Synthesis and Anti-Cancer Evaluation of Spiro-indolinone Derivatives

Liang Hong1, Jie Tong1, Guangliang Yu1, Chunqi Hu1,2*
1Yongning Pharma, Taizhou, PR China
2College of Chemistry and Chemical Engineering, Shaoxing University, Shaoxing, PR China

Abstract
A series of spiro-indolin-2-one derivatives were designed and synthesized as p53-MDM2 binding inhibitors. Though p53-MDM2 binding inhibitory and activities against p53 wild-type cell lines of most compounds were not that promising, some obtained structures showed moderate to strong inhibitory activities (IC50 <0.08 µM) against p53 mutant cell lines (SW620), suggesting that these compounds may have different modes of action to p53 pathway, further studies on treatment of p53 mutant tumors are under investigation.

Keywords: MDM2; p53; Spiro-indolinone; Anticancer

Introduction
The spiro-indolin-2-one compounds were synthesized by a series of compounds [1-3] with good p53-MDM2 binding inhibitory activity found in the structure-based design strategy of Wang and his group [4]. From the result of p53-MDM2 complex crystal structure [5], tryptophan residues on the indole ring of p53 is the most critical binding element for p53 binding MDM2 protein, which was buried in the hydrophobic pocket, and indole on the NH and MDM2 formed a hydrogen bond. Wang and his team members used computer-assisted drug screening to find compounds that mimic the indole ring and found that the structural properties of 2-indolinone were most consistent with that of indole. 6-chloro-2-indolinone was identified as a predominant fragment on the basis of previous work on peptide compounds [6,7] and Nutlin (Trp pocket with key chlorine atoms capable of occupying MDM2 protein) [8].

The Spiro cyclic backbone provides the necessary platform for introducing side chains so that the hydrophobic side chains can be inserted into Leu and Phe pockets. The crystal structures of the resulting compounds MI-63 and MDM2 were recently reported by Popowicz et al. [8]. Interestingly, the MI-63 binding pattern is mirrored as previously assumed [9-11]. As previously assumed, 6-chloro-2-indolinone occupies Trp pockets but the combination of neopentyl and substituted phenyl Mode and the previous hypothesis were reversed, suggesting that the conformation of the compound has been reversed, but the two states of MDM2 have a better inhibition. Based on the above studies, we designed a class of spiro-indolin-2-one compounds, in order to improve the class of compounds on MDM2 binding activity, and have a better anti-cancer effect (Figure 1). First, the hydrophobic groups such as methyl group, propionyl group and piperdine carboxyl group were introduced into pyrrole N to study the influence of different length or volume of hydrophobic groups on the activity. Then the effect of chlorine group were introduced into pyrrole N to study the influence of different groups such as methyl group, propionyl group and piperidine carboxyl and have a better anti-cancer effect (Figure 1). First, the hydrophobic groups were substituted phenyl to improve the class of compounds on MDM2 binding activity, but the two states of MDM2 have a better inhibition. Based on the above studies, we designed a class of spiro-indolin-2-one compounds, in order to improve the class of compounds on MDM2 binding activity, and have a better anti-cancer effect (Figure 1).

Chemistry
The synthesis route of the spiro-indolin-2-one compounds was designed via systematic literature (Scheme 1).

Salicylaldehyde or substituted salicylaldehyde(1-2) and ethyl acetocetate formed cyclization in the piperidine-catalyzed system to get acetyl coumarin compounds (6-7), And then reacted with isatin (3-5) and glycine or sarcosine (2 + 3) cyclization to form spiro intermediate (8-13). Wherein the pyrrole 1-methylated product (8-10) is directly reacted with the corresponding nitrogen-containing fragment to form (14-18); the remaining intermediates (11-13) are reacted with triphosgene and the corresponding amine to give the 4-methoxybenzyl protected intermediate (19-24), which is present in the presence of trifluoromethanesulfonic acid deprotection to get 25-30, and finally reacts with the corresponding nitrogen-containing heterocycle and fragment to yield the target product 31-43.

