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Synthesis and in vitro antiproliferative evaluation of pyrimido[5,4-*c*]quinoline-4-(3*H*)-one derivatives

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ABSTRACT

A series of pyrimido[5,4-*c*]quinoline-4-(3*H*)-one derivatives variously substituted at positions 2 and 3 were synthesized and evaluated for their in vitro antiproliferative activities against a panel of six human cancer cell lines. Biological evaluation revealed that the vast majority of derivatives exhibited moderate tumor growth inhibitory activities. In particular, compound **7e** showed effective anti-tumor activity with broad-spectrum toward numerous cell lines and the most active member in this study. This derivative displaying significant activity against KB (IC_{50}: 4.9 μM), CNE2 (IC_{50}: 13.8 μM), MGC-803 (IC_{50}: 4.8 μM), GLC-82 (IC_{50}: 7.88 μM), MDA-MB-453 (IC_{50}: 18.2 μM) and MCF-7 (IC_{50}: 10.1 μM) cell lines could be considered as the most promising and useful template for future development to obtain more potent anti-tumor agent(s).

1. Introduction

Although there has been great progress in the development of treatment and prevention for cancer, it still remains an enormous threat to people's health in the 21st century, representing the second primary cause of death in the world [1]. In the past years, considerable efforts have been made to develop innovative strategies for finding safe and effective methods of treating this disease. With the increasing understanding of the biological process involved in cancer cell survival and the discovering of new target, more and more novel chemical therapeutic drugs have been designed for treatment of cancer.

The derivatives of pyrimidoquinolines have been attracted great interest over many years due to their broad bioactivities. A great deal of investigations on the synthesis of pyrimidoquinolines was carried out [2–9], and many derivatives with excellent anti-HIV and anti-tumor activities have been obtained [10–14]. For example, 5-deazaflavins {pyrimido[4,5-b]quinoline-2,4 (3*H*,10*H*)-diones} and 2-deoxo-2-phenyl-5-deazaflavins {2-phenylpyrimido [4,5-b]quinolin-4(10*H*)-ones} as selective inhibitors of protein kinase C (PKC) exhibited effective growth inhibition against cancer cell lines such as A 431 cells and HT 1080 cells [15]; 2-amino-pyrimido[4,5-c]quinolin-1(2*H*)-ones and 2,5-diaryl-3-methylpyrimido [4,5-c]quinolin-1(2*H*)-one derivatives as a class of cytotoxic antimitotic agents have been reported in the literatures [13,16]; in addition, many attentions have also been focused on the synthesis and bioactivities of pyrimido[5,4-*c*]quinoline derivatives [17–22]. Although previous researches revealed that they exhibited several significant pharmacological activities (e.g. antioxidant [20], antimalarials [21], antiherpetic [22]), these reported pyrimido[5,4-*c*] quinoline derivatives suffered from serious limitations such as the rapid development of drug resistant. Thus, novel, potent, selective and less toxic agents containing pyrimido[5,4-*c*]quinoline system are still urgently required to triumph over the limitations.

In the present work, a novel series of pyrimido[5,4-*c*]quinoline-4-(3*H*)-one derivatives bearing variously substituted at positions 2 and 3 were synthesized and tested for in vitro antiproliferative activity. The preliminary bioassay showed that some of them have antiproliferative activity and the most active compound **7e** against KB (IC_{50}: 4.9 μM), CNE2 (IC_{50}: 13.8 μM), MGC-803 (IC_{50}: 4.8 μM), GLC-82 (IC_{50}: 7.88 μM), MDA-MB-453 (IC_{50}: 18.2 μM) and MCF-7 (IC_{50}: 10.1 μM) cell lines could be regarded as the most promising and useful template for future development to obtain more potent anti-tumor agent(s).

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2. Chemistry

The synthetic methods of pyrimido[5,4-c]quinoine derivatives using pyrimidine moiety [19,20] or quinoline moiety [21–23] as sarting materials have appeared in the literature. Here, we present three synthetic routes to novel pyrimido[5,4-c]quinoine derivatives (Scheme 1).

Firstly, a series of pyrimido[5,4-c]quinoine derivatives bearing 3-substituted groups have been synthesized. The general procedure for the synthesis of derivatives **6a**–**k** was described in Scheme 1. Ethyl 4-amino-2-methylquinoline-3-carboxylate (**2**) was prepared in 68% yield from 2-Aminobenzonitrile (**1**) by treatment with acetoacetic ester and tin tetrachloride in refluxing toluene. It was treated with triethyl orthoformate using acetic anhydride as a catalyst to yield intermediate **5**. Upon reaction with n-butylamine, n-propylamine, isopropylamine, hydrazine hydrate, glycol, ethanolamine, β-phenylethylamine, benzylamine, ethylamine, (±)-sec-butylamine and isobutylamine, yielded corresponding 3-substituted pyrimido[5,4-*c*]quinoine-4-(3*H*)-one derivatives **6a**–**k**.

Secondly, the iminophosphorane **3** was obtained in a satisfactory yield when **2** was treated with triphenylphosphine, hexachloroethane and Et_3N. As shown in Scheme 1, the iminophosphorane **3** reacted with substituted phenyl isocyanate to give carbodiimide **4** by using aza-Wittig reaction in a mild condition. The reaction of carbodiimide **4** with substituted phenols yielded **7a**–**j** in high yields under the condition of heating for 4–6 h in the presence of a catalytic amount of K_2CO_3.

Finally, carbodiimide **4** was treated with 2-methylpropan-2-amine or hydrazine hydrate to yield **8a**–**c** in satisfactory yield at room temperature for 12-13h using EtONa as a catalyst. Meanwhile, compound **8b** was reacted with triethyl orthoformate to afford the cyclized compound **9**. The preparations are summarized in Table 1.

3. Pharmacology

To evaluate the anticancer potencies of these newly synthesized pyrimido[5,4-*c*]quinoline-4(3*H*)-one derivatives, the antiproliferative activities of compounds **6a**–**k**, **7a**–**j**, **8a**–**c** and **9** were tested against six human cancer cell lines including KB human oral carcinoma cells, CNE2 human nasopharyngeal carcinoma cells, MGC-803 human gastric carcinoma cells, GLC-82 human lung carcinoma cells, MDA-MB-453 human breast carcinoma cells and MCF-7 breast adenocarcinoma cells by performing MTT assay. These results were summarized in Table 2 and presented as the concentration of drug inhibiting 50% cell growth (IC_{50}). 5-FU which is one of the most effective anticancer agents was used as the reference drug in this study.

