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Synthesis and Antitumor Activity of 4-*tert*-Butyl-5-benzyl-2-benzyliminothiazoles

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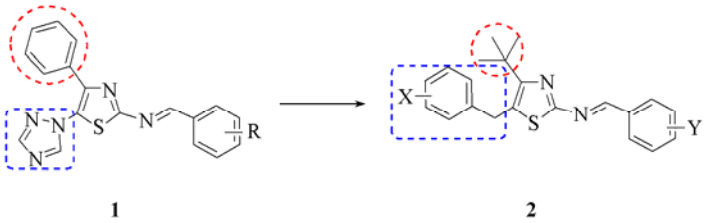
Abstract A series of novel Schiff bases including 4-*tert*-butyl-5-benzyl-2-benzyliminothiazoles was synthesized by reacting the aromatic aldehydes with the corresponding 2-aminothiazoles. The antitumor bioassay revealed that compounds **2**n and **2**m exhibited potent cytotoxicity against human cervix cancer(HeLa) cell line with IC_{50} values of 0.001 and 0.007 mmol/L, respectively. The preliminary structure-activity relationship(SAR) investigations and the apoptosis evaluation suggest that 4-*tert*-butyl-5-benzyl-2-benzyliminothiazoles may be a satisfactory backbone for antitumor activity, and compound **2**n can serve as an attractive candidate for the development of novel apoptosis in anticancer treatment.

Keywords 2-Aminothiazole; Schiff base; Antitumor activity

1 Introduction

Thiazole derivatives appear frequently in many natural and synthetic products with various pharmacological activities such as antitumor, antibacterial, antifungal, antiviral and anti-inflammatory effects[1—3]. Among them, 2-aminothiazoles have long been used as precursors for the synthesis of bioactive molecules. Previous studies indicate that organic compounds bearing 2-aminothiazoles with different pharmacophore possess potent antitumor activities[4—9]. In addition to 2-aminothiazoles, Schiff bases also show some biological activities including antibacterial, antifungal, antiviral and anticancer effects[10—12]. Reported 4-phenyl-5-(1*H*-1,2,4-traizol-1-yl) thiazolyl Schiff base derivatives(**1**), which were designed by molecular hybridization of 2-aminothiazole and Schiff base, show good efficacy against human tumors such as leukemia, stomach, and larynx cancer[13].

Inspired by these reports, we developed an idea of embedding *tert*-butyl, 1,2,4-triazole or substituted benzyl moieties embedded into thiazolyl Schiff base(Scheme 1). In our previous work[14], we reported some thiazole Schiff base compounds containing *tert*-butyl and 1,2,4-triazole and investigated their bactericidal activity. We also introduced the hydrophobic *tert*-butyl and substituted benzyl to replace the phenyl and triazole cycles in compound **1** for the synthesis of 4-*tert*-butyl-5-benzyl-2-benzyliminothiazoles(**2**), and evaluated their bactericidal activities as well as inhibitions against COX-2[15—17]. Therefore, as a continuation of our studies on thiazole derivatives, we have synthesized a series of 4-*tert*-butyl-5-benzyl-2-benzylimino-thiazoles(**2**a—**2**t) and evaluated their preliminary cytotoxicity on human cervix cancer(HeLa) cell line.



1 **2**

Scheme 1 Design of 4-*tert*-butyl-5-benzyl-2-benzyliminothiazoles(2)

2 Results and Discussion

2.1 Synthesis

The synthesis of compounds **2**l—**2**t was reported in our previous publications[15—17]. And the synthetic route of the new thiazole Schiff bases **2**a—**2**k is described in Scheme 2. The desired thiazole derivatives **2** were synthesized from 4,4-dimethyl-1-aryl-3-pentanone, which were prepared from the substituted benzaldehydes and pinacolone according to the reported method[18].

4,4-Dimethyl-1-aryl-3-pentanones were selectively brominated to *α*-bromoketones(**3**a—**3**f) in excellent yields(83%—93%) within 15—30 min at room temperature, with ionic liquid 1-buty-3-methylimidazolium tribromide([Bmin]Br_3) as solvent and brominating reagent. [Bmin]Br_3 was prepared as described

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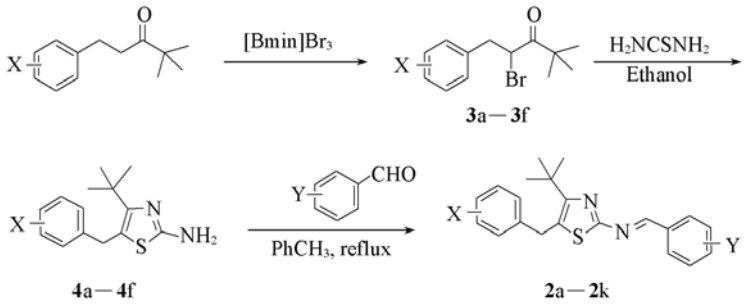