A total of 27 spiro-indolin-2-one compounds were synthesized by the method described above. The structures of the new compounds were confirmed by 1H NMR MS and elemental analysis.

Experiments

Biology

Protein inhibitory test assay: Measurements were performed using an EnVision Multilabel Plate Reader with a 480 nM excitation filter and a 535 nM emission filter. The fluorescence polarization value FP was measured by incubating the test compound (DMSO <4%) at a concentration of 10 μM with the protein (final concentration of 10 nM) -fluorescence substrate (final concentration 1 nM) for 1 h. Simultaneously, DMSO (<4%) with the same dosing volume was incubated with the protein (final concentration of 10 nM) solution and the fluorescent substrate (final concentration of 1 nM), respectively, to test FPmax and FPmin. The inhibition of p53-MDM2 binding at the concentration of 10 μM was calculated according to the following formula:

\[ \text{Inhibition\%}=1-\left(\frac{\text{FP}-\text{FP}_{\text{min}}}{\text{FP}_{\text{max}}-\text{FP}_{\text{min}}}\right) \]

Cell viability assay (SRB assay): 100 μL of cell suspension (A549, HCT116, SW620, and PC3 cells) was added to each well in a 96-well cell culture plate. The plate was incubated in a CO2 incubator for 24
Introduction of hydrophobic groups such as methyl, propionyl, piperidinecarboxyl and other hydrophobic segments, the effects on the activity were investigated.

The nitrogen-containing molecular fragments were introduced to improve the water-solubility of the compounds, and the effects on the activity were also investigated.

The cell inhibition rate was calculated by the following formula:

\[
\text{Inhibitory\%} = \left( \frac{A_{515\text{control cells}} - A_{515\text{treated cells}}}{A_{515\text{control cells}}} \right) \times 100%
\]

(A515: OD under wavelength of 515 nm)

Introduction of chloro and the effects on the activity were investigated.
IC₅₀ was calculated by the Fisher analysis. Assays were performed in triplicate to get the final data and SD values.

Synthesis of 1-(4-methoxybenzyl)indoline-2,3-dione (4)

Isatin (2.0 g, 13 mmol) was dissolved in DMF (20 mL) in an ice bath, NaOH (2.7 g, 66 mmol) was added, after stirring under ice bath for 30 min, 4-methoxybenzylchloride (9.0 mL, 66 mmol) was added, after 3 h in room temperature stirring, water was added to quench the reaction. The obtained red solid was recrystallized with ethyl acetate and petroleum ether, and get a red solid 4. Yield: 67%; M.P.: 167.6 - 168°C (Lit: 8: 119 - 121°C).

Synthesis of 6-chloro-1-(4-methoxybenzyl)indoline-2,3-dione (5)

6-Chloroindole (10.0 g, 55 mmol) was dissolved in DMSO (20 mL) in an ice bath, NaOH (2.7 g, 66 mmol) was added, after stirring under ice bath for 30 min, 4-methoxybenzylchloride (9.0 mL, 66 mmol) was added, after 3 h in room temperature stirring, water was added to quench the reaction. The obtained solid was recrystallized with ethyl acetate and petroleum ether, get a red solid 6-chloro-1-(4-methoxybenzyl) indoline; The obtained solid (5.0 g, 16 mmol) was added with ethyl acetate and petroleum ether, and get a red solid 6-chloro-1-(4-methoxybenzyl)indoline-2,3-dione. The obtained solid was recrystallized by petroleum ether/ethyl acetate to give 6-chloro-1-(4-methoxybenzyl)indoline-2,3-dione with excellent purity (over 99%). Yield: 95%; M.P.: 121 - 123°C (Lit. 8: 119 - 121°C).