4. Results and discussion

4.1. Nuclear magnetic resonance spectral studies

In this paper, IR, ^{1}H-NMR spectra, ^{13}C NMR spectra and mass spectra were used for the identification and confirmation of the newly assigned structures. Assignments of the signals are based on the chemical shifts and intensity patterns.

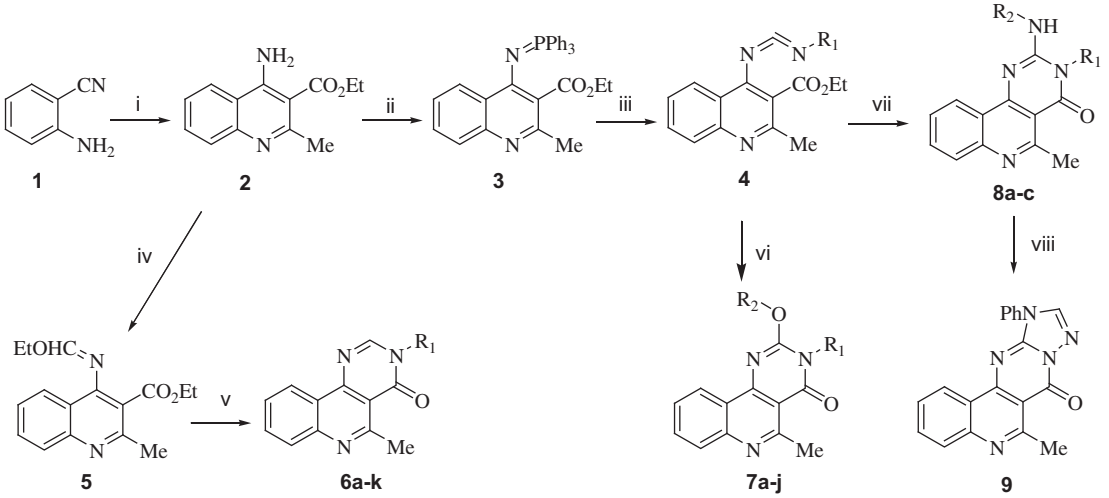
The ^{1}H-NMR spectra of all the target compounds, showed a singlet at δ_H 2.97–3.12 ppm, integrating for 3 protons: this data suggested that the signal belonged to 5-Me. Still, four downfield aromatic protons on the quinoline ring were distinctly observed in ^{1}H-NMR spectra. The structures of the compounds **6a**–**k** were confirmed in particular by the presence of a proton resonance at the 2-position as a singlet signal at δ_H 8.21–9.03 ppm in ^{1}H-NMR spectra. The H NMR spectra of compounds **8b**–**c** showed singlets for (NH_2) proton at δ_H 5.75 and another singlets for one (NH) proton at δ_H 9.81–9.98.

^{13}C NMR spectra of all the compounds were taken and the signal obtained further confirmed the proposed structures. All the compounds showed a signal at 23.0–27.5ppm due to methyl carbon. The characteristic peaks observed within the ^{13}C NMR spectra of synthesized derivatives are given in Section 6.

4.2. In vitro antiproliferative activity

The present results demonstrate that some compounds exhibited significant activity against certain cancer cell lines in comparison with 5-FU. Among them, compound **7e** showed the best inhibitory activity with IC_{50} values 4.9 μM, 13.8 μM, 4.8 μM, 7.88 μM, 18.2 μM, 10.1 μM against KB, CNE2, MGC-803, GLC-82, MDA-MB-453 and MCF-7, respectively.

Compounds **7a**, **7c** and **7e** displayed more potent inhibitory activity than compounds **7b** and **7d**, indicating that introduction of bulky and electron-withdrawing substituent such as groups bearing naphthalene, bromo and nitryl would benefit the potency.



Scheme 1. Synthesis of derivatives **6a**–**k**, **7a**–**j**, **8a**–**c** and **9**. Reagents and conditions: (i) $CH_3COCH_2COOCH_2CH_3$, $SnCl_4$, 130 °C, 6 h; (ii) PPh_3, C_2Cl_6, Et_3N, CH_3CN, rt, 24 h; (iii) R_1NCO, CH_2Cl_2, 45 °C, 12 h; (iv) $CH(OEt)_3/Ac_2O$, reflux, 6 h; (v) RNH_2/anhydrous acetonitrile, 40 °C, 8 h; (vi) R_2OH, K_2CO_3, 75 °C, 4–6 h; (vii) R_2NH_2, rt, 0.5–1 h, CH_3CH_2ONa, CH_3CH_2OH, rt, 12 h; (viii) $CH(OEt)_3$, reflux, 4 h.

Table 1
The preparation of derivatives **6a**–**k**, **7a**–**j**, **8a**–**c** and **9**.

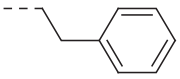
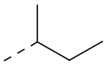
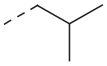
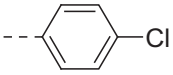
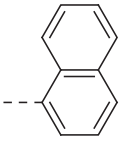
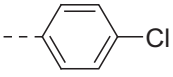
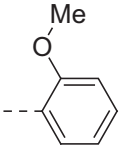
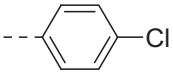
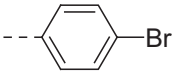
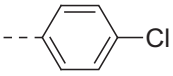
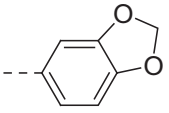
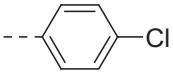
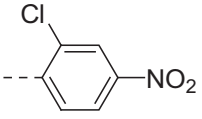
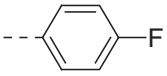
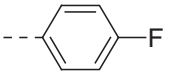
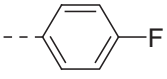
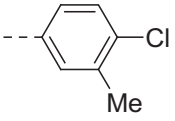
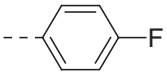
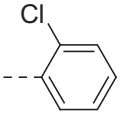
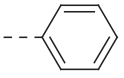
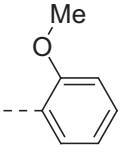
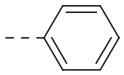
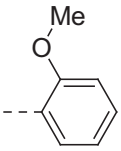
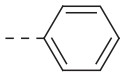
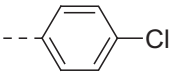
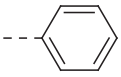
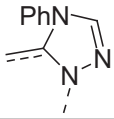
Compound	R_1	R_2	Yield (%)	Mp. (°C)
6a	*n*-Bu	–	50.0	123.4–125.1
6b	*n*-Pr	–	50.0	129.9–131.0
6c	*i*-Pr	–	46.0	119.4–121.4
6d	NH_2	–	43.0	291.0–292.0
6e	$-(CH)_2OH$	–	53.0	248.6–249.2
6f	$-(CH)_2\ NH_2$	–	51.9	232.8–235.4
6g		–	36.7	167.6–168.8
6h	Bn	–	34.7	160.0–161.2
6i	Et	–	30.7	128.1–129.0
6j		–	40.7	134.2–135.0
6k		–	50.2	138.3–140.1
7a			33.8	208.4–210.4
7b			32.2	209.2–210.3
7c			30.2	208.3–210.2
7d			29.5	270.6–272.4
7e			34.6	248.7–251.2
7f			30.5	196.0–197.5
7g			34.5	193.8–194.9
7h			33.6	207.7–208.9
7i			35.5	216.8–219.1