Scheme 2 Synthetic route of compounds 2a—2k

previously[19], and after work-up, the bromine carrier [Bmin]Br could be used to react with bromine to regenerate [Bmin]Br_3 for the next run. Next, the compounds **3**a—**3**f reacted with thiourea in ethanol to produce 4-*tert*-butyl-5-benzylthiazol-2-amine hydrobromides, which were converted to free-based 2-aminothiazoles(**4**a—**4**f) by neutralization with ammonia. To test the influence of substituents and their positions on the biological activity, the designed thiazoles **2**a—**2**k were prepared by reacting the corresponding compounds **4**a—**4**f with various substituted aromatic aldehydes under reflux in anhydrous toluene in 42%—76% yields. Difficulties induced by the poor reactivity of some starting materials were overcome *via* the use of piperidine as well as azeotropic removal of water by a Dean-Stark trap. Each of the final compounds was purified by crystallization from absolute ethanol. Owing to the presence of chromophoric groups(C═N) in the molecules, the Schiff bases formed crystals with different shades of yellow. All the new compounds were confirmed by elemental analyses and ^{1}H NMR spectroscopy. The protons of CH═N display an acute single peak between δ 8.66 and 9.35.

2.2 Structure-activity Relationship

Compounds **2**a—**2**t were studied for their cytotoxicities against HeLa cell line, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide(MTT) assay. The results(Table 1) indicate that most of compounds **2** with *p*-Cl substituent on the 5-benzyl fragment of the thiazole ring showed moderate to strong cytotoxic activities against HeLa cells. Among them, compounds **2**m and **2**n exhibited relatively higher cytotoxic activities than Cisplatin(IC_{50}=0.009 mmol/L), with IC_{50} values of 0.007 and 0.001 mmol/L, respectively. *p*-Chloro on the 5-benzyl fragment of the thiazole ring is important for the antitumor activity. Displacement of electron-withdrawing *p*-chlorobenzyl with electron-donating *p*-methoxybenzyl on the thiazole significantly decreased the cytotoxic activities(compared IC_{50} value of compound **2**m to that of compound **2**j). Moreover, chlorine substitution at the 2- and 4-positions was also investigated for compounds **2**a and **2**b. Substitution at the 2-position showed an obvious weaker activity.

For the *para*-chlorobenzyl series, it can be seen that substituent Y and its position on the phenyl ring exhibited slight variations in their cytotoxic activities(compounds **2**b—**2**e and **2**l—**2**t). Thiazoles with *para*-electron-withdrawing(4-Cl, 4-NO_2) on the phenyl ring are more active than a 4-electron-donating

Table 1 Inhibition of HeLa cell line by compounds 2a—2t and Cisplatin

Compound	X	Y	IC_{50}/(mmol·L^{-1})
2a	2-Cl	3-NO_2	0.159
2b	4-Cl	3-NO_2	0.048
2c	4-Cl	4-$N(CH_3)_2$	0.019
2d	4-Cl	2-Cl-5-NO_2	0.014
2e	4-Cl	2,4-$(MeO)_2$	0.014
2f	2-MeO	3-NO_2	0.036
2g	2-EtO	3-NO_2	0.251
2h	4-MeO	3-NO_2	0.050
2i	2-MeO	4-NO_2	>0.5
2j	4-MeO	4-NO_2	0.232
2k	2,4,5-$(MeO)_3$	3-NO_2	0.357
2l	4-Cl	2-NO_2	0.013
2m	4-Cl	4-NO_2	0.007
2n	4-Cl	4-Cl	0.001
2o	4-Cl	2-OH	0.194
2p	4-Cl	2-OH-5-NO_2	0.039
2q	4-Cl	2-OH-5-Br	0.040
2r	4-Cl	2-OH-3,5-$(Br)_2$	0.028
2s	4-Cl	2-OH-3,5-$(Cl)_2$	0.029
2t	4-Cl	2-OH-3,5-$(I)_2$	0.031
Cisplatin			0.009

[4-$N(CH_3)_2$] variant(compared IC_{50} values of compounds **2**n and **2**m to **2**c). These imply that the antitumor activity is related to the electron-withdrawing group on the phenyl ring. In addition, improved activity was achieved by 4-NO_2 variant **2**m, while 2- or 3-NO_2 variants **2**l and **2**b showed an obvious decrease in activity(compared IC_{50} value of compounds **2**m to those of compounds **2**l and **2**b). Interestingly, the antitumor property of compound **2**o with 2-OH was significantly lower than that of compound **2**l with 2-NO_2. But when electron-withdrawing groups(NO_2 or halogen atoms) were introduced into the *meta*-position of the phenyl ring in **2**o, the associated compounds **2**p—**2**t showed an obvious increase in cytotoxicity(compared IC_{50} value of compound **2**o to those of compounds **2**p, **2**q, **2**r, **2**s and **2**t).