Synthesis of 3-acetyl-2H-chromen-2-one (7)

To a refluxing mixture of salicylaldehyde (1.0 g, 14.3 mmol) in water (15 mL) was added NaH (343 mg, 14.3 mmol) was added, after stirring under ice bath for 1.5 h. After completion of the reaction, the bath, then a solution of CrO₃ (4-methoxybenzyl) indoline; The obtained solid (5.0 g, 16 mmol) was added, after 3 h in room temperature stirring, water was added to quench the reaction. The obtained solid was recrystallized by petroleum ether/ethyl acetate to get a red solid 6-chloro-1-(4-methoxybenzyl)indoline-2,3-dione. The obtained solid was recrystallized by petroleum ether/ethyl acetate to give 6-chloro-1-(4-methoxybenzyl)indoline-2,3-dione with excellent purity (over 99%). Yield: 95%; M.P.: 121 - 123°C (Lit: 8: 119 - 121°C).

Synthesis of 3-acetyl-7-chloro-2H-chromen-2-one (7)

Synthesis of 3-acetyl-7-chloro-2H-chromen-2-one was obtained by using 4-chloro-2-hydroxybenzaldehyde instead of salicylaldehyde, according above reaction. The obtained solid was recrystallized by petroleum ether/ethyl acetate to get 7 with excellent purity (over 99%). Yield: 95%; M.P.: 121 - 123°C (Lit: 8: 119 - 121°C).

General method for the synthesis of compounds (8-13)

Corresponding amino acids (2.0 mmol), 6 (or 7) (2.0 mmol) and 3 (or 4, or 5) (2.0 mmol) was refluxing in methanol (30 mL) for 15 hours. After the reaction was done, the mixture was cooled to room temperature; the solid was filtered, and recrystallized in methanol, to get a white solid 8-13.

421[M+H]+. Anal. Calcd for C₁₉H₁₄Cl₂N₂O₃: C, 62.55; H, 5.36; N, 7.24; Found: C, 62.55; H, 5.36; N, 7.24.

3'-[2-hydroxyphenyl]-1'-methyl-4'-(morpholin-4-ylcarbonyl)spiro[indole-3,2'-pyrrolidin]-2(1'H)-one (16): Yield: 85%; M.P.: 120 - 122°C; 1H NMR (500 MHz, CDCl₃) δ 7.44 (s, OH, 1H), 5.76 (d, J = 7.4 Hz, Ar-H, 1H), 7.17 (dd, J = 12.2, 4.4 Hz, Ar-H, 2H), 7.09 (m, Ar-H, 2H), 6.85 (d, J = 8.0 Hz, Ar-H, 1H), 6.71 (dd, J = 9.4, 7.8 Hz, Ar-H, 2H), 4.45 (d, J = 9.4 Hz, CH, 1H), 3.73 (m, CH₂×2, 4H), 3.49 (m, CH₂×2, 4H), 2.17 (s, CH₃, 3H); MS (ESI): 421[M+H]+. Anal. Calcd for C₁₉H₂₅N₃O₄·H₂O·C; 68.55; H, 6.71; N, 13.32; Found: C, 68.57; H, 6.73; N, 13.28.

3'-[2-hydroxyphenyl]-1'-methyl-4'-(morpholin-4-ylcarbonyl)spiro[indole-3,2'-pyrrolidin]-2(1'H)-one (16): Yield: 85%; M.P.: 120 - 122°C; 1H NMR (500 MHz, CDCl₃) δ 7.44 (s, OH, 1H), 5.76 (d, J = 7.4 Hz, Ar-H, 1H), 7.17 (dd, J = 12.2, 4.4 Hz, Ar-H, 2H), 7.09 (m, Ar-H, 2H), 6.85 (d, J = 8.0 Hz, Ar-H, 1H), 6.71 (dd, J = 9.4, 7.8 Hz, Ar-H, 2H), 4.45 (d, J = 9.4 Hz, CH, 1H), 3.73 (m, CH₂×2, 4H), 3.49 (m, CH₂×2, 4H), 2.17 (s, CH₃, 3H); MS (ESI): 408[M+H]+. Anal. Calcd for C₁₉H₂₄ClN₃O₄·H₂O·C; 66.55; H, 6.71; N, 13.32; Found: C, 66.57; H, 6.73; N, 13.28.