Table 1 (*continued*)

Compound	R_1	R_2	Yield (%)	Mp. (°C)
7j			30.9	225.6–226.9
8a			30.6	240.7–242.6 °C
8b	$-NH_2$		40.0	285.7–287.3
8c	$-NH_2$		42.1	286.8–290.0
9			37.7	335.0–336.0

Compared compound **8b** (MGC-803 IC_{50}: >50μM) with **8c** (MGC-803 IC_{50}: 14.7μM), it can be found that the 4-chloro at 2 position would enhance antiproliferative activity. Meanwhile, the cyclized compound **9** showed weak antiproliferative activity.

Table 2
Antiproliferative activities of compounds **6a-k**, **7a-j**, **8a-c** and **9** against KB, CNE2, MGC-803, GLC-82, MDA-MB-453, and MCF-7 cells.

Compound	In vitro antiproliferative IC_{50}[a] (μM)					
	KB	CNE2	MGC-803	GLC-82	MDA-MB-453	MCF-7
6a	>50	>50	>50	>50	>50	>50
6b	>50	>50	>50	>50	>50	>50
6c	>50	>50	>50	>50	>50	>50
6d	>50	>50	>50	>50	>50	>50
6e	>50	>50	>50	>50	>50	>50
6f	39.8	>50	39.9	>50	45.7	43.5
6g	>50	>50	>50	35.2	49.5	>50
6h	>50	26.5	>50	>50	>50	>50
6i	>50	>50	>50	>50	>50	>50
6j	>50	>50	>50	>50	>50	>50
6k	>50	>50	>50	>50	>50	>50
7a	33.9	47.0	18.5	42.1	>50	37.9
7b	39.2	>50	>50	>50	49.7	34
7c	29.5	28.7	15.3	>50	26.6	35.7
7d	30.9	47.7	>50	>50	>50	>50
7e	4.9	13.8	4.8	7.88	18.2	10.1
7f	nt[b]	nt	nt	nt	nt	nt
7g	>50	>50	41.4	>50	>50	>50
7h	>50	>50	>50	>50	>50	>50
7i	>50	>50	>50	>50	>50	35.5
7j	22.8	42.9	>50	>50	>50	>50
8a	>50	>50	>50	>50	>50	>50
8b	>50	>50	>50	>50	>50	>50
8c	30.3	>50	14.7	>50	>50	>50
9	>50	>50	>50	>50	>50	>50
5-FU	9.85	17.2	16.6	15.5	>50	9.67

Antiproliferative activity was determined by MTT assay as shown in Experimental 6.2 part. All data are presented as mean values of three independent experiments. Coefficients of variation were <10%.

[a] IC_{50}: concentration of the tested compound that inhibits 50% of cell growth.

[b] nt: not test.

5. Conclusion

In summary, we have synthesized a novel series of pyrimido [5,4-*c*]quinoline-4-(3*H*)-one derivatives. Compounds **7a**, **7c** and **7e** showed potential antiproliferative activity with broad-spectrum against several human cancer cell lines. Compound **7e** which was the most active members in this study displayed more or similar potent antiproliferative activities against cancer cell lines in comparison with 5-FU. These findings have encouraged us to continue the development and testing of novel pyrimidoquinoline derivatives and to conduct further studies to investigate SAR and their mechanisms of action.

6. Experimental protocols

6.1. Chemistry

6.1.1. General

Melting points were measured on an electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on an NE XUS-470 infrared spectrometer as KBr pellets with absorption in cm^{-1}. ^{1}H-NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or CD_3COCD_3 as solvent on a Varian Mercury 400 (or 600) spectrometer and resonances are given in ppm (d) relative to TMS. MS spectra were measured with a Finnigan MS spectrometer. All of the solvents and materials were reagent grade and purified as required. All compounds were routinely checked by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., P. R. China). Column chromatography was performed using silica gel (200–300 mesh) from Qingdao Haiyang Chemical Group Co., China.

6.1.2. General procedure for the preparation of 3-substituted 5-methylpyrimido[5,4-c]- quinolin-4(3H)-ones (*6a–k*)

2-Aminobenzonitrile (**1**; 2.36 g, 20 mmol) and $SnCl_4$ (3.7 ml, 32 mmol) were add to a stirred solution of ethyl acetoacetate (2.6 ml, 20 mmol) in anhydrous toluene (50 ml). The reaction mixture was stirred under nitrogen at room temperature for 0.5 h,

and then heated at reflux for 6 h (130 °C). The mixture was added to sat.aq. Na_2CO_3 solution (150 ml, pH 10), and the resulting suspension was extracted with AcOEt (3 × 50 ml). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure, the obtained residue was subjected to column chromatography (silica gel, eluent: petroleum ether-acetone, 7:3) to afford **2** in 68% yield.

To a solution of ethyl 4-amino-2-methylquinoline-3-carboxylate **2** (0.46 g, 2 mmol) in 20 ml triethyl orthoformate, 1 ml of acetic anhydride was added under nitrogen at temperature. The solution was stirred and refluxed for 6 h. At which time the reaction was completed, the solution was concentrated under vacuum. Removal of the solvent gave intermediate **5**, which was used directly without further purification.

