp: 102–104°C; IR ν_{max} (KBr)/cm^{-1}: 3613, 3018, 2976, 1627, 1571, 1487, 1359, 1325; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.46–7.38 (m, 3H, Ar-H), 7.18 (d, J = 7.6 Hz, 1H, Ar-H), 2.38 (s, 3H, CH_3), 2.17 (s, 6H, CH_3CO + Ar-CH_3); EIMS *m/z* (%): 246 [M^+](100), 231(75), 203(27), 170(28), 132(68), 118(40), 91(63), 71(59); HRMS (APCI): calcd for $C_{13}H_{14}N_2OS+H$ 247.0905. Found 247.0884.

5-Acetyl-4-methyl-2-(*p*-methylphenylamino)-thiazole (3h). White crystal, mp: 190–192°C (lit. [27]: 189°C); IR ν_{max} (KBr)/cm^{-1}: 3281, 3131, 1610, 1483, 1372, 1323, 1309, 1239; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.22–7.05 (m, 4H, Ar-H), 2.58 (s, 3H, CH_3), 2.44 (s, 3H, CH_3CO), 2.36 (s, 3H, Ar-CH_3); EIMS *m/z* (%): 246 [M^+](100), 231(96), 203(11), 162(10), 132(13), 118(18), 91(15), 71(10).

5-Acetyl-4-methyl-2-imino-3-(*p*-methylphenyl)-2,3-dihydrothiazole (4h). Pale yellow crystal, mp: 114–116°C; IR ν_{max} (KBr)/cm^{-1}: 3300, 3211, 3037, 1621, 1586, 1384, 1358, 1323; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.34 (d, J = 8.0 Hz, 2H, Ar-H), 7.12 (d, J = 8.4 Hz, 2H, Ar-H), 2.42 (s, 3H, CH_3), 2.34 (s, 3H, CH_3CO), 2.21 (s, 3H, Ar-CH_3); EIMS *m/z* (%): 246 [M^+](100), 231(21), 203(10), 132(93), 91(63), 65(30); HRMS (APCI): calcd for $C_{13}H_{14}N_2OS+H$ 247.0905. Found 247.0913.

5-Acetyl-4-methyl-2-phenylamino-thiazole (3i). Pale yellow crystal, mp: 154–155°C (lit. [27]: 147°C); IR ν_{max} (KBr)/cm^{-1}: 3280, 3203, 3140, 3096, 1617, 1558, 1483, 1332; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.42 (t, J = 8.0 Hz, 2H, Ar-H), 7.34 (d, J = 8.0 Hz, 2H, Ar-H), 7.19 (t, J = 7.2 Hz, 1H, Ar-H), 2.60 (s, 3H, CH_3), 2.46 (s, 3H, CH_3CO); EIMS *m/z* (%): 232 [M^+](100), 217(35), 189(47), 148(57), 118(36), 104(77), 77(90), 51(15).

5-Acetyl-4-methyl-2-imino-3-phenyl-2,3-dihydrothiazole (4i). Pale yellow crystal, mp: 128–129°C (lit. [29]: 128–129°C; IR ν_{max} (KBr)/cm^{-1}: 3325, 3284, 3054, 1586, 1496, 1327, 1093, 1022; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.56 (t, J = 7.4 Hz, 2H, Ar-H), 7.50 (t, J = 7.4 Hz, 1H, Ar-H), 7.26 (d, J = 7.2 Hz, 2H, Ar-H), 2.35 (s, 3H, CH_3), 2.21 (s, 3H, CH_3CO); EIMS *m/z* (%): 232 [M^+](92), 231(100), 217(12), 189(13), 130(7), 118(94), 91(7), 77(89); HRMS (APCI): calcd for $C_{12}H_{12}N_2OS+H$ 233.0749. Found 233.0760.

Biological section. In vitro *cervical cancer cell antiproliferation assay*. The human cervical cancer cell lines (Hela and Siha derived from Shanghai Institutes for Biological Science, Chinese Academy of Sciences) were cultivated at 37°C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, purchased from Gibco) supplemented with 800 (U/v) penicillin, 0.1% (w/v) streptomycin, and 10% (v/v) fetal bovine serum for 3–5 days. Human cervical cancer cells, treated with trypsin-EDTA solution, were seeded into 96-well flat bottom plates at 10^6 cells per well and incubated in a 5% CO_2 incubator at 37°C for 24 h. Cultures were treated with compounds prepared in different concentrations. Mitochondrial metabolism was measured as a marker for cell growth by adding 10 microliters per well MTT (5 mg/mL in medium, Sigma) with 3 h of incubation at 37°C. Crystals formed were dissolved in 150 μL of DMSO. The absorbance was determined using a microplate reader at 490 nm. The absorbance data were converted into a cell proliferation percentage, compared to DMSO-treated cells, to determine the IC_{50}s.

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