HETEROCYCLES, Vol. 104, No. 7, 2022, pp. -. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 23rd March, 2022, Accepted, 18th April, 2022, Published online, 22nd April, 2022
DOI: 10.3987/COM-22-14658

EFFICIENT SYNTHESIS OF NOVEL SPIRO[INDOLINE-3,5'-PYRANO-[2,3-*d*]PYRIMIDIN]-2-ONE DERIVATIVES AND ANTITUMOR ACTIVITY EVALUATION

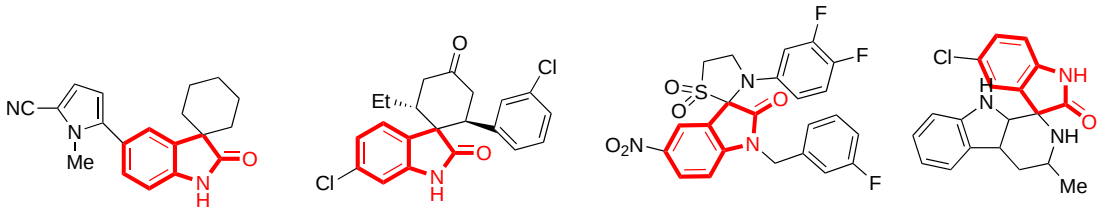
Zhenhua Li,* Guoqing Huang, Dayou Rong, Yingyan Cao, and Ronghui Hu

College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, P.R. China; E-Mail: lizhenhua@zjut.edu.cn

Abstract – An efficient method for synthesis of the spiro-[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one derivatives from 2'-amino-2-oxo-spiro[indoline-3,4'-pyran]-3'-carbonitriles using bis(trichloromethyl) carbonate (BTC) and triphenylphosphine oxide (TPPO) was developed. A series of target compounds with broad substrate scope were synthesized in moderate to good yields. In addition, the antitumor activities against four cancer cell lines A549, HepG-2, MCF-7, and HeLa were evaluated using 5-FU and cisplatin as reference, all compounds showed good antitumor activity compared with the standard drugs.

INTRODUCTION

In recent decades, spirocyclic compounds have attracted great interest in medicinal chemistry due to their numerous biological activities attributed primarily to their versatility and structural similarity of important pharmacophore centers.[1] Considering the frequent occurrence of spirocyclic motifs in natural products and the unrivalled success in drug discovery,[2] spiro centers were introduced into the novel compounds, making them more natural like, which were expected to increase the possibility of discovering new drug lead compounds.[3] It was worth mentioning that spirooxindoles had various biological activities,[4] such as anti-HIV,[5] anticancer,[6] anti-tuberculosis,[7] antimalarial[8] and MDM2 inhibitors,[9] which allowed them becoming the focus of modern organic, medicinal and natural product chemistry (Figure 1).



progesterone receptor agonist | MDM2-p53 interction inhibitor | *M. tuberculosis* inhibitor | antimalarial

Figure 1. Structures of 2-oxindoles derivatives with biological effects

Incidentally, pyrimidines were a class of *N*-heterocyclics widely found in biological molecules and commercial drugs (Amuvatinib,[10] Gefitinib,[11] Erlotinib,[12] et al.). Among them, pyrano[2,3–*d*]pyrimidines were dominant structural motif in natural products, including alkaloids, carbohydrates, pheromones and iridoids,[13] which exhibited miscellaneous biological activities such as anticancer,[14] antimicrobial[15] and antioxidant activities (Figure 2).[16]

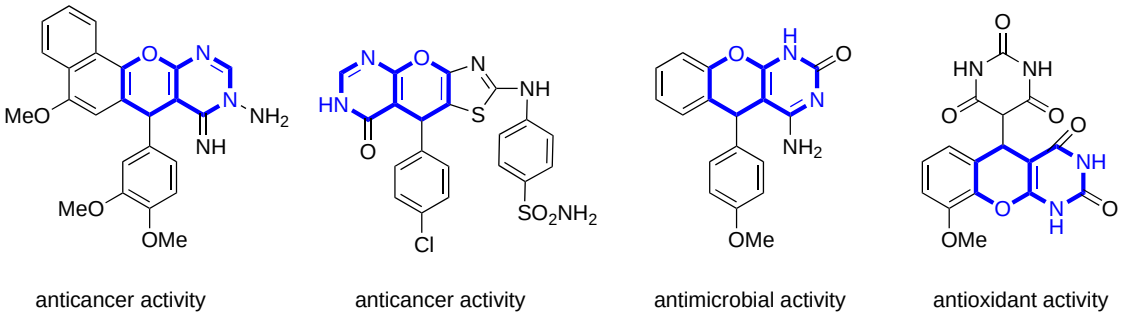
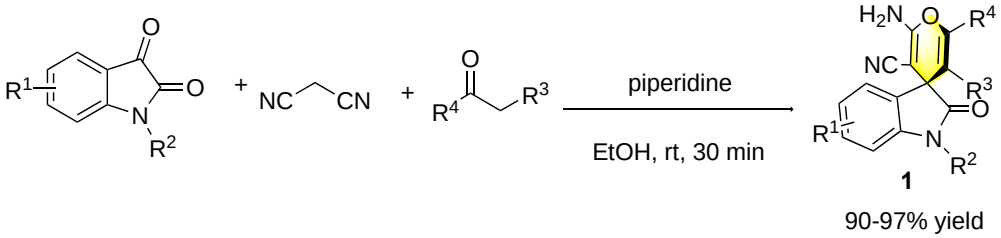


Figure 2. Structures of pyrano[2,3−*d*]pyrimidines derivatives with biological effects

Molecular hybridization strategy indicated that a single structural motif embedded in two or more bioactive skeletons exhibited enhanced biological activity. On this basis, we continued preliminary study[17] and synthesized a series of spirooxindoles fused pyrano[2,3-*d*]pyrimidine derivatives for further screening of antitumor activity. Starting from isatins, a series of substrates were synthesized by one-pot method. Subsequently, a direct cyclization of 2'-amino-2-oxyspiro[indoline-3,4-pyran]-3'-carbonitrile into spiro[indoline-3,5'-pyran[2,3-*d*]pyrimidin]-2-one mediated by bis(trichloromethyl) carbonate (BTC) and triphenylphosphine oxide (TPPO) was developed, which was an efficient protocol for the synthesis of pyrano[2,3-*d*]pyrimidines with spirooxindole. This new type of spirocyclic derivatives are expected to serve as a bridge between chemistry and medicine, providing a class of potential alternatives for antitumor drugs in the future.

RESULTS AND DISCUSSION

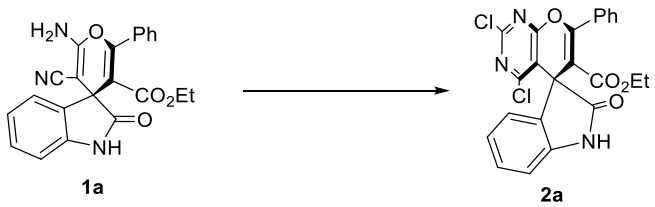
Initially, 2'-amino-2-oxo[indole-3,4'-pyran]-3'-carbonitrile derivatives were synthesized by isatin, malononitrile, 1,3-dicarbonyl compound or *α*-cyanoketone as raw materials in anhydrous ethanol using piperidine as catalyst in a three-component reaction system (Scheme 1).



Scheme 1. One-pot synthesis of 2'-amino-2-oxospiro[indoline-3,4'-pyran]-3'-carbonitriles

Next, we commenced our study by selecting **1a** as the model substrates. Based on the study of BTC/TPPO reaction in previous work,[17] the initial molar ratio of BTC/TPPO was determined to be 1:3. Intriguingly, the reaction proceeded smoothly and the desired product **2a** was obtained in 50% yield (Table 1, entry 1). In addition, the yield of **2a** was significant increased to 81% when the temperature increased from 90 ℃ to 110 ℃ (Table 1, entry 2). Perhaps due to the increase of active sites and the aggravation of side reactions caused by the continuous increase of temperature, the yield of **2a** was decreased (Table 1, entries 3-4). Prolonging the reaction time, the substrate was also more likely to be converted into byproducts, resulting in a bit decrease in the yield of **2a**, which could be found that the suitable time was 3 h (Table 1, entries 2, 5 and 6). What's more, continuously changing the amount of BTC/TPPO was not beneficial to increase yield (Table 1, entries 7-8). Taking into account the non-negligible solvent effect, different solvents were investigated. The desired product was obtained with 61% in toluene, while other solvents were ineffective. (Table 1, entries 9-11).