To the solution of **5** prepared above in anhydrous acetonitrile (10 ml) was added substituted primary amine (3 ml). The mixture was stirred for 8 h at 40 °C and filtered, the filtrate was condensed and the residue was recrystallized from dichloromethane/petroleum ether to give pure 3-substituted-5-methylpyrimido[5,4-*c*]quinolin-4(3*H*)-ones **6a**–**k**.

*6.1.2.1. 3-Butyl-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6a**).* Pale yellow powder, yield 50%, m.p. 123.4–125.1 °C; IR(KBr): 1681 cm^{-1} (C═O); ^1H NMR (400 MHz, $CDCl_3$): 0.95 (t, J = 6.8, 3H, Me), 1.40 (m, 2H, CH_2), 1.76 (m, 2H, CH_2), 3.11 (s, 3H, Me), 3.95 (t, J = 7.2, 2H, CH_2), 7.55 (t, J = 8.0, 1H, Ar-H), 7.77 (t, J = 8.0, 1H, Ar-H), 7.99 (d, J = 8.0, 1H, Ar-H), 8.21 (s, 1H, CH), 8.68 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 13.6, 19.9, 27.5, 31.2, 47.4, 112.7, 123.1, 124.4, 126.4, 128.3, 132.1, 148.4, 150.4, 153.8, 160.0, 160.3; EI MS: *m/z* 267 ($[M]^+$, 72), 266 (17), 250 (5), 238 (11), 225 (51), 211 (100), 194 (12), 183 (23); HREIMS *m/z* 267.1373 (calcd for $C_{16}H_{17}N_3O$, 267.1373).

*6.1.2.2. 5-Methyl-3-propylpyrimido[5,4-c]quinolin-4(3H)-one (**6b**).* Pale yellow powder, yield 50%, m.p. 129.9–131.0 °C; IR(KBr): 1676 cm^{-1} (C═O); ^1H NMR (400 MHz, DMSO-d_6): 0.93 (t, J = 6.8, 3H, Me), 1.77 (m, 2H, CH_2), 3.00 (s, 3H, Me), 4.00 (t, J = 7.6, 2H, CH_2), 7.68 (t, J = 8.0, 1H, Ar-H), 7.89 (t, J = 8.0, 1H, Ar-H), 7.96 (d, J = 8.0, 1H, Ar-H), 8.70 (d, J = 8.0, 1H, Ar-H), 8.78 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 10.5, 21.4, 26.8, 47.7, 111.7, 122.3, 123.8, 126.1, 127.7, 131.6, 147.4, 152.2, 152.7, 158.6, 159.3; EI MS: *m/z* 254 ($[M+1]^+$, 13), 253 (75), 225 (6), 212 (14), 211 (100), 194 (8), 183 (29); HREIMS *m/z* 253.1218 (calcd for $C_{15}H_{15}N_3O$, 253.1218).

*6.1.2.3. 3-Isopropyl-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6c**).* Pale yellow powder, yield 46.0%, m.p. 119.4–121.4 °C; IR(KBr): 1673 cm^{-1} (C═O); ^1H NMR (400 MHz, DMSO-d_6): 1.49 (d, J = 6.8, 6H, 2Me), 3.01 (s, 3H, Me), 5.05 (m, 1H, CH), 7.68 (t, J = 8.0, 1H, Ar-H), 7.87 (t, J = 8.0, 1H, Ar-H), 7.95 (d, J = 8.0, 1H, Ar-H), 8.70 (d, J = 8.0, 1H, Ar-H), 8.85 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 20.9, 20.9, 26.9, 46.2, 111.5, 122.2, 123.7, 126.1, 127.7, 131.6, 147.4, 149.7, 152.0, 158.8, 159.0; EI MS: *m/z* 254 ($[M+1]^+$, 12), 253 (76), 225 (4), 212 (15), 211 (100), 194 (5), 183 (36); HREIMS *m/z* 253.1214 (calcd for $C_{15}H_{15}N_3O$, 253.1214).

*6.1.2.4. 5-Methyl-3-aminopyrimido[5,4-c]quinolin-4(3H)-one (**6d**).* Pale yellow powder, yield 43.0%, m.p. 291.0–292.0 °C; IR(KBr): 1682 cm^{-1} (C═O), 3300 cm^{-1}, 3176 cm^{-1} (NH_2); ^1H NMR (400 MHz, DMSO-d_6): 3.06 (s, 3H, Me), 6.04 (s, 2H, NH_2), 7.70 (t, J = 8.0, 1H, Ar-H), 7.91 (t, J = 8.0, 1H, Ar-H), 8.00 (d, J = 8.0, 1H, Ar-H), 8.76 (d, J = 8.0, 1H, Ar-H), 8.83 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.3, 111.5, 122.5, 123.8, 126.1, 127.8, 131.5, 147.5, 151.3, 152.3, 158.6, 159.2; EI MS: *m/z* 227 ($[M+1]^+$, 12), 226 (93), 211 (4), 198 (14), 197 (100), 169 (11), 142 (14); HREIMS *m/z* 226.0848 (calcd for $C_{12}H_{10}N_4O$, 226.0849).

*6.1.2.5. 3-(2-hydroxyethyl)-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6e**).* Pale yellow powder, yield 53.0%, m.p. 248.6–249.2 °C; IR(KBr): 1681 cm^{-1} (C═O), 3409 cm^{-1} (–OH); ^1H NMR (400 MHz, DMSO-d_6): 3.03 (s, 3H, Me), 3.72 (br s, 2H, CH_2), 4.11 (br s, 2H, CH_2), 5.01 (s, 1H, OH), 7.69 (t, J = 8.0, 1H, Ar-H), 7.90 (t, J = 8.0, 1H, Ar-H), 7.99 (d, J = 8.0, 1H, Ar-H), 8.73 (d, J = 8.0, 1H, Ar-H), 8.67 (s, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 159.8, 159.0, 153.2, 153.1, 147.8, 128.1, 132.1, 126.5, 122.7, 124.2, 112.2, 49.2, 57.9, 27.2; EI MS: *m/z* 256 ($[M+1]^+$, 10), 255 ($[M]^+$, 63), 212 (64), 211 (100), 194 (19), 183 (40), 169 (5), 167 (10); HREIMS *m/z* 255.1013 (calcd for $C_{14}H_{13}N_3O_2$, 255.1012).