In summary, we have discerned that: 4-*tert*-butyl-5-benzyl-2-benzyliminothiazole is a satisfactory backbone for antitumor activity; the existence of 4-chloro on the 5-benzyl fragment of the thiazole ring is important for the antitumor activity of the compounds; introducing electron-withdrawing substituent on the phenyl ring improves the potency of the compounds. Compound **2**n was selected for a further mechanism study for its outstanding activity against HeLa cells.

2.3 Hoechst33342/PI Double-staining Assay

To investigate the induction of apoptosis or necrosis of compound **2**n, the morphological change of HeLa cells treated with compound **2**n at 0.1 mmol/L for 24 h was observed by Hoechst33342/PI double staining. As we know, Hoechst 33342(bisbenzimidazole dye) can penetrate through the plasma membrane and stain DNA in live cells, while PI(propidium iodide) can only enter the cells with a damaged cell membrane, such as necrotic cells or late apoptotic cells. So under light irradiation, the cell status can be confirmed according to the fluorescence color and the morphological changes of cells. As shown in Fig.1, the cells treated with compound **2**n displayed the early phenomena of apoptosis, such as congregated chromatin and nucleolus pyknosis, emitting bright blue fluorescence under ultraviolet light irradiation. The late apoptotic cells also showed light red fluorescence under green light because of the damaged cell membranes, which was in accordance with the character of apoptosis. Meanwhile, untreated HeLa cells stained with uniform blue fluorescence showed the chromatin equably distributed in the nucleoli. And some cells emitted bright red fluorescence under green light irradiation because of the uptake of PI, which indicates necrotic cells. Based on these morphologic findings, compound **2**n appears to cause the apoptosis of HeLa cells.

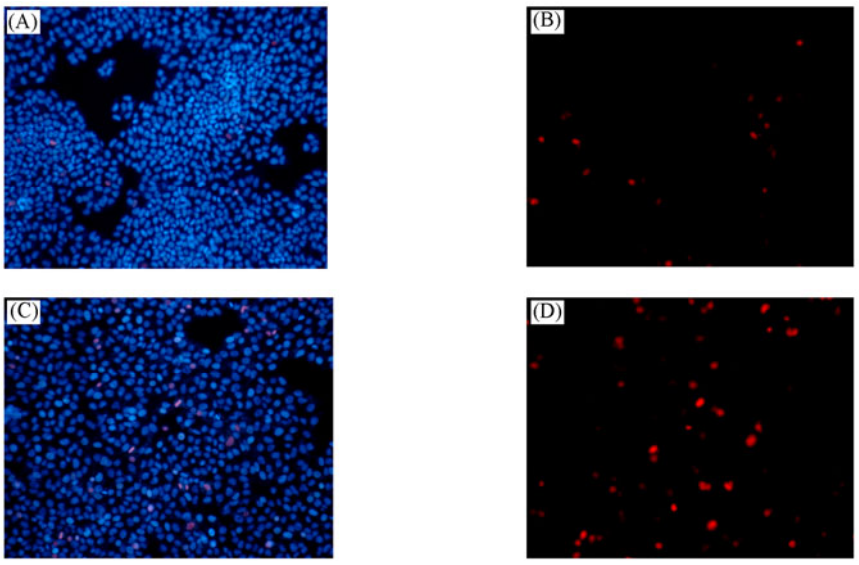

Fig.1 Effects of compound 2n on apoptosis and necrosis induction in HeLa cells

Nuclear morphology in HeLa cells was analyzed by fluorescence microscopy upon Hoechst33342/PI-staining 24 h after treatment with compound **2**n(0.1 mmol/L). (A) Control group under ultraviolet light; (B) control group under green light; (C) compound **2**n under ultraviolet light; (D) compound **2**n under green light.

3 Experimental

3.1 General

Reagents were commercial grade that were used as supplied. Reactions were monitored by thin-layer chromatography(TLC) with F_{254} silica gel precoated sheets, and spots were visualized with the help of ultraviolet(UV) light. Solvents were pre-dried and distilled prior to use. Melting points were measured on an X-4 digital melting point apparatus and the thermometer was uncorrected. The ^{1}H NMR spectra were recorded on a Bruker advanced instrument(400 MHz) in $CDCl_3$ (TMS) solution. Elemental analyses were determined on a VARIO EL III elemental analyzer.