General method for the synthesis of compounds 14-18

8-10 (50 mg, 0.16 mmol) was dissolved in THF (6 mL), corresponding amine (0.48 mmol) was added, after refluxing for min, the solvent was removed by rotary evaporation and the residue was extracted by ethyl acetate and water, the organic layer was kept and washed with brine (3x10 mL), dried with sodium sulfate, then removed the solvent, the obtained solid was recrystallized in ethyl acetate/petroleum ether and get a white solid 14-18.
6-chloro-3′-(4-chloro-2-hydroxyphenyl)-1′-methyl-4′-(morpholin-4-ylcarbonyl)spiro[indole-3,2′-pyrrolidin]-2′(1'H)-one (18): Yield: 60%; M.P.: 165 - 168°C; 1H NMR (500 MHz, MeOD) δ 7.42 (m, Ar-H, 1H), 7.31 (s, Ar-H, 1H), 7.08 (t, J=6.4 Hz, Ar-H, 1H), 6.65 (dd, J=10.7, 4.3 Hz, Ar-H, 2H), 6.63 (m, Ar-H, 1H), 4.46 (dd, J=10.3 Hz, CH, 1H), 3.85 (t, J=14.5 Hz, CH, 2H), 3.73 (m, CH, 1H), 3.07 (dd, J=11.3, 6.1 Hz, CH, 2H); MS (ESI): 416[M+H]⁺. Anal. Calcld for C₁₇H₁₅ClN₂O₄: C, 60.83; H, 4.44; N, 10.07; Found: C, 60.61; H, 4.53; N, 10.12.

General method for the synthesis of compound 25 or 28

Compounds 25 or 28 were obtained by two steps. First, the obtained 11-13 (0.47 mmol) were dissolved in methylene chloride (15 mL), then propionyl chloride (0.94 mmol) was added, after stirring till the starting material was finished, the solvent was removed, and the residue was dissolved in methylene chloride (10 mL) again, then triluoromethanesulfonic acid (0.47 mmol) was added dropwise over night in room temperature. After the reaction was done, the solvent was removed, extracted with methylene chloride and water, keep the organic, and washed with brine, dried with sodium sulfate. Remove the solvent and the obtained solid was crystallized with ethyl estate and petroleum ether to get a white solid 25 or 28.

2-propionyl-2,3,3a,9b-tetrahydro-4H-spiro[chromeno[3,4-c]pyrrole-1,3′-indole]-2′(1'H)-dione (25): Yield: 45%; M.P.: > 250°C; 1H NMR (500 MHz, CDCl₃) δ 7.26 (m, Ar-H, 2H), 7.12 (t, J=7.5 Hz, Ar-H, 1H), 7.08 (m, Ar-H, 2H), 6.86 (dd, J=7.5, 1.0 Hz, CH, 1H), 6.74 (d, J=7.7 Hz, Ar-H, 1H), 6.34 (m, Ar-H, 1H), 4.85 (dd, J=10.4 Hz, CH, 1H), 4.18 (dd, J=10.6, 6.9 Hz, CH, 1H), 3.96 (d, J=8.8 Hz, CH, 1H), 3.48 (m, CH, 1H), 2.53 (m, Ar-H, 1H), 2.35 (dd, J=16.6, 7.4 Hz, CH, 1H), 1.08 (dd, J=9.4, 5.4 Hz, CH₃, 3H); MS (ESI): 363[M+H]⁺. Anal. Calcld for C₁₉H₁₇NO₅; C, 69.60; H, 5.33; N, 10.10; Found: C, 69.61; H, 5.59; N, 10.09.