*6.1.2.6. 3-(2-aminoethyl)-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6f**).* Pale yellow powder, yield 51.9%, m.p. 232.8–235.4 °C; IR(KBr): 1669 cm^{-1} (C═O), 3356 cm^{-1} (NH_2); ^1H NMR (400 MHz, DMSO-d_6): 3.02 (s, 3H, Me), 2.89 (br s, 2H, CH_2), 4.00 (br s, 2H, CH_2), 7.68 (t, J = 8.0, 1H, Ar-H), 7.89 (t, J = 8.0, 1H, Ar-H), 7.98 (d, J = 8.0, 1H, Ar-H), 8.74 (d, J = 8.0, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 153.3, 159.9, 159.1, 128.1, 132.0, 126.5, 124.2, 122.8, 147.8, 153.1, 112.4, 49.5, 39.7, 27.3; EI MS: *m/z* 254 ($[M]^+$, 5), 253 (2), 226 (6), 225 (43), 212 (100), 197 (23); HREIMS *m/z* 254.1167 (calcd for $C_{14}H_{14}N_4O$, 254.1167).

*6.1.2.7. 5-Methyl-3-phenethylpyrimido[5,4-c]quinolin-4(3H)-one (**6g**).* White solid, yield 36.7%, m.p. 167.6–168.8 °C; IR(KBr): 1690 cm^{-1} (C═O); ^1H NMR (400 MHz, DMSO-d_6): 3.04 (s, 3H, Me), 3.07 (t, J = 6.8, 2H, CH_2), 4.27 (t, J = 6.4, 2H, CH_2), 7.23–7.31 (m, 5H, Ar-H), 7.66 (t, J = 8.0, 1H, Ar-H), 7.89 (t, J = 8.0, 1H, Ar-H), 7.98 (d, J = 8.0, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 8.66 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.5, 33.8, 47.5, 111.8, 122.4, 123.8, 125.9, 126.2, 127.7, 128.1, 128.4, 128.4, 131.5, 137.5, 147.6, 151.8, 151.8, 152.8, 158.7, 159.4; EI MS: *m/z* 316 ($[M+1]^+$, 5), 315 (23), 212 (15), 211 (100), 183 (17), 104 (24); HREIMS *m/z* 315.1380 (calcd for $C_{20}H_{17}N_3O$, 315.1379).

*6.1.2.8. 3-Benzyl-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6h**).* Pale yellow powder, yield 34.7%, m.p. 160.0–161.2 °C; IR(KBr): 1680 cm^{-1} (C═O); ^1H NMR (400 MHz, DMSO-d_6): 3.01 (s, 3H, Me), 5.27 (s, 2H, CH_2), 7.31–7.42 (m, 5H, Ar-H), 7.70 (t, J = 8.0, 1H, Ar-H), 7.90 (t, J = 8.0, 1H, Ar-H), 7.99 (d, J = 8.0, 1H, Ar-H), 9.03 (s, 1H, Ar-H), 8.76 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.4, 49.1, 112.0, 122.4, 123.9, 126.0, 127.4, 127.4, 127.8, 128.3, 128.3, 131.6, 136.0, 147.7, 152.0, 152.0, 152.9, 158.7, 159.4; EI MS: *m/z* 302 ($[M+1]^+$, 23), 301 ($[M]^+$, 100), 224 (11), 211 (14), 91 (90); HREIMS *m/z* 301.1210 (calcd for $C_{19}H_{15}N_3O$, 301.1211).

*6.1.2.9. 3-Ethyl-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6i**).* Pale yellow crystal, yield 30.7%, m.p. 128.1–129.0 °C; IR(KBr): 1684 cm^{-1} (C═O); ^1H NMR (400 MHz, DMSO-d_6): 1.35 (t, J = 6.0, 3H, Me), 3.03 (s, 3H, Me), 4.09 (q, J = 6.8, 2H, CH_2), 7.68 (t, J = 8.0, 1H, Ar-H), 7.90 (t, J = 8.0, 1H, Ar-H), 7.99 (d, J = 8.0, 1H, Ar-H), 8.83 (s, 1H, CH), 8.74 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 14.0, 26.7, 41.6, 111.9, 122.5, 123.9, 126.1, 127.8, 131.7, 147.7, 152.0, 152.9, 158.8, 159.4; EI MS: *m/z* 240 ($[M+1]^+$, 17), 239 ($[M]^+$, 100), 210 (8), 211 (61), 183 (26); HREIMS *m/z* 239.1064 (calcd for $C_{14}H_{13}N_3O$, 239.1063).

*6.1.2.10. 3-sec-Butyl-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6j**).* Red powder, yield 40.7%, m.p.134.2–135.0 °C; IR(KBr): 1688 cm^{-1} (C═O); ^1H NMR (400 MHz, DMSO-d_6): 0.82 (t, J = 6.8, 3H, Me), 1.03 (d, J = 6.4, 3H, Me), 1.33 (m, 2H, CH_2), 4.34 (m, 1H, CH), 7.43 (t, J = 8.0, 1H, Ar-H), 7.67 (t, J = 8.0, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.29 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 14.1, 26.8, 60.6, 102.2, 117.1, 122.9, 122.9, 124.5, 124.5, 128.1, 131.1, 131.1, 147.0, 153.1, 157.8, 168.5; EI MS: *m/z* 267 ($[M]^+$, 4), 230 (59), 185 (24), 184 (100); HREIMS *m/z* 267.1366 (calcd for $C_{16}H_{17}N_3O$, 267.1367).

*6.1.2.11. 3-Isobutyl-5-methylpyrimido[5,4-c]quinolin-4(3H)-one (**6k**).* Pale yellow crystal, yield 50.2%, m.p. 138.3–140.1 °C;

IR(KBr): 1679 cm^{-1} (C=O); ^1H NMR (400 MHz, DMSO-d_6): 0.93 (d, J = 6.0, 6H, 2Me); 2.15 (m, 1H, CH); 3.01 (s, 3H, Me); 3.87 (d, J = 6.4, 2H, CH_2); 7.68 (t, J = 8.0, 1H, Ar-H); 7.89 (t, J = 8.0, 1H, Ar-H); 7.98 (d, J = 8.0, 1H, Ar-H); 8.73 (d, J = 8.0, 1H); 8.77 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 19.2, 19.2, 26.8, 53.0, 111.7, 122.3, 123.8, 123.8, 126.1, 127.7, 131.6, 147.4, 152.3, 152.6, 158.7, 159.4; EI MS: m/z 268 ([M+2]$^+$, 6), 267 (M$^+$, 40), 210 (20), 211 (100), 183 (25); HREIMS m/z 267.1371 (calcd for $C_{16}H_{17}N_3O$, 267.1372).