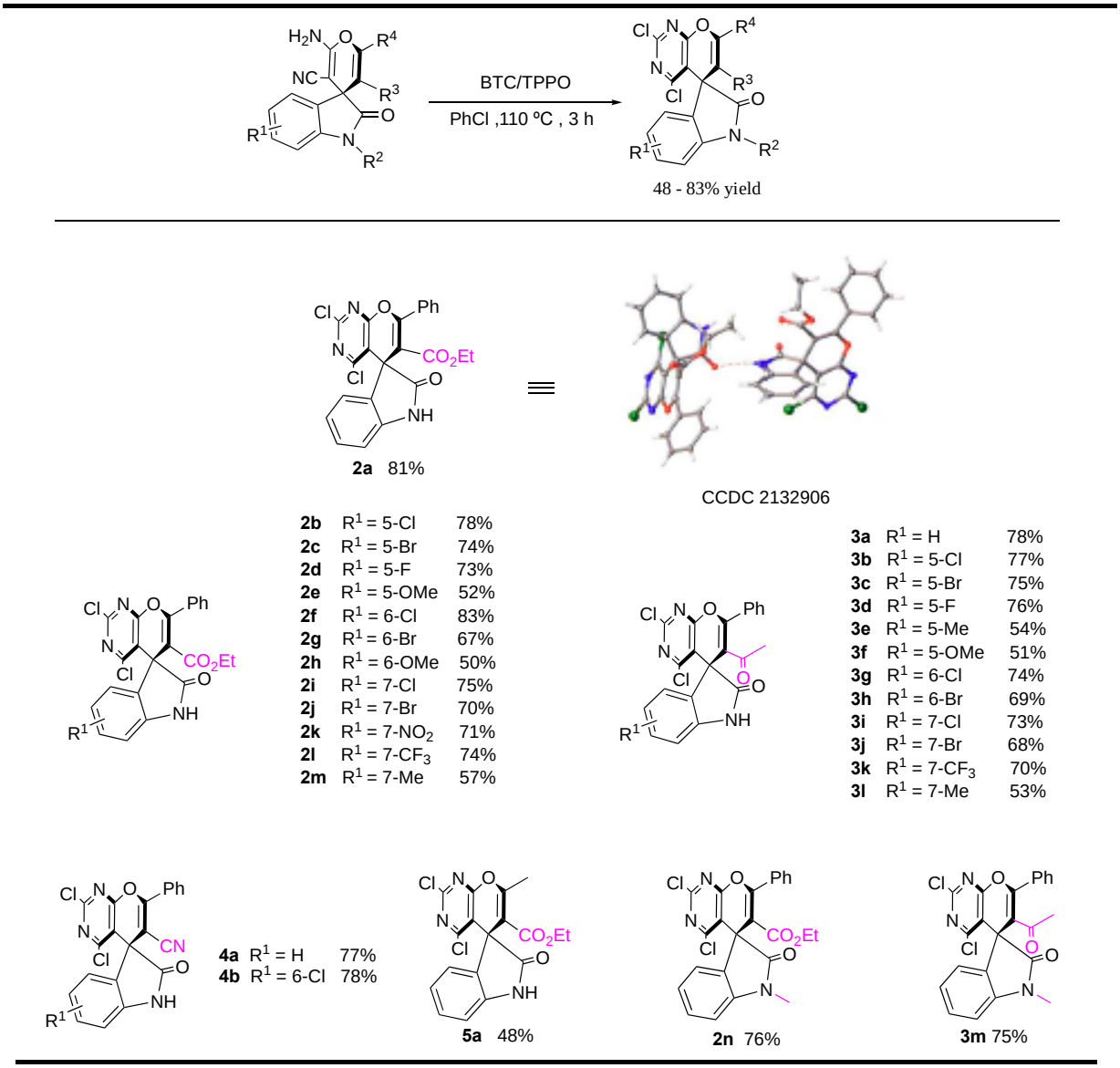
Table 1. Optimization of Reaction Conditions [a]



Entry	equiv (1a:BTC: TPPO)	solvent	T (°C)	t (h)	Yield [b](%)
1	1:1:3	PhCl	90	3	50
2	1:1:3	PhCl	110	3	81
3	1:1:3	PhCl	120	3	78
4	1:1:3	PhCl	140	3	74
5	1:1:3	PhCl	110	4	75
6	1:1:3	PhCl	110	2	60
7	1:2:3	PhCl	110	3	79
8	1:1:4	PhCl	110	3	74
9	1:1:3	toluene	110	3	61
10	1:1:3	THF	110	3	trace
11	1:1:3	MeCN	110	3	trace

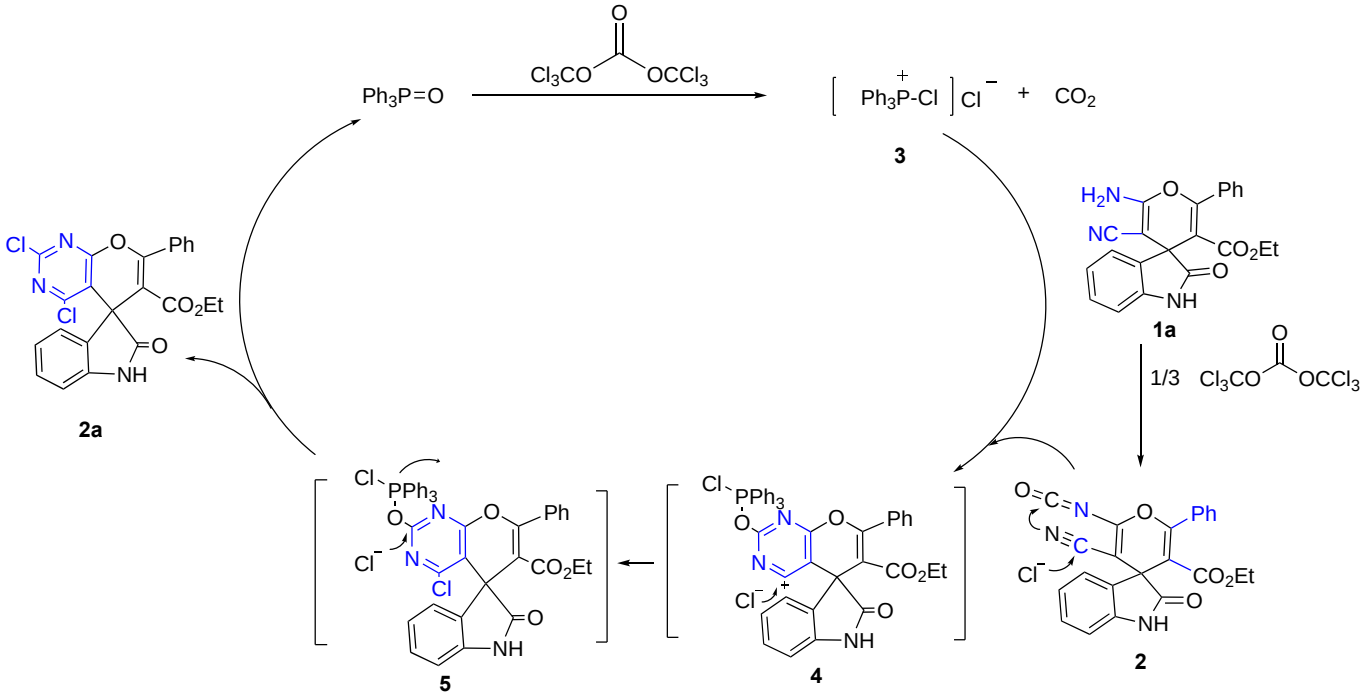
[a] Reaction conditions unless specified otherwise: BTC and TPPO in solvent were premixed under an ice bath, and the mixture was stirred at room temperature for 30 min. Then, **1a** was added, and the mixture was heated to the specified temperature. [b] Isolated yield based on **1a**.