3.2 Synthesis

3.2.1 General Procedure for Preparation of Compounds 3b—3f

The crimson [Bmin]Br_3 ionic liquid(4.48 g, 0.02 mol) was added slowly in the corresponding 4,4-dimethyl-1-aryl-3-pentanone(0.02 mol) with stirring at room temperature. The mixture was allowed to stir for 10—30 min, and was extracted with ethyl acetate(30 mL) three times. Then, the ionic liquid was recycled and the combined organic layer was washed with water, dried over Na_2SO_4 and evaporated. Finally, the residue was purified by silica gel chromatography eluted with the mixture of petroleum ether:AcOEt(volume ratio 1:9) to give the desired product. The synthesis of compound **3**a has been reported previously[19]. The recycled ionic liquid was washed with ethyl acetate and ether, dried in vacuum and reacted with bromine to give [Bmin]Br_3.

2-Bromo-1-(2-chlorophenyl)-4,4-dimethylpentan-3-one (**3**b), a colorless transparent liquid, yield 93%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.01[s, 9H, $C(CH_3)_3$], 3.37(dd, J=13.6, 6.6 Hz, 1H, CH_2), 3.46(dd, J=13.6, 8.6 Hz, 1H, CH_2), 4.99(dd, J=8.6, 6.6 Hz, 1H, CHBr), 7.16—7.19(m, 3H, 4,5,6-H of C_6H_4), 7.34(d, J=6.8 Hz, 1H, 3-H of C_6H_4).

2-Bromo-1-(4-methoxyphenyl)-4,4-dimethylpentan-3-one (**3**c), reaction time 20 min, a colorless transparent liquid, yield 87%. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 0.97[s, 9H, $C(CH_3)_3$], 3.12(dd, J=13.2, 5.4 Hz, 1H, CH_2), 3.42(dd, J=13.2, 9.8 Hz, 1H, CH_2), 3.77(s, 3H, OCH_3), 4.71(dd, J=9.8, 5.4 Hz, 1H, CHBr), 6.78(d, J=8.8 Hz, 2H, 2,6-H of C_6H_4), 7.06(d, J=8.8 Hz, 2H,

3,5-H of C_6H_4).

2-Bromo-1-(2-methoxyphenyl)-4,4-dimethylpentan-3-one (**3**d), reaction time 30 min, a white solid, yield 91%; m. p. 49—51 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.00[s, 9H, $C(CH_3)_3$], 3.26(dd, J=11.2, 6.8 Hz, 1H, CH_2), 3.32(dd, J=11.2, 8.4 Hz, 1H, CH_2), 3.86(s, 3H, OCH_3), 5.01(dd, J=6.8, 8.4 Hz, 1H, CHBr), 6.83—7.26(m, 4H, C_6H_4).

2-Bromo-1-(2-ethoxyphenyl)-4,4-dimethylpentan-3-one(**3e**), reaction time 30 min, a colorless transparent liquid, yield 83%. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.04[s, 9H, $C(CH_3)_3$], 1.46 (t, J=7.2 Hz, 3H, CH_3), 3.24(dd, J=13.2, 6.8 Hz, 1H, CH_2), 3.32(dd, J=13.2, 7.6 Hz, 1H, CH_2), 4.03—4.10(m, 2H, OCH_2), 5.05(dd, J=6.8, 7.6 Hz, 1H, CHBr), 6.81—7.22(m, 4H, C_6H_4).

2-Bromo-4,4-dimethyl-1-(2,4,5-trimethoxyphenyl)-pentan-3-one(**3f**), reaction time 30 min, a white solid, yield 91%; m. p. 93—95 °C. ^{1}H NMR($CDCl_3$, 600 MHz), δ: 1.01[s, 9H, $C(CH_3)_3$], 3.20—3.28(m, 2H, CH_2), 3.80(s, 3H, OCH_3), 3.85(s, 3H, OCH_3), 3.87(s, 3H, OCH_3), 4.92—4.96(m, 1H, CHBr), 6.49(d, J=4.2 Hz, 1H, 3-H of C_6H_2), 6.61(d, J=6.0 Hz, 1H, 6-H of C_6H_2).

*3.2.2 General Procedure for Preparation of Compounds **4b—4f***

A mixture of 1-aryl-2-bromo-4,4-dimethylpentan-3-one(**1**, 0.05 mol) and thiourea(0.05 mol) in ethanol(100 mL) was refluxed for 3 h. Then, the solvent was cooled, neutralized with ammonia water until pH=8—9 and left overnight. The resulting precipitate was filtered out and dried, affording compound **4** as a white solid. The aminothiazole hydrobromides **4**·HBr was obtained by cooling, filtering and drying the reaction mixture without neutralization. The synthesis of compound **4**a has been previously reported[19].