*6.1.3. General procedure for the preparation of 3-substituted phenyl-5-methyl-2- aryloxypyrimido[5,4-c]quinolin-4(3H)-one (**7a**–**j**)*

To a solution of **2** (1.15 g, 5 mmol) in CH_2Cl_2 (60 ml) was added Ph_3P (2.62 g, 10 mmol), C_2Cl_6 (2.37 g, 10 mmol) and, in this order, Et_3N (5.0 ml). The mixture was stirred for 24 h at room temperature. Then, the solution was concentrated, and the obtained residue was subjected to column chromatography (silica gel, eluent: Petroleum ether-Acetone, 8:2) to afford the **3** in 70% yield.

To a solution of iminophosphorane **3** (1.1 g, 2 mmol) in dry methylene chloride (20 ml) was added isocyanatobenzene (0.24 g, 2 mmol), 1-fluoro-4-isocyanatobenzene (0.27 g, 2 mmol) or 1-chloro-4-isocyanatobenzene (0.30 g, 2 mmol) under nitrogen at 45 °C. After the reaction mixture was stirred for 12 h, the solvent was removed under vacuum and Et_2O/petroleum ether (1:2 30 ml) was added to precipitate triphenylphosphine oxide. Removal of the solvent gave carbodiimides **4**, which were used directly without further purification.

To the solution of **4** (2 mmol) in CH_3CN (15 ml) was added substituted phenol (2 mmol) and cat solid K_2CO_3 (0.024 g, 0.2 mmol). The mixture was stirred for 4–6 h at 75 °C and then filtered, the filtrate was condensed and the residue was purified by silica gel column chromatography using a 7:3 mixture of petroleum ether-acetone as the eluent. The solvent was evaporated to dryness and the residue recrystallised from ethanol, giving the expected compounds.

*6.1.3.1. 3-(4-chlorophenyl)-5-methyl-2-(naphthalen-1-yloxy)pyrimido-[5,4-c]quinolin-4(3H)- one (**7a**).* Red solid, yield 33.8%, m.p. 208.4–210.4 °C; IR(KBr): 1684.3 cm^{-1} (C=O); ^1H NMR (400 MHz, DMSO-d_6): 2.99 (s, 3H, Me), 7.40 (t, J = 8.0, 1H, Ar-H), 7.52–7.82 (m, 12H, Ar-H), 7.97 (d, J = 7.6, 1H, Ar-H), 8.04 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.1, 109.5, 118.1, 120.8, 121.9, 123.3, 125.2, 125.7, 125.8, 126.1, 126.3, 126.4, 126.4, 127.6, 127.7, 128.0, 129.1, 130.2, 131.8, 133.5, 133.8, 133.9, 146.7, 147.7, 152.5, 154.8, 158.5, 161.3; EI MS: m/z 465 ([M+2]$^+$, 8), 463 (M$^+$, 23), 320 (11), 310 (100), 211 (13), 155 (33), 128 (10), 75 (2); HREIMS m/z 463.1069 (calcd for $C_{28}H_{18}ClO_2N_3$, 463.1071).

*6.1.3.2. 2-(2-methoxyphenoxy)-3-(4-chlorophenyl)-5-methylpyri-mido[5,4-c]quinolin-4(3H)- one (**7b**).* Yellow solid, yield 32.2%, m.p. 209.2–210.3 °C; IR(KBr): 1698.5 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): 3.13 (s, 3H, Me), 3.79 (s, 3H, Me), 7.06 (d, J = 8.0, 2H, Ar-H), 7.19 (d, J = 8.0, 1H, Ar-H), 7.34 (t, J = 8.0, 1H, Ar-H), 7.42–7.47 (m, 3H, Ar-H), 7.54–7.64 (m, 2H, Ar-H), 7.78 (t, J = 8.0, 1H, Ar-H), 7.99 (d, J = 8.0, 1H, Ar-H), 8.18 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 26.6, 56.0, 109.6, 112.7, 120.8, 122.6, 122.9, 124.6, 126.1, 127.4, 127.7, 129.5, 129.8, 132.5, 133.0, 135.2, 140.5, 148.4, 150.8, 153.9, 154.9, 160.1, 162.2; EI MS: m/z 445 ([M+2]$^+$, 9), 443 (M$^+$, 22), 353 (35), 351 (100), 320 (20), 289 (31), 261 (13), 211 (13), 184 (13), 155 (43), 149 (27), 125 (20), 57 (9); HREIMS m/z 443.1032 (calcd for $C_{25}H_{18}ClO_3N_3$, 443.1033).

*6.1.3.3. 2-(4-bromophenoxy)-3-(4-chlorophenyl)-5-methylpyrimido [5,4-c]quinolin-4(3H)-one (**7c**).* White solid, yield 30.2%, m.p. 208.3–210.2 °C; IR(KBr): 1698.5 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): 3.11 (s, 3H, Me), 7.17 (d, J = 8.0, 2H, Ar-H), 7.25–7.28 (m, 2H, Ar-H), 7.40 (d, J = 8.0, 2H, Ar-H), 7.51–7.64 (m, 3H, Ar-H), 7.82 (t, J = 8.0, 1H, Ar-H), 8.03 (d, J = 8.0, 1H, Ar-H), 8.28 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 27.0, 109.7, 119.6, 122.7, 122.9, 123.4, 124.5, 126.0, 126.3, 128.3, 129.4, 129.8, 130.0, 132.3, 132.8, 133.0, 135.2, 135.4, 148.8, 153.7, 155.4, 159.9, 160.2, 162.0; EI MS: m/z 495 ([M + 4]$^+$, 7), 493 ([M + 2]$^+$, 29), 491 (M$^+$, 21), 351 (67), 320 (38), 259 (100), 211 (14), 155 (85), 128 (31); HREIMS m/z 491.0042 (calcd for $C_{24}H_{15}ClBrO_2N_3$, 491.0041).