With optimal conditions established, the substrate scope was investigated (Scheme 2). Electron-withdrawing groups showed negligible influence on the reaction efficiency, affording the corresponding pyrano[2,3−*d*]pyrimidines derivatives in 70%–78% yields, such as halogen and trifluoromethyl groups (**2b-2d, 2i-2l**). Particularly, the yield of 6-chlorine was 83% (**2f**). Unfortunately, electron-donating groups, such as methyl and methoxy, showed poor tolerability for product formation (**2e, 2h, 2m, 3e, 3f, and 3l**). In addition, The substituents at the C-5 position of pyran ring, such as ester, carbonyl and cyano group were well-tolerated (**2a, 3a, 4a**). It should be noted that benzene ring at the C-6 position of the pyran ring affected significantly the reaction process, the lower yield using -Me instead of –Ph may be due to the weakening of conjugate effect (**5a**). Furthermore, N-Me of substrates also gained desired products with satisfied yields (**2n, 3m**).



Scheme 2. Substrate scope of spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one derivatives

On the basis of the previous literatures,[17,18] a plausible mechanism was been proposed (**Scheme 3**). Firstly, after the mixture of BTC and TPPO, the nucleophilic attacked from TPPO to BTC to generate dichlorotriphenylphosphine salt **3**. After the substrate addition, the amino-group of **1a** was reacted with BTC to form an intermediate **2**. Then, with the temperature progressing. The closure of the pyrimidine ring was triggered by nucleophilic attack of chlorine on the cyano group. Intermediate **2** was captured by the dichlorotriphenylphosphine salt **3** to form an O-P bond, giving Intermediate **4**. Finally, the first chlorination occurred at the C-4 position, and the oxygen atom was replaced by a chlorine anion to obtain the product **2a** through the S_NAr mechanism.



Scheme 3. Plausible mechanism

The biological activities of some spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one derivatives were investigated, and the results showed that these compounds exhibited cytotoxicity against A549, Hep-G2, MCF-7 and HeLa cell lines by using cisplatin and 5-FU as reference. As indicated in Table 2, generally, the synthesized compounds had a broad-spectrum of anticancer activity, showing favorable inhibition against the tested cancer cell lines than reference drug. The activities are varied due to the variation in the substituent of the aromatic ring. From the comparison of compounds **2f** and **2g**, it is clear that bromo substitution on aryl ring is more active than chloro substitution as shown in Table 2. In the case of the 6-chloro substitution compounds **2f** and **4b** the IC_{50} values show that compound **4b** is the more active, which also reveals that cyano substitution on pyran ring induces more activity than ester substitution. Specifically, compound **2b** exhibited highest cytotoxic activity against MCF-7 with IC_{50} = 11.78 μM, compound **4b** showed the best inhibition activities against A549 (IC_{50} = 10.84 μM), Hep-G2 (IC_{50} = 7.43

μM) and HeLa (IC_{50} = 10.86 μM). The pyrimidine moiety located in the spirooxindole skeleton is beneficial for the activity. The new chemical entities as promising anticancer agents.

In summary, an efficient method for synthesis the of spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one from 2'-amino-2-oxospiro[indoline-3,4'-pyran]-3'-carbonitrile derivatives using BTC/TPPO was developed. A new class of compounds with good functional group tolerance were synthesized efficiently and easily with moderate to good yields. The novel spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one derivatives were evaluated for their in vitro anticancer activity against human cancer cell line (A549, HepG-2, MCF-7 and HeLa). Some of these compounds exhibited significant anticancer activities. Further bioassay, optimization and structure–activity relationships of the title compounds were underway.

Table 2. In vitro cytotoxicity of spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one derivatives

Compound	[a]IC_{50} (μM) ± SD			
	A549	HepG-2	MCF-7	HeLa
2b	16.68 ± 0.42	10.93 ± 0.58	**11.78 ± 1.15**	11.68 ± 0.17
2c	15.89 ± 1.95	16.95 ± 0.26	20.92 ± 1.90	18.59 ± 0.88
2d	13.22 ± 0.26	11.53 ± 0.73	17.73 ± 1.13	13.51 ± 0.36
2e	18.71 ± 0.61	11.32 ± 0.52	20.55 ± 0.29	21.91 ± 1.19
2f	19.31 ± 0.95	11.49 ± 0.21	17.41 ± 0.17	13.71 ± 0.95
2g	12.35 ± 0.21	8.64 ± 0.64	12.98 ± 0.29	11.70 ± 0.49
2i	14.52 ± 0.44	14.12 ± 0.33	17.50 ± 0.85	15.91 ± 0.29
2k	17.56 ± 1.66	17.73 ± 0.39	19.28 ± 1.15	16.20 ± 0.65
2l	13.68 ± 0.75	14.23 ± 1.21	15.56 ± 1.38	13.54 ± 0.88
3d	18.04 ± 0.48	12.92 ± 0.15	18.05 ± 0.20	20.77 ± 0.03
3g	19.77 ± 1.90	16.63 ± 0.43	27.20 ± 1.58	26.42 ± 1.23
3h	20.64 ± 0.08	13.51 ± 0.42	21.52 ± 0.06	24.43 ± 1.33
3i	13.96 ± 0.81	14.75 ± 1.68	18.66 ± 0.78	19.31 ± 1.35
3j	14.69 ± 0.45	10.21 ± 0.35	15.41 ± 2.38	13.97 ± 1.55
3k	16.31 ± 0.61	11.99 ± 0.23	17.56 ± 0.12	18.66 ± 0.70
3l	20.71 ± 1.06	14.12 ± 0.12	22.66 ± 1.49	23.28 ± 0.76
4b	**10.84 ± 0.389**	**7.43 ± 0.38**	14.26 ± 0.46	**10.86 ± 0.43**
5-FU	196.82 ± 14.75	121.92 ± 2.98	177.87 ± 10.61	136.04 ± 0.20
cisplatin	64.23 ± 4.17	60.08 ± 2.85	77.85 ± 1.21	55.97±0.35

[a]IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control

EXPERIMENTAL

Synthetic reagents are commercially available and can be used without further purification. Analytical TLC (thin-layer chromatography) was performed with 0.25 mm silica gel G with a 254 nm fluorescent indicator. Melting points (mp) were obtained on a digital melting point apparatus WRS-1B and uncorrected. NMR spectra were recorded with a Brüker 400 and 600 MHz spectrometer for ^{1}H NMR, 101 and 151 MHz for ^{13}C NMR, and 565 MHz for ^{19}F NMR. TMS was used as an internal standard for ^{1}H and ^{13}C NMR, ^{1}H, ^{13}C and ^{19}F. The followed abbreviations are used to describe peak patterns where

appropriate: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple, dd = oublet of doublet. Coupling constants (*J*) are reported in Hertz (Hz). High-resolution mass spectra were measured with HRMS-ESI-Q-TOF. Infrared (IR) data were recorded as films on potassium bromide plates on a NICOLET iS50 FT-IR spectrometer. Purification of products was accomplished by column chromatography (*n*-hexane/EtOAc =5:1) on silica gel.

General Procedure for the Synthesis of 2'-amino-2-oxospiro[indoline-3,4'-pyran]-3'-carbonitrile (1)

To a stirred mixture of isatin (147 mg, 1 mmol) and malononitrile (66 mg, 1 mmol) in 10 mL anhydrous EtOH, a catalytic amount of piperidine (8 mg, 10 mol%) and ethyl benzoylacetate (130 mg, 1 mmol) were added. The reaction mixture was stirred at room temperature for about 1 h. After complete conversion as indicated from the color change and from thin-layer chromatography, the precipitated solid was filtered and washed with EtOH to furnish analytically pure product.