4-*tert*-Butyl-5-(2-chlorobenzyl)thiazol-2-amine(**4**b), **4**b·HBr yield 45%; m. p. 249.0—254.0 °C. Compound **4**b yield 41%; m. p. 136.9—138.5 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.32[s, 9H, $C(CH_3)_3$], 4.20(s, 2H, CH_2), 4.90(br, 2H, NH_2), 7.16—7.37(m, 4H, 3,4,5,6-H of C_6H_4).

4-*tert*-Butyl-5-(4-methoxybenzyl)thiazol-2-amine(**4**c), **4**c·HBr yield 79%; m. p. 159.4—160.8 °C. Compound **4**c yield 72%; m. p. 118.8—120.5 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.34[s, 9H, $C(CH_3)_3$], 3.79(s, 2H, OCH_3), 4.07(s, 2H, CH_2), 4.90(br, 2H, NH_2), 6.83(d, J=8.8 Hz, 2H, 3,5-H of C_6H_4), 7.11(d, J=8.8 Hz, 2H, 2,6-H of C_6H_4).

4-*tert*-Butyl-5-(2-methoxybenzyl)thiazol-2-amine(**4**d), **4**d·HBr yield 78%; m. p. 191—193 °C. Compound **4**d yield 68%; m. p. 119.2—129.0 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.34[s, 9H, $C(CH_3)_3$], 3.83(s, 3H, OCH_3), 4.09(s, 2H, CH_2), 4.83(br, 2H, NH_2), 6.84—7.24(m, 4H, 3,4,5,6-H of C_6H_4).

4-*tert*-Butyl-5-(2-ethoxybenzyl)thiazol-2-amine(**4**e), **4**e·HBr yield 41%; m. p. 200—202 °C. Compound **4**e yield 21%; m. p. 131—134 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.36[s, 9H, $C(CH_3)_3$], 1.41(t, J=6.8 Hz, 3H, CH_3), 4.05(q, J=6.8 Hz, 2H, OCH_2), 4.09(s, 2H, CH_2), 4.91(br, 2H, NH_2), 6.83—7.26(m, 4H, 3,4,5,6-H of C_6H_4).

4-*tert*-Butyl-5-(2,4,5-trimethoxybenzyl)thiazol-2-amine(**4**f), **4**f·HBr yield 24%; m. p. 222.7—223.3 °C. Compound **4**f yield 21.9%; m. p. 154.6—155.0 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.35[s, 9H, $C(CH_3)_3$], 3.78, 3.81, 3.89(3×s, 9H, 3×OCH_3), 4.03(s, 2H, CH_2), 4.74(br, 2H, NH_2), 6.52(s, 1H, 3-H of C_6H_2), 6.67(s, 1H, 5-H of C_6H_2).

*3.2.3 General Procedure for Preparation of Compounds **2a—2k***

A mixture of benzaldehyde(1 mmol), piperidine(0.2 mL) and toluene(5 mL) was added dropwise to a stirred solution of 4-*tert*-butyl-5-benzylthiazol-2-amine(1 mmol) in toluene(5 mL) at room temperature. Then the mixture was heated under reflux for 2 h(monitored by TLC). After cooling, the resulting precipitate was collected by vacuum filtration and recrstallized from anhydrous ethanol to give the desired product as a yellow solid.

4-*tert*-Butyl-5-(2-chlorobenzyl)-*N*-(3-nitrobenzylidene)-thiazol-2-amine(**2**a), yield 62%; m. p. 167—170 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.47(s, 9H, 3×CH_3), 4.40(s, 2H, CH_2), 7.18—7.42(m, 4H, C_6H_4), 7.66(t, J=8.0 Hz, 1H, 5-H of 3-$O_2NC_6H_4$), 8.29(d, J=8.0 Hz, 1H, 4-H of 3-$O_2NC_6H_4$), 8.34(ddd, J=8.0, 2.4, 2.0 Hz, 1H, 6-H of 3-$O_2NC_6H_4$), 8.77(t, J=1.8 Hz, 1H, 2-H of 3-$O_2NC_6H_4$), 8.89(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{21}H_{20}ClN_3O_2S$: C 60.94, H 4.87, Cl 8.57, N 10.15, S 7.75; found: C 60.63, H 4.72, Cl 8.34, N 10.26, S 7.63.