2-(piperidin-1-ylcarbonyl)-2,3,3a,9b-tetrahydro-4H-spiro[chromeno[3,4-c]pyrrole-1,3′-indole]-2′(1'H)-dione (26): Yield: 28%; M.P.: 118 - 120°C; 1H NMR (500 MHz, CDCl₃) δ 7.29 (s, OH, 1H), 7.27 (m, Ar-H, 2H), 7.12 (t, J=7.5 Hz, Ar-H, 1H), 7.06 (d, J=8.2 Hz, Ar-H, 1H), 6.88 (s, Ar-H, 1H), 6.82 (t, J=7.4 Hz, Ar-H, 1H), 6.70 (d, J=7.7 Hz, Ar-H, 1H), 6.24 (d, J=7.3 Hz, Ar-H, 1H), 4.59 (dd, J=10.1, 1.8 Hz, CH, 1H), 4.34 (m, CH, 1H), 3.84 (d, J=9.6 Hz, CH, 1H), 3.51 (t, J=7.9 Hz, CH, 1H), 3.34 (m, CH₂x2, 4H), 1.66 (m, CH₂x3, 6H); MS (ESI): 418[M+H]⁺. Anal. Calcld for C₁₉H₂₁NO₅; C, 69.05; H, 5.55; N, 10.07; Found: C, 69.15; H, 5.59; N, 10.09.

2-(piperidin-1-ylcarbonyl)-2,3,3a,9b-tetrahydro-4H-spiro[chromeno[3,4-c]pyrrole-1,3′-indole]-2′(1'H)-dione (29): Yield: 18%; M.P.: > 250°C; 1H NMR (500 MHz, CDCl₃) δ 7.16 (d, J=6.7 Hz, Ar-H, 1H), 7.04 (t, J=7.5 Hz, Ar-H, 2H), 6.98 (d, J=2.0 Hz, Ar-H, 1H), 6.85 (m, Ar-H, 1H), 6.72 (dd, J=8.0, 2.0 Hz, Ar-H, 1H), 6.63 (d, J=8.0 Hz, 1H), 6.07 (d, J=8.2 Hz, Ar-H, 1H), 4.42 (dd, J=10.3, 1.9 Hz, CH, 1H), 4.21 (dd, J=10.1, 8.4 Hz, CH, 1H), 3.70 (d, J=9.6 Hz, CH, 1H), 3.46 (m, CH, 1H), 3.34 (m, CH₂x2, 4H), 1.66 (m, CH₂x3, 6H); MS (ESI): 452[M+H]⁺. Anal. Calcld for C₁₂H₁₂NO₅; C, 63.79; H, 4.91; N, 9.30; Found: C, 63.89; H, 4.99; N, 9.31.

7-chloro-2-(piperidin-1-ylcarbonyl)-2,3,3a,9b-tetrahydro-4H-spiro[chromeno[3,4-c]pyrrole-1,3′-indole]-2′(1'H)-dione (30): Yield: 30%; M.P.: > 250°C; 1H NMR (500 MHz, CDCl₃) δ 6.18 (d, J=6.7 Hz, Ar-H, 2H), 7.04 (t, J=7.5 Hz, Ar-H, 1H), 6.99 (d, J=2.0 Hz, Ar-H, 1H), 6.85 (s, Ar-H, 1H), 6.72 (dd, J=8.2, 2.0 Hz, Ar-H, 1H), 6.63 (d, J=8.0 Hz, Ar-H, 1H), 6.07 (d, J=8.2 Hz, Ar-H, 1H), 4.48 (dd, J=10.3, 1.9 Hz, CH, 1H), 4.22 (dd, J=10.1, 8.4 Hz, CH, 1H), 3.72 (d, J=9.6 Hz, CH, 1H), 3.46 (m, CH, 1H), 3.28 (m, CH₂x2, 4H), 1.58 (m, CH₂x2, 6H); MS (ESI): 452[M+H]⁺. Anal. Calcld for C₁₂H₁₂ClNO₅; C, 63.79; H, 4.91; N, 9.30; Found: C, 63.81; H, 4.89; N, 9.27.

General method for the synthesis of compounds 31-43

25-30 (0.16 mmol) was dissolved in THF (6 mL), corresponding amine (0.48 mmol) was added, after refluxing for min, the solvent was removed by rotary evaporation and the residue was extracted by ethyl estate and water, the organic layer was kept and washed with brine (3x10 mL), dried with sodium sulfate, then removed the solvent, the obtained solid was recrystallized in ethyl estate/petroleum ether and get a white solid 31-43.