General Procedure for the Synthesis of 2',4'-dichlorospiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (2)

After complete addition, the mixture was stirred for 30 min at room temperature. Then ethyl 2'-amino-3'-cyano-2-oxo-6'-phenylspiro[indoline-3,4’-pyran]-5'-carboxylate **1a** (387 mg, 1 mmol) was added, and the mixture was heated to 110 °C until completion of reaction (followed by TLC, *n*-hexane/EtOAc = 5:1). After cooling down, the mixture was then poured into the ice water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified over column chromatography (*n*-hexane/EtOAc = 5:1) to afford the pure produce 2',4'-dichlorospiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one. Triphenylphosphine oxide was recovered by column chromatography (*n*-hexane/EtOAc = 1:1).

Ethyl 2',4'-dichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2a):

White solid, 81% yield, mp 170 - 172 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.56 (d, *J* = 6.8 Hz, 2H), 7.49 – 7.37 (m, 3H), 7.33 – 7.26 (m, 1H), 7.13 (d, *J* = 7.4 Hz, 1H), 7.08 – 7.01 (m, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 3.81 – 3.60 (m, 2H), 0.68 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.27, 164.84, 164.65, 162.22, 159.13, 153.92, 141.97, 131.81, 130.95, 130.93, 130.28, 128.38, 128.32, 124.98, 123.51, 111.68, 110.38, 108.79, 61.52, 51.32, 13.09. IR (KBr): V_{max} = 3187, 1728, 1712, 1663, 1566, 1524, 1473, 1367, 1308, 1263, 1199, 1096, 1081, 1033, 957, 928, 854, 786, 754, 697 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{15}Cl_2N_3O_4$ $[M + H]^+$ 468.0508, found 468.0512.

Ethyl 2',4',5-trichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2b):

White solid, 78% yield, mp 225 - 227 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.55 (dd, *J* = 7.0, 1.6 Hz, 2H), 7.51 – 7.45 (m, 1H), 7.42 (dd, *J* = 8.2, 6.8 Hz, 2H), 7.27 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.11 (d, *J* = 2.0 Hz, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 3.82 – 3.64 (m, 2H), 0.70 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.09, 164.80, 164.51, 162.15, 159.46, 154.62, 140.64, 132.42, 131.69, 131.11, 130.34, 128.80, 128.44, 128.40, 125.28, 111.42, 111.09, 108.13, 61.68, 51.40, 13.08. IR (KBr): V_{max} = 3258, 1740, 1717, 1665, 1571, 1526, 1476, 1445, 1388, 1367, 1310, 1265, 1195, 1140, 1086, 1020, 959, 855, 834, 786, 751, 697 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}Cl_3N_3O_4$ $[M + H]^+$ 502.0033, found 502.0031.

Ethyl 5-bromo-2',4'-dichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2c) :

White solid, 74% yield, mp 239 - 241 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.55 (dd, *J* = 7.2, 1.8 Hz, 2H), 7.51 – 7.46 (m, 1H), 7.45 – 7.40 (m, 3H), 7.24 (d, *J* = 1.8 Hz, 1H). 6.82 (d, *J* = 8.2 Hz, 1H), 3.83 – 3.63 (m, 2H), 0.70 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.89, 164.79, 164.50, 162.13, 159.47, 154.64, 141.11, 133.21, 132.77, 131.69, 131.10, 128.43, 128.40, 128.03, 115.88, 111.84, 111.09, 108.13, 61.66, 51.31, 13.08. IR (KBr): V_{max} = 3338, 1982, 1741, 1697, 1647, 1567, 1522, 1474, 1438, 1386, 1368, 1243, 1221, 1190, 1020, 1000, 956, 934, 885, 853, 784, 738, 698, 654 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}BrCl_2N_3O_4$ $[M + H]^+$ 545.9532, found 545.9528.

Ethyl 2',4'-dichloro-5-fluoro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2d) :

White solid, 73% yield, mp 220 - 222 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.55 (dd, *J* = 7.0, 1.6 Hz, 2H), 7.48 (td, *J* = 7.2, 1.4 Hz, 1H), 7.42 (dd, *J* = 8.2, 6.8 Hz, 2H), 7.04 – 6.98 (m, 1H), 6.92 – 6.84 (m, 2H), 3.78 – 3.67 (m, 2H), 0.70 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.22, 164.75, 164.54, 162.17, 159.63 (d, *J* = 243 Hz), 159.43, 154.44, 138.09 (d, *J* = 2.2 Hz), 132.17 (d, *J* = 7.5 Hz), 131.70, 131.06, 128.42, 128.38, 116.84 (d, *J* = 23.3 Hz), 112.89 (d, *J* = 25.0 Hz), 111.16, 111.08 (d, *J* = 7.8 Hz), 108.26, 61.62, 58.45, 13.08. ^{19}F NMR (565 MHz, $CDCl_3$) δ -118.51. IR (KBr): V_{max} = 3328, 2981, 1724, 1662, 1629, 1569, 1487, 1456, 1386, 1366, 1308, 1265, 1222, 1187, 1094, 1079, 1021, 956, 939, 855, 804, 769, 699, 648 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}Cl_2FN_3O_4$ $[M + H]^+$ 486.0332, found 486.0326.

Ethyl 2',4'-dichloro-5-methoxy-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2e) :

Yellow solid, 52% yield, mp 210 - 212 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.48 – 7.42 (m, 1H), 7.42 – 7.37 (m, 2H), 6.86 – 6.76 (m, 2H), 6.70 (d, *J* = 2.2 Hz, 1H), 3.78 (m,

2H), 3.73 (s, 3H), 0.71 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.21, 164.73, 164.61, 162.27, 159.11, 156.52, 153.93, 135.41, 132.08, 131.82, 130.92, 128.39, 128.37, 114.97, 111.78, 111.73, 110.88, 108.79, 61.50, 58.40, 55.85, 13.11. IR (KBr): V_{max} = 3419, 2935, 1720, 1663, 1608, 1569, 1524, 1491, 1439, 1386, 1347, 1306, 1267, 1204, 1080, 1032, 957, 855, 794, 699 cm^{-1}.HRMS (ESI): calcd for $C_{24}H_{17}Cl_2N_3O_5$ $[M + H]^+$ 498.0564, found 498.0561.

Ethyl 2',4',6-trichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2f) :

White solid, 83% yield, mp 218 - 220 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 7.54 (dd, *J* = 7.0, 1.8 Hz, 2H), 7.50 – 7.46 (m, 1H), 7.42 (dd, *J* = 8.2, 6.6 Hz, 2H), 7.05 (d, *J* = 2.4 Hz, 2H), 6.95 (d, *J* = 1.6 Hz, 1H), 3.82 – 3.65 (m, 2H), 0.70 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.13, 164.90, 164.52, 162.14, 159.38, 154.61, 143.07, 136.10, 131.72, 131.09, 129.31, 128.43, 128.39, 125.81, 123.51, 111.19, 111.04, 108.20, 61.69, 50.87, 13.09. IR (KBr): V_{max} = 3313, 2969, 7939, 1705, 1633, 1570, 1525, 1480, 1446, 1389, 1368, 1302, 1237, 1222, 1180, 1135, 1082, 1032, 960, 929, 856, 814, 751, 738, 687 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}Cl_3N_3O_4$ $[M + H]^+$ 502.0034, found 502.0030.

Ethyl 6-bromo-2',4'-dichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2g) :

White solid, 67% yield, mp 220 - 222 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.54 (dd, *J* = 6.8, 1.6 Hz, 2H), 7.53 – 7.44 (m, 1H), 7.42 (dd, *J* = 8.2, 6.6 Hz, 2H), 7.20 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.10 (d, *J* = 1.8 Hz, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 3.82 – 3.70 (m, 2H), 0.72 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.88, 164.89, 164.50, 162.14, 159.39, 154.69, 143.12, 131.71, 131.11, 129.82, 128.44, 128.40, 126.46, 126.12, 123.99, 113.77, 111.10, 108.06, 61.70, 50.89, 13.10. IR (KBr): V_{max} = 3338, 1982, 1741, 1697, 1647, 1567, 1522, 1474, 1438, 1386, 1368, 1243, 1221, 1190, 1020, 1000, 956, 934, 885, 853, 784, 738, 698, 654 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}BrCl_2N_3O_4$ $[M + H]^+$ 545.9521, found 545.9517.