4-*tert*-Butyl-5-(4-chlorobenzyl)-*N*-(3-nitrobenzylidene)-thiazol-2-amine(**2**b), yield 63%; m. p. 126—128 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.46(s, 9H, 3×CH_3), 4.30(s, 2H, CH_2), 7.16(d, J=8.4 Hz, 2H, 2,6-H of 4-ClC_6H_4), 7.31(d, J=8.4 Hz, 2H, 3,5-H of 4-ClC_6H_4), 7.66(t, J=8.0 Hz, 1H, 5-H of 3-$O_2NC_6H_4$), 8.29(d, J=7.6 Hz, 1H, 4-H of 3-$O_2NC_6H_4$), 8.35(dd, J=8.0, 1.2 Hz, 1H, 6-H of 3-$O_2NC_6H_4$), 8.78(d, J=1.6 Hz, 1H, 2-H of 3-$O_2NC_6H_4$), 8.90(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{21}H_{20}ClN_3O_2S$: C 60.94, H 4.87, Cl 8.57, N 10.15, S 7.75; found: C 60.78, H 4.96, Cl 8.75, N 10.02, S 7.52.

4-*tert*-Butyl-5-(4-chlorobenzyl)-*N*-(4-(dimethylamino)benzylidene)thiazol-2-amine(**2**c), yield 76%; m. p. 165—167 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.43(s, 9H, 3×CH_3), 3.07[s, 6H, $N(CH_3)_2$], 4.25(s, 2H, CH_2), 6.69[d, J=8.8 Hz, 2H, 3,5-H of 4-$N(CH_3)_2C_6H_4$], 7.15(d, J=8.4 Hz, 2H, 2,6-H of 4-ClC_6H_4), 7.28(d, J=8.4 Hz, 2H, 3,5-H of 4-ClC_6H_4), 7.80[d, J=8.8 Hz, 2H, 2,6-H of 4-$N(CH_3)_2C_6H_4$], 8.51(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{23}H_{26}ClN_3S$: C 67.05, H 6.36, Cl 8.61, N 10.20, S 7.78; found: C 66.78, H 6.52, Cl 8.77, N 10.03, S 7.46.

4-*tert*-Butyl-N-(2-chloro-5-nitrobenzylidene)-5-(4-chlorobenzyl)thiazol-2-amine(**2**d), yield 43%; m. p. 99.2—102.3 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.44[s, 9H, $(CH_3)_3$], 4.28(s, 2H, CH_2), 7.14(d, J=8.4 Hz, 2H, 3,5-H of C_6H_4), 7.28(d, J=8.4 Hz, 2H, 2,6-H of C_6H_4), 7.58(d, J=8.4 Hz, 1H, 3-H of C_6H_3), 8.22(dd, J=8.4, 2.8 Hz, 1H, 4-H of C_6H_3), 9.11(d, J=2.8 Hz, 1H, 6-H of C_6H_3), 9.24(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{21}H_{19}Cl_2N_3O_2S$: C 56.25, H 4.27, Cl 15.81, N 9.37, S 7.15; found: C 56.43, H 4.12, Cl 15.53, N 9.26, S 7.28.

4-*tert*-Butyl-5-(4-chlorobenzyl)-*N*-(3,4-dimethoxybenzylidene)thiazol-2-amine(**2**e), yield 73%; m. p. 122—125 °C.

^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.45[s, 9H,$(CH_3)_3$], 3.95(s, 3H, 4-OCH_3), 3.96(s, 3H, 3-OCH_3), 4.28(s, 2H, CH_2), 6.92(d, J=8.4 Hz, 1H, 5-H of C_6H_3), 7.16(d, J=8.4 Hz, 2H, 2,6-H of 4-ClC_6H_4), 7.30(d, J=8.4 Hz, 2H, 3,5-H of 4-ClC_6H_4), 7.33(dd, J=8.4, 2.0 Hz, 1H, 6-H of C_6H_3), 7.68(d, J=2.0 Hz, 1H, 2-H of C_6H_3), 8.59(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{23}H_{25}ClN_2O_2S$: C 64.40, H 5.87, Cl 8.26, N 6.53, S 7.47; found: C 64.32, H 5.65, Cl 8.42, N 6.38, S 7.23.

4-*tert*-Butyl-5-(2-methoxybenzyl)-*N*-(3-nitrobenzylidene)-thiazol-2-amine(**2**f), yield 61%; m. p. 147—150 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.48(s, 9H, 3×CH_3), 3.84(s, 3H, OCH_3), 4.28(s, 2H, CH_2), 6.92—7.27(m, 4H, 2-$MeOC_6H_4$), 7.64(t, J=7.8 Hz, 1H, 5-H of 3-$O_2NC_6H_4$), 8.27(d, J=7.6 Hz, 1H, 4-H of 3-$O_2NC_6H_4$), 8.30—8.35(m, 1H, 6-H of 3-$O_2NC_6H_4$), 8.76(t, J=1.8 Hz, 1H, 2-H of 3-$O_2NC_6H_4$), 8.84(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{22}H_{23}N_3O_3S$: C 64.53, H 5.66, N 10.26, S 7.83; found: C 64.41, H 5.75, N 10.38, S 7.69.