3′-(2-hydroxyphenyl)-2-oxo-1′-propionyl-1,2-dihydropyridino[3,2′-pyrrolidin]-4′-carboxamide (31): Yield: 85%; M.P.: 124 - 126°C; 1H NMR (500 MHz, CDCl₃) δ 7.24 (s, NH, 2H), 7.12 (t, J=7.6 Hz, Ar-H, 1H), 7.18 (m, Ar-H, 2H), 6.86 (m, Ar-H, 4H), 6.74 (d, J=7.7 Hz, Ar-H, 1H), 6.36 (m, Ar-H, 1H), 4.81 (dd, J=10.4 Hz, 1.9 Hz, 1H).
Hz, CH, 1H), 4.18 (dd, J=10.6, 6.9 Hz, CH, 1H), 3.96 (d, J=8.8 Hz, CH, 1H), 3.48 (m, CH, 1H), 2.51 (dd, J=16.6, 7.4 Hz, CH, 1H), 2.33 (dd, J=16.6, 7.4 Hz, CH, 1H), 1.08 (dd, J=9.4, 5.4 Hz, CH, 3H); MS (ESI): 380[M+H]+. Anal. Calcd for C_{19}H_{23}N_{3}O_{2}: C, 66.48; H, 5.58; N, 11.08; Found: C, 66.44; H, 5.56; N, 11.18.

3'- (2-hydroxyphenyl)-4'- (methyl-4'-carbonyl)-1'- propionylspiro[indole-3,2'-pyrrolidin]-2'(1H)-one (32): Yield: 78%; M.P.: 131 - 133°C; 'H NMR (500 MHz, CDCl3) δ 7.26 (m, Ar-H, 2H), 7.12 (t, J=7.5 Hz, Ar-H, 1H), 7.08 (m, Ar-H, 2H), 6.86 (td, J=7.5, 1.0 Hz, Ar-H, 1H), 6.74 (d, J=7.7 Hz, Ar-H, 1H), 6.34 (m, Ar-H, 1H), 4.85 (d, J=10.4 Hz, CH, 1H), 4.18 (dd, J=10.6, 6.9 Hz, CH, 1H), 3.70 (m, CH3×3, 6H), 3.53 (m, CH2×2, 4H), 2.53 (dd, J=16.6, 7.4 Hz, CH, 1H), 2.35 (dd, J=16.6, 7.4 Hz, CH, 1H), 1.08 (dd, J=9.4, 5.4 Hz, CH, 3H); MS (ESI): 450[M+H]+. Anal. Calcd for C_{26}H_{30}N_{4}O_{5}: C, 65.84; H, 6.55; N, 11.37; Found: C, 65.86; H, 6.59; N, 11.33.

6-chloro-3'- (2-hydroxyphenyl)-2'-oxo-1'-propionyl-1,2- dihydrospiro[indole-3,2'-pyrrolidin]-4'carbonamide (38): Yield: 89%; M.P.: 124 - 126°C; 'H NMR (500 MHz, CDCl3) δ 7.48 (d, J=8.0 Hz, Ar-H, 1H), 7.30 (m, Ar-H, 2H), 7.00 (dd, J=8.3, 2.1 Hz, Ar-H, 2H), 6.76 (d, J=1.8 Hz, Ar-H, 1H), 6.31 (d, J=8.3 Hz, Ar-H, 1H), 4.15 (m, CH, 1H), 3.98 (d, J=10.9 Hz, CH, 1H), 3.89 (s, CH, 1H), 3.79 (t, J=9.2 Hz, CH, 1H), 3.46 (dd, J=9.3, 4.4 Hz, CH, 1H), 3.17 (d, J=5.0 Hz, CH, 1H), 2.53 (dd, J=16.6, 7.4 Hz, CH, 1H), 2.35 (dd, J=16.6, 7.4 Hz, CH, 1H), 1.13 (t, J=7.5 Hz, CH3, 3H); MS (ESI): 414[M+H]+. Anal. Calcd for C_{19}H_{13}ClN_{3}O_{2}, C, 60.95; H, 4.87; N, 10.15; Found: C, 60.85; H, 4.90; N, 10.21.