Ethyl 2',4'-dichloro-6-methoxy-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2h) :

Yellow solid, 50% yield, mp 198 - 200 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.55 (d, *J* = 7.0 Hz, 2H), 7.49 – 7.44 (m, 1H), 7.41 (dd, *J* = 8.2, 6.8 Hz, 2H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.54 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.49 (d, *J* = 2.2 Hz, 1H), 3.80 (s, 3H), 3.79 – 3.66 (m, 2H), 0.71 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.41, 164.96, 164.58, 162.23, 161.49, 158.98, 153.75, 143.03, 131.91, 130.86, 128.39, 128.34, 125.78, 123.12, 111.85, 109.01, 108.09, 97.38, 61.46, 55.55, 50.74, 13.13. IR (KBr): V_{max} = 3414, 2927, 1719, 1654, 1632, 1601, 1567, 1523, 1504, 1462, 1385, 1366, 1306, 1222, 1157, 1080, 1023, 960, 856, 699 cm^{-1}.HRMS (ESI): calcd for $C_{24}H_{17}Cl_2N_3O_5$ $[M + H]^+$ 498.0578, found 498.0575.

Ethyl 2',4',7-trichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2i) :

White solid, 75% yield, mp 210 - 212 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.80 – 7.73 (m, 2H), 7.74 – 7.66 (m, 1H), 7.64 (dd, *J* = 8.2, 6.6 Hz, 2H), 7.53 (dd, *J* = 7.2, 2.2 Hz, 1H), 7.31 – 7.16 (m, 2H), 3.96 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.57, 164.49, 164.44, 162.17, 159.40, 154.63, 141.48, 132.90, 132.01, 131.73, 131.04, 128.43, 128.40, 124.65, 123.76, 111.27, 108.25, 103.19, 61.60, 52.43, 13.11. IR (KBr): V_{max} = 3261, 2983, 1754, 1714, 1650, 1569, 1531, 1474, 1455, 1344, 1309, 1281, 1250, 1171, 1118, 1034, 1012, 966, 858, 791, 749, 708 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}Cl_3N_3O_4$ $[M + H]^+$ 502.0012, found 502.0006.

Ethyl 7-bromo-2',4'-dichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2j) :

White solid, 70% yield, mp 196 - 198 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.57 – 7.50 (m, 2H), 7.52 – 7.41 (m, 4H), 7.07 (d, *J* = 7.4 Hz, 1H), 7.01 – 6.92 (m, 1H), 3.82 – 3.65 (m, 2H), 0.72 (t, *J* = 7.2Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.89, 164.79, 164.50, 162.13, 159.47, 154.64, 141.11, 133.21, 132.77, 131.69, 131.10, 128.43, 128.40, 128.03, 115.88, 111.84, 111.09, 108.13, 61.66, 51.31, 13.08. IR (KBr): V_{max} = 3198, 1736, 1716, 1661, 1614, 1567, 1522, 1473, 1446, 1383, 1310, 1272, 1248, 1183, 1116, 1082, 1022, 959, 940, 853, 754, 725, 698, 655 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}BrCl_2N_3O_4$ $[M + H]^+$ 545.9530, found 545.9528.

Ethyl 2',4'-dichloro-7-nitro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2k) :

White solid, 71% yield, mp 256 - 258 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.16 (dd, *J* = 8.7, 1.1 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 2H), 7.49 (d, *J* = 7.4 Hz, 1H), 7.46 – 7.40 (m, 3H), 7.19 (dd, *J* = 8.6, 7.2 Hz, 1H), 3.71 (m, 2H), 0.67 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.54, 164.54, 164.41, 161.93, 159.79, 156.08, 139.33, 134.35, 131.66, 131.26, 131.19, 130.58, 128.47, 128.46, 125.13, 123.03, 110.65, 107.43, 61.73, 50.18, 13.06. IR (KBr): V_{max} = 3299, 2980, 1763, 1715, 1653, 1626, 1528, 1467, 1365, 1309, 1248, 1169, 1155, 1088, 1010, 972, 953, 929, 855, 786, 749, 706 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{14}Cl_2N_4O_6$ $[M + H]^+$ 513.0362, found 513.0359.

Ethyl 2',4'-dichloro-2-oxo-7'-phenyl-7-(trifluoromethyl)spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2l):

White solid, 74% yield, mp 193 - 195 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.57 – 7.51 (m, 3H), 7.50 – 7.45 (m, 1H), 7.42 (dd, *J* = 8.2, 6.6 Hz, 2H), 7.32 (d, *J* = 7.4 Hz, 1H), 7.20 – 7.14 (m, 1H), 3.96 – 3.34 (m, 2H), 0.71 (t, *J* = 7.2 Hz, 3H). ^{19}F NMR (565 MHz, $CDCl_3$) δ -60.68. ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.09, 164.51, 164.41, 162.00, 159.53, 155.11, 139.52, 139.51, 132.51, 131.67, 131.11, 128.42, 128.34, 126.88 (q, *J* = 3.9 Hz), 123.60 (q, *J* = 271.9 Hz), 123.31, 112.47 (q, *J* = 33.6 Hz). 111.04, 107.95,

61.63, 50.29, 13.03. IR (KBr): V_{max} = 3224, 1739, 1717, 1662, 1620, 1569, 1521, 1459, 1369, 1309, 1221, 1187, 1172, 1087, 1034, 963, 946, 854, 804, 749, 700, 660 cm^{-1}. HRMS (ESI): calcd for $C_{24}H_{14}Cl_2F_3N_3O_4$ $[M + H]^+$ 536.0365, found 536.0367.

Ethyl 2',4'-dichloro-7-methyl-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2m):

White solid, 57% yield, mp 205 - 207 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.54 (s, 1H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.49 – 7.43 (m, 1H), 7.43 – 7.38 (m, 2H), 7.11 (dd, *J* = 7.0, 1.8 Hz, 1H), 7.00 – 6.92 (m, 2H), 3.77 – 3.62 (m, 2H), 2.28 (s, 3H), 0.68 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.20, 164.68, 164.65, 162.26, 159.01, 153.49, 140.71, 131.87, 131.54, 130.88, 130.66, 128.39, 128.33, 123.44, 122.32, 119.91, 111.86, 109.06, 61.45, 51.80, 16.47, 13.08. IR (KBr): V_{max} = 3431, 2924, 1721, 1663, 1567, 1523, 1465, 1446, 1387, 1309, 1210, 1187, 1082, 1041, 966, 858, 772, 751, 701 cm^{-1}. HRMS (ESI): calcd for $C_{24}H_{17}Cl_2N_3O_4$ $[M + H]^+$ 482.0632, found 482.0630.

Ethyl 2',4'-dichloro-1-methyl-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (2n):

White solid, 76% yield, mp 190 - 192 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.48 (m, 2H), 7.39 (td, *J* = 16.4, 15.2, 7.6 Hz, 4H), 7.15 – 7.10 (m, 1H), 7.08 – 7.01 (m, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 3.74 – 3.54 (m, 2H), 3.33 (s, 3H), 0.63 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.25, 164.76, 164.64, 161.84, 158.98, 154.37, 144.83, 131.97, 130.86, 130.46, 130.31, 128.39, 128.33, 124.66, 123.46, 111.86, 108.81, 108.42, 61.30, 50.65, 27.02, 13.11. IR (KBr): V_{max} = 3418, 1716, 1667, 1609, 1567, 1522, 1492, 1469, 1447, 1388, 1368, 1311, 1256, 1220, 1180, 1125, 1089, 1059, 1019, 954, 926, 855, 805, 776, 762, 696 cm^{-1}. HRMS (ESI): calcd for $C_{24}H_{17}Cl_2N_3O_4$ $[M + H]^+$ 482.0655, found 482.0650.

6'-Acetyl-2',4'-dichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3a):

White solid, 78% yield, mp 180 - 182 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.56 – 7.48 (m, 1H), 7.44 – 7.33 (m, 2H), 7.22 – 7.13 (m, 1H), 7.13 – 7.06 (m, 1H), 7.00 – 6.92 (m, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 1.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.28, 176.06, 164.64, 162.34, 159.08, 151.00, 141.31, 137.13, 133.99, 130.89, 130.13, 129.32, 128.87, 124.67, 123.33, 112.19, 111.51, 110.51, 51.53, 19.49. IR (KBr): V_{max} = 3424, 2924, 1733, 1659, 1524, 1483, 1472, 1448, 1381, 1349, 1291, 1219, 1160, 1129, 1090, 1028, 973, 910, 881, 854, 816, 754, 703, 687 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{13}Cl_2N_3O_4$ $[M + H]^+$ 4378.0399, found 438.0407.