4-*tert*-Butyl-5-(2-ethoxybenzyl)-*N*-(3-nitrobenzylidene)-thiazol-2-amine(**2**g), yield 50%; m. p. 129—132 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.40(t, J=7.2 Hz, 3H, CH_3), 1.47(s, 9H, 3×CH_3), 4.04(q, J=7.2 Hz, 2H, OCH_2), 4.24(s, 2H, CH_2), 6.76—7.14(m, 4H, 2-$EtOC_6H_4$), 7.62—7.67(m, 1H, 5-H of 3-O_2NC_6H4), 8.28(d, J =8.0 Hz, 1H, 4-H of 3-$O_2NC_6H_4$), 8.33(m, 1H, 6-H of 3-$O_2NC_6H_4$), 8.76(t, J=1.6 Hz, 1H, 2-H of 3-$O_2NC_6H_4$), 8.88(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{23}H_{25}N_3O_3S$: C 65.23, H 5.95, N 9.92, S 7.57; found: C 65.12, H 6.07, N 9.75, S 7.43.

4-*tert*-Butyl-5-(4-methoxybenzyl)-*N*-(3-nitrobenzylidene)-thiazol-2-amine(**2**h), yield 64%; m. p. 130—133 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.47(s, 9H, 3×CH_3), 3.81(s, 3H, OCH_3), 4.26(s, 2H, CH_2), 6.87(d, J=8.0 Hz, 2H, 3,5-H of 4-$MeOC_6H_4$), 7.23(d, J=8.0 Hz, 2H, 2,6-H of 4-$MeOC_6H_4$), 7.66(t, J=8.0 Hz, 1H, 5-H of 3-$O_2NC_6H_4$), 8.28(d, J=8.0 Hz, 1H, 4-H of 3-$O_2NC_6H_4$), 8.33(d, J=8.0 Hz, 1H, 6-H of 3-$O_2NC_6H_4$), 8.76(t, J=1.6 Hz, 1H, 2-H of 3-$O_2NC_6H_4$), 8.59(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{22}H_{23}N_3O_3S$: C 64.53, H 5.66, N 10.26, S 7.83; found: C 64.44, H 5.52, N 10.45, S 7.63.

4-*tert*-Butyl-5-(2-methoxybenzyl)-*N*-(4-nitrobenzylidene)-thiazol-2-amine(**2**i), yield 72%; m. p. 161—163 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.48(s, 9H, 3×CH_3), 3.84(s, 3H, OCH_3), 4.28(s, 2H, CH_2), 6.92—7.29(m, 4H, 2-$MeOC_6H_4$), 8.09(d, J=8.8 Hz, 2H, 2,6-H of 4-$O_2NC_6H_4$), 8.30(d, J=8.8 Hz, 2H, 3,5-H of 4-$O_2NC_6H_4$), 8.84(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{22}H_{23}N_3O_3S$: C 64.53, H 5.66, N 10.26, S 7.83; found: C 64.36, H 5.49, N 10.42, S 7.56.

4-*tert*-Butyl-5-(4-methoxybenzyl)-*N*-(4-nitrobenzylidene)-thiazol-2-amine(**2**j), yield 56%; m. p. 135—137 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 1.47(s, 9H, 3×CH_3), 3.81(s, 3H, OCH_3), 4.26(s, 2H, CH_2), 6.88(d, J=8.4 Hz, 2H, 3,5-H of 4-$MeOC_6H_4$), 7.16(d, J=8.4 Hz, 2H, 2,6-H of 4-$MeOC_6H_4$), 8.10(d, J=8.8 Hz, 2H, 2,6-H of 4-$O_2NC_6H_4$), 8.30(d, J=8.8 Hz, 2H, 3,5-H of 4-$O_2NC_6H_4$), 8.86(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{22}H_{23}N_3O_3S$: C 64.53, H 5.66, N 10.26, S 7.83; found: C 64.72, H 5.56, N 10.38, S 7.67.