6-chloro-3'- (2-hydroxyphenyl)-2'-oxo-1'-propionyl-1,2- dihydrospiro[indole-3,2'-pyrrolidin]-4'carbonamide (34): Yield: 70%; M.P.: 154 - 156°C; 'H NMR (500 MHz, CDCl3) δ 8.07 (s, OH, 1H), 7.36 (d, J=7.3 Hz, Ar-H, 1H), 7.08 (dd, J=15.3, 7.7 Hz, Ar-H, 2H), 7.00 (t, J=7.7 Hz, Ar-H, 2H), 6.68 (dd, J=19.5, 7.8 Hz, Ar-H, 2H), 6.56 (d, J=7.6 Hz, Ar-H, 1H), 4.78 (dd, J=19.8, 9.0 Hz, CH, 1H), 4.45 (d, J=11.9 Hz, CH, 1H), 4.00 (dd, J=17.5, 8.9 Hz, CH, 2H), 3.70 (ddd, J=15.5, 8.8, 5.2 Hz, CH, 4H), 3.59 (dd, J=10.2, 5.1 Hz, CH, 2H), 3.42 (m, CH, 2H), 3.33 (m, CH, 4H), 2.91 (m, CH, 2H), 1.66 (m, CH, 4H); MS (ESI): 505[M+H]+. Anal. Calcd for C_{28}H_{25}N_{4}O_{4}C_{6}, 66.34; H, 6.03; N, 12.89; Found: C, 66.42; H, 6.09; N, 12.87.

3'- (2-hydroxyphenyl)-4'- (morpholin-4-ylcarbonyl)-1'- (piperidin-1-ylcarbonyl)spiro[indole-3,2'-pyrrolidin]-2'(1H)-one (33): Yield: 85%; M.P.: 195 - 198°C; 'H NMR (500 MHz, MeOD) δ 7.45 (d, J=7.1 Hz, Ar-H, 1H), 6.98 (dd, J=7.9, 6.9 Hz, Ar-H, 2H), 6.82 (t, J=7.0 Hz, Ar-H, 1H), 6.73 (d, J=7.6 Hz, Ar-H, 1H), 6.48 (m, Ar-H, 2H), 5.94 (d, J=7.4 Hz, Ar-H, 1H), 4.62 (t, J=10.0 Hz, CH, 1H), 4.43 (dd, J=17.9, 9.8 Hz, CH, 1H), 4.36 (d, J=8.0 Hz, CH, 1H), 3.91 (t, J=9.7 Hz, CH, 1H), 3.40 (m, CH2, 2H), 3.30 (m, CH2, 1H), 1.58 (d, J=5.4 Hz, CH, 6H); MS (ESI): 435[M+H]+. Anal. Calcd for C_{24}H_{26}N_{3}O_{4}: C, 66.80; H, 6.05; N, 9.35; Found: C, 66.82; H, 6.07; N, 9.25.

N,N'-diethyl-3'- (2-hydroxyphenyl)-4'- (methyl-4'- ycarbonyl)-2'-oxo-1,2-dihydro-1'H-spiro[indole-3,2'-pyrrolidin] -1'-carboxamide (37): Yield: 60%; M.P.: 159 - 166°C; 'H NMR (500 MHz, CDCl3) δ 6.37 (t, J=7.0 Hz, Ar-H, 1H), 7.16 (m, Ar-H, 2H), 7.03 (dd, J=13.2, 7.2 Hz, Ar-H, 2H), 6.77 (m, Ar-H, 2H), 6.60 (d, J=7.7 Hz, Ar-H, 1H), 4.74 (d, J=10.0 Hz, CH, 1H), 4.46 (dd, J=11.8, 5.2 Hz, CH, 1H), 3.99 (dd, J=17.3, 9.0 Hz, CH, 2H), 3.76 (m, CH3×3, 6H), 3.48 (m, CH3×3, 6H), 3.16 (dd, J=14.3, 7.2 Hz, CH2, 2H), 1.12 (t, J=7.1 Hz, CH2×2, 6H); MS (ESI): 493[M+H]+. Anal. Calcd for C_{19}H_{25}N_{3}O_{4}, C, 65.84; H, 6.55; N, 11.37; Found: C, 65.86; H, 6.59; N, 11.33.