6'-Acetyl-2',4',5-trichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3b):

White solid, 77% yield, mp 180 - 182 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.75 (dd, *J* = 8.2, 1.2 Hz, 2H), 7.58 – 7.52 (m, 1H), 7.44 – 7.39 (m, 2H), 7.14 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.08 (d, *J* = 2.0 Hz, 1H), 6.72 (d, *J* = 8.2 Hz, 1H), 1.88 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.21, 175.84, 164.52, 162.26, 159.40, 151.54, 139.85, 137.00, 134.22, 132.39, 130.12, 129.29, 128.99, 128.65, 125.07, 111.69,

111.51, 110.89, 51.63, 19.56. IR (KBr): V_{max} = 3416, 2920, 1749, 1669, 1618, 1561, 1524, 1478, 1447, 1381, 1347, 1291, 1275, 1207, 1133, 1098, 1027, 973, 905, 884, 817, 788, 722, 699 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{12}Cl_3N_3O_3$ $[M + H]^+$ 471.9968, found 471.9965.

6'-Acetyl-5-bromo-2', 4'-dichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3c): White solid, 75% yield, mp 192 - 194 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.81 – 7.77 (m, 2H), 7.63 – 7.57 (m, 1H), 7.50 – 7.44 (m, 2H), 7.33 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.26 (d, *J* = 1.8 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 1H), 1.93 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.19, 175.77, 164.53, 162.24, 159.41, 151.53, 139.82, 137.02, 134.20, 132.40, 130.12, 129.29, 128.99, 128.66, 125.08, 111.70, 111.48, 110.89, 51.62, 19.54. IR (KBr): V_{max} = 3415, 1750, 1669, 1595, 1524, 1475, 1447, 1381, 1316, 1291, 1274, 1221, 1188, 1133, 1108, 1027, 973, 881, 815, 718, 699 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{12}BrCl_2N_3O_3$ $[M + H]^+$ 515.9412, found 515.9410.

6'-Acetyl-2', 4'-dichloro-5-fluoro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3d): White solid, 76% yield, mp 225 - 227 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.75 (dd, *J* = 8.2, 1.2 Hz, 2H), 7.55 (td, *J* = 7.4, 1.4 Hz, 1H), 7.44 – 7.38 (m, 2H), 6.92 – 6.81 (m, 2H), 6.73 (dd, *J* = 8.4, 4.0 Hz, 1H), 1.88 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.12, 176.13, 164.55, 162.28, 159.36, 159.28 (d, *J* = 243 Hz), 151.46, 137.38 (d, *J* = 2.2 Hz), 137.01, 134.18, 132.17 (d, *J* = 7.6 Hz), 129.28, 128.99, 116.70 (d, *J* = 23.4 Hz), 112.55 (d, *J* = 25.2 Hz), 111.75, 111.28 (d, *J* = 7.9 Hz), 110.98, 51.95, 19.52. ^{19}F NMR (565 MHz, $CDCl_3$) δ -118.50. IR (KBr): V_{max} = 3215, 1720, 1660, 1593, 1526, 1487, 1449, 1383, 1349, 1267, 1223, 1197, 1185, 1101, 1028, 971, 889, 824, 824, 781, 709, 694 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{12}Cl_2FN_3O_3$ $[M + H]^+$ 456.0221, found 456.0215.

6'-Acetyl-2',4'-dichloro-5-methyl-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3e): White solid, 54% yield, mp 223 - 225 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.78 – 7.73 (m, 2H), 7.52 (td, *J* = 7.2, 1.2 Hz, 1H), 7.39 (m, 2H), 6.96 (d, *J* = 8.0 Hz, 1H), 6.89 (d, *J* = 1.8 Hz, 1H), 6.66 (d, *J* = 7.8 Hz, 1H), 2.20 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.19, 175.84, 164.66, 162.34, 159.01, 150.59, 138.63, 137.12, 133.93, 133.01, 130.95, 130.43, 129.36, 128.77, 125.44, 112.27, 111.60, 110.10, 51.60, 21.03, 19.37. IR (KBr): V_{max} = 3203, 1744, 1723, 1666, 1596, 1524, 1492, 1448, 1382, 1315, 1291, 1217, 1145, 1106, 1092, 1026, 973, 887, 814, 774, 694 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{15}Cl_2N_3O_3$ $[M + H]^+$ 452.0531, found 452.0525.

6'-Acetyl-2',4'-dichloro-5-methoxy-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3f): White solid, 51% yield, mp 212 - 214 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.54 – 7.47 (m, 1H), 7.41 – 7.36 (m, 2H), 6.67 (d, *J* = 11.6 Hz, 3H), 3.68 (s, 3H), 1.88 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.07, 175.99, 164.63, 162.39, 159.07, 156.32, 150.82, 137.11, 134.70, 133.98, 131.99, 129.34, 128.83, 114.86, 112.18, 111.52, 111.50, 111.02, 58.43, 55.76, 19.42. IR (KBr): V_{max} = 3412, 2927, 1732, 1659, 1595, 1524, 1492, 1438, 1380, 1315, 1290, 1222, 1202, 1150, 1131, 1105,

1027, 973, 887, 817, 795, 704, 694 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{15}Cl_2N_3O_4$ $[M + H]^+$ 467.0452, found 467.0451.

6'-Acetyl-2',4',6-trichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one(3g):

White solid, 74% yield, mp 203 - 205 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.62 – 7.50 (m, 1H), 7.49 – 7.38 (m, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.95 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.82 (d, *J* = 1.8 Hz, 1H), 1.87 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.34, 176.00, 164.52, 162.23, 159.30, 151.72, 142.51, 137.14, 135.87, 134.17, 129.31, 129.26, 129.01, 125.45, 123.35, 111.87, 111.20, 111.07, 51.11, 19.63. IR (KBr): V_{max} =3420, 1728, 1665, 1601, 1563, 1521, 1460, 1381, 1348, 1293, 1273, 1218, 1160, 1104, 1026, 960, 916, 890, 827, 792, 120, 681 cm^{-1}.HRMS (ESI): calcd for $C_{22}H_{12}Cl_3N_3O_3$ $[M + H]^+$ 471.9989, found 471.9987.

6'-Acetyl-6-bromo-2',4'-dichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3h):

White solid, 69% yield, mp 198 - 200 °C. ^1H NMR (600 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.73 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.58 – 7.52 (m, 1H), 7.44 – 7.38 (m, 2H), 7.10 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.96 (d, *J* = 7.6 Hz, 2H), 1.87 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.39, 176.23, 164.52, 162.24, 159.28, 151.80, 142.80, 137.14, 134.19, 129.86, 129.28, 129.02, 126.22, 125.71, 123.78, 114.10, 111.78, 111.03, 51.24, 19.65. IR (KBr): V_{max} = 3266, 2924, 1735, 1656, 1523, 1480, 1448, 1380, 1315, 1291, 1245, 1131, 1106, 1028, 1000, 973, 892, 856, 805, 786, 747, 702 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{12}BrCl_2N_3O_3$ $[M + H]^+$ 515.9411, found 515.9410.

6'-Acetyl-2',4',7-trichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3i):

White solid, 73% yield, mp 227 - 229 °C. ^1H NMR (600 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.75 – 7.71 (m, 2H), 7.56 – 7.51 (m, 1H), 7.43 – 7.37 (m, 2H), 7.19 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.00 (d, *J* = 7.4 Hz, 1H), 6.96 – 6.89 (m, 1H), 1.87 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.08, 174.85, 164.47, 162.28, 159.31, 151.51, 139.33, 137.14, 134.05, 132.08, 130.04, 129.25, 128.95, 124.11, 122.86, 115.60, 111.86, 111.05, 52.39, 19.54. IR (KBr): V_{max} = 3158, 1726, 1662, 1597, 1565, 1519, 1449, 1375, 1352, 1294, 1220, 1191, 1120, 1025, 971, 930, 899, 824, 791, 739, 711, 685 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{12}Cl_3N_3O_3$ $[M + H]^+$ 471.9969, found 471.9965.