4-*tert*-Butyl-*N*-(3-nitrobenzylidene)-5-(2,4,5-trimethoxybenzyl)thiazol-2-amine(**2**k), yield 51%; m. p. 137—139 °C. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 1.47(s, 9H, 3×CH_3), 3.81, 3.82, 3.92(3×s, 9H, 3×OCH_3), 4.21(s, 2H, CH_2), 6.56(s, 1H, 3-H of C_6H_2), 6.69(s, 1H, 6-H of C_6H_2), 7.66(t, J=8.0 Hz, 1H, 5-H of 3-$O_2NC_6H_4$), 8.28(d, J=7.6 Hz, 1H, 4-H of 3-$O_2NC_6H_4$), 8.33(ddd, J=8.0, 2.4, 2.0 Hz, 1H, 6-H of 3-$O_2NC_6H_4$), 8.76(t, J=1.8 Hz, 1H, 2-H of 3-$O_2NC_6H_4$), 8.84(s, 1H, N═CH). Elemental anal.(%) calcd. for $C_{24}H_{27}N_3O_5S$: C 61.39, H 5.80, N 8.95, S 6.83; found: C 61.48, H 5.82, N 8.84, S 6.56.

The general experimental procedure of compounds **2**l—**2**t was recorded in ref.[20].

3.3 Cytotoxicity Study Using HeLa Cell

3.3.1 Biological Materials

Each of compounds **2**a—**2**t and Cisplatin were respectively dissolved in 0.1 mL of DMSO(Sigma, USA) to make a final concentration of 0.01 mol/L and were stored at 4 °C. The solutions of each of compounds **2**a—**2**t and Cisplatin were freshly diluted with an RPMI-1640(Dingguo, Beijing, China) to the appropriate final concentrations when needed. HeLa cell line was purchased from Cell Bank of Xiangya School of Medicine, Central South University of China. Cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated calf serum(Invitrogen, USA), 100 U/mL penicillin G, and 100 U/mL streptomycin(pH=7.4) in a water-jacketed CO_2 incubator(Dingguo, Beijing, China) with a humidified atmosphere of 5% CO_2 at 37 °C. MTT(Amresco, USA) was dissolved in 0.01 mol/L PBS and was stored at 4° away from light.

3.3.2 MTT Assay of Proliferation Inhibition

Assays of cytotoxicity were conducted in 96-well, flat-bottomed microtitre plates. The supplement culture medium (RPMI 1640, 100 μL) with HeLa cells(1×10^5 cells per mL) was added to the wells and incubated for 48 h at 37 °C in atmosphere containing 5% CO_2. Then graded amounts of each of compounds **2**a—**2**t and Cisplatin were added to the wells in 10 μL of the PBS free culture medium and the plates were incubated in a 5% CO_2 humidified atmosphere for 48 h. Four replica wells were used for controls. Compounds **2**a—**2**t and Cisplatin were tested at concentrations ranging between 0 and 0.5 mmol/L. After cultured for 48 h, 20 μL of MTT(in 2.5 mg/mL PBS) was added to each well, and cells were incubated at 37 °C in atmosphere containing 5% CO_2 for 4 h. The resulting formazan crystals were then dissolved in 100 μL of DMSO and the absorbance was read at 570 nm with a Multiskan MK3 microplate reader. Assays were performed in triplicate on three independent experiments. Inhibition ratio(IR, %) was calculated as: (1–average absorbance of treated group/average absorbance of control group)×100%.

The IC_{50} value was defined as the concentration that caused 50% inhibition of cell proliferation, and was calculated by means of SPSS statistical software.

3.3.3 Hoechst33342 and PI Double Staining

Apoptotic or necrotic cell death was characterized by Hoechst33342 and propidium iodide(PI, Sigma-Aldrich) double staining. Twenty-four hours after compound **2**n(0.1 mmol/L) treatment, cells were stained with 10 mg/L

Hoechst33342 and 10 mg/L PI for 30 min at 37 °C. Cells were then washed with PBS three times. Stained cells were viewed under a fluorescence microscope(Nikon80i, Japan).

4 Conclusions

In this work, we synthesized a series of novel 4-*tert*-butyl-5-benzyl-2-benzyliminothiazoles by reacting the aromatic aldehydes with the corresponding 2-aminothiazoles. The antitumor bioassay revealed that some of the compounds possess excellent antitumor activity against HeLa cell line, especially compound **2**n may be an effective anticancer drug candidate as a potent apoptosis inducer. Although these findings are preliminary, the results of structure-activity relationship(SAR) investigations suggest that in the novel derivatives of 2-aminothiazole(**2**), the existence of 4-chloro on the 5-benzyl fragment of the thiazole ring and electron-withdrawing substitutions on the phenyl ring can lead to promising agents with antitumor activity. Moreover, it can be deduced that 4-*tert*-butyl-5-benzyl-2-benzylimino-thiazoles may be a satisfactory backbone for antitumor activity. Further structural modifications and biological evaluations of these compounds are currently in progress.

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