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(m, CH2×3, 6H); MS (ESI): 469[M+H]+. Anal. Calcd for C28H31ClN4O5:
C, 62.39; H, 5.80; N, 10.39; Found: C, 62.41; H, 5.77; N, 10.43.

3'-[4-chloro-2-hydroxyphenyl]-4'-[(4-methylpiperazin-1-yl)
carbonyl]-1'-(piperidin-1-ylcarbonyl)spiro[indole-3,2'-pyrrolidin]-
2(1H)-one (43): Yield: 68%; M.P.: 209 - 211°C; 1H NMR (500 MHz,
CDCl3) δ 7.27 (m, Ar-H, 2H), 7.01 (m, Ar-H, 2H), 6.75 (m, Ar-H, 1H),
6.60 (d, J=7.7 Hz, Ar-H, 1H), 6.54 (s, Ar-H, 1H), 4.66 (s, CH, 1H), 4.55
(d, J=12.1 Hz, CH, 1H), 3.96 (dd, J=12.4, 8.9 Hz, CH, 2H), 3.63 (m, CH,
2H), 3.24 (dd, J=28.4, 9.4 Hz, CH, 4H), 2.23 (s, CH3, 3H), 2.17 (m, CH,
4H), 1.54 (d, J=39.5 Hz, CH, 8H); MS (ESI): 552[M+H]+. Anal. Calcd
for C29H34ClN5O4: C, 63.09; H, 6.21; N, 12.69; Found: C, 63.13; H,
6.25; N, 12.72.

Results and Discussion

Based on the inhibition data of p53-MDM2 binding and the
in vitro antitumor activity of the compounds in Table 1, the following
conclusions can be drawn:

Some of the compounds showed moderate to strong inhibition of
p53-MDM2 binding. The activity of lactone ring - opening compounds
(14-18, 31-43) was significantly higher than that of lactone compounds
(9-10, 25-30). Among them, the inhibitory activity of the indole-
6-phenylcyclopropene derivatives (16-18, 38-43) on p53-MDM2
binding was not superior to the non-chlorinated derivatives (14-15,
31-37). From the tumor cell proliferation inhibitory activity point of
view, the introductions of chlorine atoms help to improve activity. The
activity of the N-piperidinecarboxyl-substituted compounds (40-43)
on the pyrrole ring is generally higher than that of the N-propionyl-
substituted compounds (40-43) in the presence of the chlorine atom,
Compounds (38-39) and N-methyl substituted compounds (16-18).
The obtained compounds showed weak inhibitory activity against wild-
type p53-expressing cell lines HCT116 and A549, as well as that against
p53-deficient PC3 cell lines, and some of the compounds exhibited
excellent effects on the p53 mutant SW620 cell line (28, 42, IC50 < 0.08
μM), suggesting that the action of these compounds may be related to

Table 1: The inhibitory of p53-MDM2 and anti-cancer activities of spiro-indolinone derivatives.
other targets on the p53 pathway, which is worthy of further study on the treatment of p53 mutant tumors.

Conclusions

A series of spiro-indolin-2-one compounds have been designed and synthesized based on the known spiro-indolin-2-ones and MDM2 proteins as p53-MDM2 binding inhibitors, 27 novel molecules were synthesized and their structures were confirmed by 1H NMR, MS and elementary analysis. The inhibitory activity of p53-MDM2 binding assay showed that some of the compounds had moderate inhibitory activity but showed weaker activity on wild-type p53 tumor cell lines. These compounds have potent inhibitory activity on p53 mutant cell line SW620 and may be involved in other p53-related signalling pathways or activation of p53 function, which is worth further study.

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