*6.1.3.4. 2-(benzo[d] [1,3]dioxol-6-yloxy)-3-(4-chlorophenyl)-5-mehylpyrimido[5,4-c]quinolin-4 (3H)-one (**7d**).* White solid, yield 29.5%, m.p. 270.6–272.4 °C; IR(KBr): 1697.8 cm^{-1} (C=O); ^1H NMR (400 MHz, acetone-d_6): 3.00 (s, 3H, Me), 6.12 (s, 2H, CH_2), 6.83 (d, J = 8.0, 1H, Ar-H), 6.95 (d, J = 8.0, 1H, Ar-H), 7.29 (d, J = 8.0, 1H, Ar-H), 7.54 (m, 1H, Ar-H), 7.69 (d, J = 8.0, 2H, Ar-H), 7.72 (d, J = 8.0, 2H, Ar-H), 7.85 (t, J = 8.0, 1H, Ar-H), 7.94 (d, J = 8.0, 1H, Ar-H), 8.31 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.7, 102.0, 103.8, 108.0, 109.7, 114.2, 122.3, 124.0, 126.4, 128.1, 129.4, 130.5, 130.5, 132.4, 132.4133.7, 133.9, 145.3, 145.7, 147.7, 148.1, 152.9, 155.5, 159.0, 161.6; EI MS: m/z 459 ([M+2]$^+$, 13), 457 (M$^+$, 29), 320 (23), 304 (100), 211 (3), 155 (43); HREIMS m/z 457.0827 (calcd for $C_{25}H_{16}ClO_4N_3$, 457.0827).

*6.1.3.5. 2-(2-Chloro-4-nitrophenoxy)-3-(4-chlorophenyl)-5-methyl-pyrimido[5,4-c]quinolin-4- (3H)-one (**7e**).* White powder, yield 34.6%, m.p. 248.7–251.2 °C; IR(KBr): 1695 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): 3.12 (s, 3H, Me), 7.48 (m, 3H, Ar-H), 7.61 (m, 3H, Ar-H), 7.81 (d, J = 8.0, 1H, Ar-H), 8.08 (d, J = 8.0, 2H, Ar-H), 8.33 (d, J = 8.0, 1H, Ar-H), 8.45 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 26.4, 109.9, 120.0, 122.4, 123.3, 124.2, 124.7, 126.1, 126.6, 127.7, 128.3, 128.7, 129.4, 130.1, 132.2, 132.9, 135.9, 146.1, 148.4, 152.0, 153.0, 153.2, 160.0, 161.7; EI MS: m/z 494 ([M+2]$^+$, 59), 492 ([M]$^+$, 92), 459 (20), 457 (60), 341 (20), 339 (63), 322 (32), 320 (100), 293 (14), 211 (10), 155 (99), 143 (14), 127 (53), 114 (11); HREIMS m/z 492.0388 (calcd for $C_{24}H_{14}Cl_2N_4O_4$, 492.0388).

*6.1.3.6. 2-(4-fluorophenoxy)-3-(4-fluorophenyl)-5-methylpyrimido [5,4-c]quinolin-4(3H)-one (**7f**).* Pale yellow powder, yield 30.5%, m.p.196.0–197.5 °C; IR(KBr): 1694 cm^{-1} (C=O); ^1H NMR (400 MHz, acetone-d_6): 2.97 (s, 3H, Me), 7.29–7.89 (m, 10H, Ar-H), 7.59 (t, J = 8.0, 1H, Ar-H), 8.24 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ_C 26.7, 109.7, 116.1, 116.2, 116.4, 122.3, 123.7, 123.8, 123.9, 126.4, 128.1, 130.7, 130.8, 131.1, 132.4, 147.5, 148.1, 152.7, 155.5, 158.7, 158.9, 160.9, 161.7, 163.3; EI MS: m/z 416 ([M+1]$^+$, 21), 415 (M$^+$, 71), 350 (28), 349 (83), 322 (9), 321 (30), 305 (12), 304 (47), 279 (24), 278 (100), 227 (12), 211 (21), 185 (15), 184 (59), 156 (8), 155 (46), 143 (8), 128 (9); HREIMS m/z 415.1124 (calcd for $C_{24}H_{15}F_2N_3O_2$, 415.1125).

*6.1.3.7. 2-(4-Chloro-3-methylphenoxy)-3-(4-fluorophenyl)-5-methyl-pyrimido[5,4-c]quinolin-4 (3H)-one (**7g**).* Pale yellow powder, yield 34.5%, m.p.193.8–194.9 °C; IR(KBr): 1694 cm^{-1} (C=O); ^1H NMR (400 MHz, DMSO-d_6): 2.37 (s, 3H, Me), 2.97 (s, 3H, Me), 7.30 (d, J = 8.0, 1H, Ar-H), 7.36–7.61 (m, 5H, Ar-H), 7.72 (br s, 2H, Ar-H), 7.86 (t, J = 8.0, 1H, Ar-H), 7.96 (d, J = 8.0, 1H, Ar-H), 8.15 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 19.0, 25.9, 109.4, 115.6, 115.8, 120.5, 122.0, 123.5, 123.6, 125.9, 127.7, 129.3, 130.2, 130.4, 130.7, 131.8, 136.7, 147.9, 149.7, 152.4, 154.6, 158.5, 160.5, 161.2, 163.0; EI MS: m/z 445 ([M]$^+$, 50), 308 (100), 304 (47), 273 (52), 211 (12), 155 (75), 128 (20), 114 (8), 57 (4); HREIMS m/z 445.0997 (calcd for $C_{25}H_{17}ClFN_3O_2$, 445.0997).