6'-Acetyl-7-bromo-2',4'-dichloro-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3j):

White solid, 68% yield, mp 225 - 227 °C. ^1H NMR (600 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.73 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.58 – 7.47 (m, 1H), 7.44 – 7.38 (m, 2H), 7.33 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.04 (d, *J* = 7.4 Hz, 1H), 6.88 (dd, *J* = 8.2, 7.6 Hz, 1H), 1.88 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.09, 174.47, 164.46, 162.27, 159.33, 151.54, 140.94, 137.18, 134.04, 132.81, 131.97, 129.23, 128.95, 124.47, 123.44, 111.93, 111.08, 103.37, 52.72, 19.54. IR (KBr): V_{max} = 3157, 1727, 1660, 1565, 1519, 1448, 1385, 1352, 1294, 1218, 1191, 1132, 1097, 1026, 970, 898, 881, 822, 780, 752, 683 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{12}BrCl_2N_3O_3$ $[M + H]^+$ 515.9412, found 515.9410.

6'-Acetyl-2',4'-dichloro-7'-phenyl-7-(trifluoromethyl)spiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3k):

White solid, 70% yield, mp 198 - 200 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.71 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.57 – 7.51 (m, 1H), 7.45 – 7.37 (m, 3H), 7.28 (d, *J* = 7.6 Hz, 1H), 7.11 – 7.06 (m, 1H), 1.90 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.08, 174.99, 164.56, 162.10, 159.46, 151.99, 138.91, 138.89, 137.13, 134.10, 132.49, 129.18, 128.97, 128.02, 126.80 (q, *J* = 4.1 Hz), 123.44(q, *J* = 272 Hz), 112.60 (q, *J* = 33.6 Hz), 111.74, 110.84, 50.61, 19.57. ^{19}F NMR (565 MHz, $CDCl_3$) δ -60.79. IR (KBr): V_{max} = 3187, 1735, 1687, 1663, 1624, 1597, 1566, 1522, 1458, 1395, 1381, 1339, 1317, 1296, 1221, 1169, 1123, 1096, 1027, 972, 934, 902, 885, 866, 784, 747, 689 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{12}Cl_2F_3N_3O_3$ $[M + H]^+$ 505.0223, found 505.0216.

6'-Acetyl-2',4'-dichloro-7-methyl-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3l):

White solid, 53% yield, mp 226 - 228 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.71 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.53 – 7.47 (m, 1H), 7.39 – 7.33 (m, 2H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 7.4 Hz, 1H), 6.90 – 6.84 (m, 1H), 2.15 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.10, 176.76, 164.64, 162.35, 158.95, 150.54, 140.13, 137.18, 133.84, 131.40, 130.56, 129.27, 128.76, 123.20, 122.02, 119.91, 112.40, 111.70, 51.99, 19.33, 16.38. IR (KBr): V_{max} = 3164, 1718, 1685, 1663, 1627, 1565, 1521, 1448, 1376, 1350, 1289, 1220, 1158, 1129, 1033, 971, 904, 885, 869, 834, 781, 751, 684 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{15}Cl_2N_3O_3$ $[M + H]^+$ 452.0530, found 452.0526.

6'-Acetyl-2',4'-dichloro-1-methyl-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidin]-2-one (3m):

White solid, 75% yield, mp 227 - 229 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 7.6 Hz, 2H), 7.56 – 7.47 (m, 1H), 7.43 – 7.32 (m, 2H), 7.26 (d, *J* = 15.4 Hz, 1H), 7.12 (d, *J* = 7.4 Hz, 1H), 7.03 – 6.95 (m, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 3.18 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.54, 174.31, 164.67, 161.96, 158.97, 151.47, 144.27, 137.43, 133.79, 130.43, 130.17, 129.11, 128.82, 124.38, 123.32, 112.50, 111.72, 108.65, 50.96, 26.89, 19.50. IR (KBr): V_{max} = 3417, 1714, 1661, 1611, 1570, 1530, 1494, 1472, 1380, 1320, 1296, 1271, 1225, 1162, 1127, 1100, 1082, 1024, 970, 904, 866, 806, 751, 706, 688 cm^{-1}. HRMS (ESI): calcd for $C_{23}H_{15}Cl_2N_3O_3$ $[M + H]^+$ 452.0531, found 452.0528.

2', 4'-Dichloro-2-oxo-7'-phenylspiro [indoline-3,5'-pyrano[2,3-*d*] pyrimidine]-6'-carbonitrile (4a):

White solid, 77% yield, mp 240 - 242 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.91 (dd, *J* = 7.4, 1.8 Hz, 2H), 7.62 – 7.54 (m, 1H), 7.51 (dd, *J* = 8.2, 6.6 Hz, 2H), 7.40 (td, *J* = 7.6, 1.6 Hz, 1H), 7.20 – 7.08 (m, 2H), 7.02 (d, *J* = 7.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.05, 163.86, 162.57, 159.97, 159.86, 140.57, 132.69, 131.11, 130.17, 128.86, 128.79, 128.30, 124.77, 124.39, 114.14, 110.96, 109.60, 89.57, 51.22. IR (KBr): V_{max} =3250, 2227, 1730, 1616, 1570, 1489, 1455, 1380, 1350, 1301, 1258, 1203, 1129, 1070, 1018, 960, 928, 849, 743, 690, 660 cm^{-1}. HRMS (ESI): calcd for $C_{21}H_{10}Cl_2N_2O_2$ $[M + H]^+$ 421.0253, found 421.0254.

2',4',6-Trichloro-2-oxo-7'-phenylspiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carbonitrile(4b):

White solid, 78% yield, mp 273 - 275 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.59 (s, 1H), 7.84 (dd, *J* = 7.2, 1.6 Hz, 2H), 7.70 – 7.64 (m, 1H), 7.61 (ddd, *J* = 8.2, 5.8, 1.8 Hz, 3H), 7.19 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.06 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.46, 164.43, 161.45, 160.28, 158.73, 144.11, 135.56, 133.11, 129.67, 129.51, 129.45, 128.69, 127.91, 123.55, 114.82, 111.06, 110.54, 89.87, 50.95. IR (KBr): V_{max} = 3147, 1732, 1615, 1571, 1519, 1487, 1458, 1382, 1352, 1302, 1257, 1207, 1131, 1070, 1018, 958, 927, 848, 744, 692, 661 cm^{-1}. HRMS (ESI): calcd for $C_{21}H_9Cl_3N_4O_2$ $[M + H]^+$ 454.9854, found 454.9850.

Ethyl 2',4'-dichloro-7'-methyl-2-oxospiro[indoline-3,5'-pyrano[2,3-*d*]pyrimidine]-6'-carboxylate (5a):

White solid, 48% yield, mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 7.57 – 7.46 (m, 1H), 7.30 – 7.22 (m, 2H), 7.15 (d, *J* = 7.8 Hz, 1H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.67 (s, 3H), 1.25 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.30, 164.26, 163.92, 162.06, 158.92, 156.97, 142.24, 131.68, 130.10, 124.69, 123.52, 111.90, 110.12, 107.52, 61.56, 50.55, 19.46, 13.51. IR (KBr): V_{max} = 3415, 2932, 1727, 1710, 1618, 1566, 1523, 1474, 1366, 1344, 1300, 1107, 971, 855, 801 cm^{-1}. HRMS (ESI): calcd for $C_{18}H_{13}Cl_2N_3O_4$ $[M + H]^+$ 406.0353, found 406.0356.

In vitro anticancer screening

Cell Counting Kit‑8 (CCK‑8) assay:

Human lung cancer A549, human liver cancer HepG-2, human cervical cancer Hela and human breast cancer MCF-7 cells in logarithmic phase were taken and prepared into 5×10^4/mL single cell suspension, and 100 μL to 96-well flat-bottomed culture plates were added. After 24 h of incubation in 37 °C and 5% CO_2 incubator, 100 μL of the above compound solutions with different concentrations (0.80 μg/mL to 100 μg/ml) were added, and two duplicate wells were made for each drug concentration. After culturing for 48 h in 37 °C and 5% CO_2 incubator, 10 μL of CCK8 application solution was added, and the cultivation was continued for 2 h, use Power Wave XS type full-wavelength microplate reader at 450 nm wavelength for colorimetric, measure the OD value of each sample, use the average OD of 2 wells. The inhibition rates of different compounds on human lung cancer A549, human liver cancer HepG-2, human cervical cancer Hela and human breast cancer MCF-7 cells were calculated.

ACKNOWLEDGEMENTS

We are grateful for financial support by the Zhejiang Province Public Welfare Technology Application Research Project under Grant Number LGG21B060004.

REFERENCES

1. Y. Zheng, C. M. Tice, and S. B. Singh, *Bioorg. Med. Chem. Lett*., 2014, **24**, 3673; S. Stotani, C. Lorenz, M. Winkler, F. Medda, E. Picazo, R. Ortega Martinez, A. Karawajczyk, J. Sanchez-Quesada, and F. Giordanetto, *ACS Comb. Sci*., 2016, **18**, 330; K. Hiesinger, D. Dar'in, E. Proschak, and M. Krasavin, *J. Med. Chem.*, 2020, **64**, 150.
2. B. L. DeCorte, *J. Med. Chem*., 2016, **59**, 9295.
3. M. Lahlou, *Pharmacol. Pharm.*, 2013, **04**, 17.
4. A. Fensome, W. R. Adams, A. L. Adams, T. J. Berrodin, J. Cohen, C. Huselton, A. Illenberger, J. C. Kern, V. A. Hudak, M. A. Marella, E. G. Melenski, C. C. McComas, C. A. Mugford, O. D. Slayden, M. Yudt, Z. Zhang, P. Zhang, Y. Zhu, R. C. Winneker, and J. E. Wrobel, *J. Med. Chem.*, 2008, **51**, 1861.
5. G. Kumari, Nutan, M. Modi, S. K. Gupta, and R. K. Singh, *Eur. J. Med. Chem*., 2011, **46**, 1181.
6. M. M.-C. Lo, C. S. Neumann, S. Nagayama, E. O. Perlstein, and S. L. Schreiber, *J. Am. Chem. Soc.*, 2004, **126**, 16077; K. Ding, Y. Lu, Z. Nikolovska-Coleska, Su Qiu, Y. Ding, W. Gao, J. Stuckey, K. Krajewski, P. P. Roller, Y. Tomita, D. A. Parrish, J. R. Deschamps, and S. Wang, *J. Am. Chem. Soc.*, 2005, **127**, 10130.
7. V. V. Vintonyak, K. Warburg, H. Kruse, S. Grimme, K. Hübel, D. Rauh, and H. Waldmann, *Angew. Chem. Int. Ed.*, 2010, **49**, 5902.
8. R. Matthias, M. Case, Y. Bryan K. S, L. Marcus C. S, Z. Bin, R. Bruce, S. Patrick, D. M. Plouffe, N. V. Dharia, J. Tan, K. R. Spencer, G. E. González-Páez, S. B. Lakshminarayana, A. Goh, R. Suwanarusk, T. Jegla, E. K. Schmitt, H. Beck, R. Brun, F. Nosten, L. Renia, V. Dartois, T. H. Keller, and D. A. Fido, *Science.*, 2010, **329**, 1175; B. K. S. Yeung, B. Zou, M. Rottmann, S. B. Lakshminarayana, S. H. Ang, S. Y. Leong, J. Tan, J. Wong, S. Keller-Maerki, C. Fischli, A. Goh, E. K. Schmitt, P. Krastel, E. Francotte, K. Kuhen, D. Plouffe, K. Henson, T. Wagner, E. A. Winzeler, F. Petersen, R. Brun, V. Dartois, T. T. Diagana, and T. H. Keller, *J. Med. Chem.*, 2010, **53**, 5155.
9. K. Ding, Y. Lu, Z. Nikolovska-coleska, G. Wang, S. Qiu, S. Shangary, W. Gao, D. Qin, J. Stuckey, K. Krajewski, P. P. Roller, and S. Wang, *J. Med. Chem.*, 2006, **49**, 3432.
10. S. H. Myers, V. G. Brunton, and A. Unciti-Broceta, *J. Med. Chem.*, 2015, **59**, 3593.
11. A .G. Sacher, K. M. Komatsubara, and G. R. Oxnard, *J. Thorac. Oncol.*, 2017, **12**, 1344.
12. F. Gass-Jégu, A. Gschwend, A.-C. Gairard-Dory, B. Mennecier, M. Tebacher-Alt, B. Gourieux, and É. Quoix, *Lung Cancer*, 2016, **99**, 76.
13. A. V. K, K. Rakesh, K. Anju, and B. Rita, *Tetrahedron.*, 1900, **46**, 3953; B. Arthur D, S. Jaewon. L, and A. Gary L, *J. Org. Chem.*, 1976, **41**, 1095.
14. F. F. Alblewi, R. M. Okasha, Z. M. Hritani, H. M. Mohamed, M. A. A. El-Nassag, A. H. Halawa, A.

Mora, A. M. Fouda, M. A. Assiri, A. M. Al-Dies, T. H. Afifi, and A. M. El-Agrody, *Bioorg. Chem.*, 2019, **87**, 560; F. F. Alblewi, R. Okasha, A. Eskandrani, T. Afifi, H. Mohamed, A. Halawa, A. Fouda, A. Al-Dies, A. Mora, and A. El-Agrody, *Molecules.*, 2019, **24**, 1060; A. H. Halawa, M. M. Elaasser, A. M. El Kerdawy, A. M. A. I. A. El-Hady, H. A. Emam, and A. M. El-Agrody, *Med. Chem. Res.*, 2017, **26**, 2624; M. M. Ghorab, F. A. Ragab, H. I. Heiba, and R. M. El-Hazek, *Eur. J. Med. Chem.*, 2011, **46**, 5120.

15. M. Cherif, M. Horchani, Y. O. Al-Ghamdi, S. G. Almalki, Y. E. Alqurashi, H. Ben Jannet, and A. Romdhane, *J. Mol. Struct.*, 2020, **1220**, 128685; M. Thangaraj, B. Ranjan, R. Muthusamy, A. Murugesan, and R. M. Gengan, *J. Heterocycl. Chem.*, 2019, **56**, 867; I. A. Radini, H. M. Hamed, M. A. Y. Kharir, and A. H. F. A. Elwahab, *Eur. J. Org. Chem.*, 2017, **8**, 240; L. Suresh, Y. Poornachandra, S. Kanakaraju, C. Ganesh Kumar, and G. V. P. Chandramouli, *Org. Biomol. Chem.*, 2015, **13**, 7294.
16. Z. Ebrahimi, A . Davoodnia, A. Motavalizadehkakhky, and J. Mehrzad, *Org. Prep. Proced. Int.*, 2019, **51**, 357; R. M. Naidu Kalla, R. S. Karunakaran, M. Balaji, and I. Kim, *ChemistrySelect.*, 2019, **4**, 644; T. E. Ali, D. A. Bakhotmah, and M. A. Assiri, *Synth. Commun.*, 2020, **50**, 3314.
17. Z. Li, D. Wu, and W. Zhong, *Heterocycles*, 2012, **85**, 1417; Y. Zou, Z. Li, and W. Su, *J. Chem. Res.*, 2014, **38**, 143; Z. H. Li, Z. J. Jiang, Q. L. Shao, J. J. Qin, Q. F. Shu, W. H. Lu, and W. K. Su, *J. Org. Chem.*, 2018, **83**, 6423; J. Qin, Z. Li, S. Ma, L. Ye, G. Jin, and W. Su, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2020, **195**, 1007.
18. Y. S. Li, X. R. Liang, and W. K. Su, *Org. Prep. Proced. Int.*, 2003, **35**, 613; D. Y. Chi, J. H. Lee, B. S. Lee, H. Shin, and D. H. Nam, *Synlett*, 2006, 0065; W. Zhong, L. Hong, and Y. Zheng, *Lett. Org. Chem.*, 2010, **7**, 229.