*6.1.3.8. 2-(2-chlorophenoxy)-3-(4-fluorophenyl)-5-methylpyrimido [5,4-c]quinolin-4(3H)-one (**7h**).* Pale yellow crystal, yield 33.6%, m.p.207.7–208.9 °C; IR(KBr): 1674 cm^{-1} (C=O); ^1H NMR (400 MHz,

Acetone-d_6): 3.01 (s, 3H, Me), 7.42–7.49 (m, 4H, Ar-H), 7.56 (m, 2H, Ar-H), 7.66 (d, J = 8.0, 1H, Ar-H), 7.78–7.85 (m, 3H, Ar-H), 7.97 (d, J = 8.0, 1H, Ar-H), 8.15 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, Acetone-d_6): δ_C 27.1, 110.8, 116.9, 117.2, 123.5, 124.9, 125.1, 126.8, 126.8, 127.3, 128.8, 129.2, 131.2, 131.6, 131.7, 132.1, 133.0, 148.5, 149.6, 153.8, 160.1, 162.4, 162.6, 164.9; EI MS: *m/z* 431 ([M]$^+$, 42), 396 (100), 321 (12), 304 (42), 294 (30), 259 (21), 201 (19), 155 (54), 128 (15), 77 (4); HREIMS *m/z* 431.0845 (calcd for $C_{24}H_{15}ClFN_3O_2$, 431.0844).

*6.1.3.9. 2-(2-methoxyphenoxy)-5-methyl-3-phenylpyrimido[5,4-c] quinolin-4(3H)-one (**7i**).* White powder, yield 35.5%, m.p. 216.8–219.1 °C; IR(KBr): 1694 cm^{-1} (C=O); ^{1}H NMR (400 MHz, Acetone-d_6): 3.01 (s, 3H, Me), 3.82 (s, 3H, $-OCH_3$) , 7.08 (t, J = 8.0, 1H, Ar-H), 7.24 (d, J = 8.0, 1H, Ar-H), 7.31 (d, J = 8.0, 1H, Ar-H), 7.37 (t, J = 8.0, 1H, Ar-H), 7.47 (t, J = 8.0, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.65 (br s, 4H, Ar-H), 7.82 (t, J = 8.0, 1H, Ar-H), 7.95 (d, J = 8.0, 1H, Ar-H), 8.18 (d, J = 8.0, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.7, 56.2, 109.6, 113.4, 118.2, 120.8, 122.3, 122.7, 123.7, 126.3, 127.6, 128.1, 128.5, 128.8, 129.1, 129.3, 132.4, 134.9, 140.3, 148.1, 150.7, 152.9, 155.2, 159.1, 161.6; EI MS: *m/z* 410 ([M+1]$^+$, 24), 409 ([M]$^+$, 85), 291 (11), 290 (62), 289 (100), 261 (45), 211 (13), 155 (74), 128 (20), 77 (13); HREIMS *m/z* 409.1426 (calcd for $C_{25}H_{19}N_3O_2$, 409.1426).

*6.1.3.10. 2-(2,4-dichlorophenoxy)-3-phenyl-5-methyl-3H-pyrimido [5,4-c]quinolin-4-one (**7j**).* White powder, yield 30.9%, m.p. 225.6–226.9 °C; IR(KBr): 1698 cm^{-1} (C=O); ^{1}H NMR (600 MHz, Acetone-d_6): δ_H 3.11 (3H, s), 7.26–7.63 (9H, m, 9 × Ar-H), 7.76 (1H, td, J = 8.4,1.2Hz Ar-H), 7.98 (1H, d, J = 9.0Hz, Ar-H), 8.16 (1H, d, J = 9.0Hz, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.7, 109.9, 122.2, 123.7, 125.7, 126.6, 127.0, 128.2, 128.5, 128.8, 129.3, 129.5, 129.9, 131.5, 132.6, 146.2, 148.1, 152.5, 154.2, 159.0, 161.5; EI MS: *m/z* 449 ([M+2]$^+$, 29), 448 ([M+1]$^+$, 11), 447 ([M]$^+$, 47), 414 (31), 413 (26), 412 (100), 330 (48), 329 (13), 328 (78), 293 (34), 286 (50), 211 (12), 155 (83), 143 (14), 128 (23), 114 (9), 101 (7), 77 (12); HREIMS *m/z* 447.0533 (calcd for $C_{24}H_{15}Cl_2N_3O_2$, 447.0534).

*6.1.4. Synthesis of 2-(tert-butylamino)-3-phenyl-5-methyl-3H-pyrimido[5,4-c]quinolin-4-one (8a), 2-(4-chlorophenylamino)-5-methyl-3-aminopyrimido[5,4-c]quinolin-4(3H)-one (**8b**) and 2-(phenylamino)-5-methyl-3-aminopyrimido[5,4-c]quinolin-4(3H)-one (**8c**)*

2-methylpropan-2-amine or hydrazine hydrate (2 mmol) was added into the solution of **4** in CH_2Cl_2 (10 ml). After the reaction mixture was stirred continuously for 0.5–1 h at room temperature, the solvent was removed and 10 ml of anhydrous ethanol with several drops of sodium ethoxide in ethanol were added. After stirring for another 12 h at room temperature, the solution was concentrated and the residue was purified by silica gel column chromatography using a 7:3 mixture of petroleum ether-acetone as the eluent to give pure compounds **8a**–**c**.

*6.1.4.1. 2-(tert-butylamino)-3-phenyl-5-methyl-3H-pyrimido[5,4-c] quinolin-4-one (**8a**).* Light yellow powder, yield 30.6%, m.p.240.7–242.6 °C; IR(KBr): 1681 cm^{-1} (C=O); ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 1.51 (9H, s), 3.04 (3H, s), 7.34 (2H, d, J = 7.2 Hz, 2 × Ar-H), 7.59 (4H, m, 4 × Ar-H), 7.77 (1H, t, J = 7.2 Hz, Ar-H), 7.97 (1H, d, J = 8.4 Hz, Ar-H), 8.70 (1H, d, J = 7.8 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 27.1, 29.0, 53.2, 107.5, 123.5, 124.8, 125.4, 128.1, 128.7, 130.1, 130.9, 131.7, 134.6, 151.5, 155.3, 160.5, 162.2; EI MS: *m/z* 359 ([M + 1]$^+$, 9), 358 ([M]$^+$, 35), 302 (60), 301 (100), 155 (10), 77(5); HREIMS *m/z* 358.1799 (calcd for $C_{22}H_{22}N_4O$, 358.1798).

*6.1.4.2. 2-(4-chlorophenylamino)-5-methyl-3-aminopyrimido[5,4-c] quinolin-4(3H)-one (**8b**).* White solid, yield 40.0%, m.p. 285.7–287.3 °C; IR(KBr): 1675 cm^{-1} (C=O), 3325 cm^{-1} (NH_2); ^{1}H NMR (400 MHz, DMSO-d_6): 2.97 (s, 3H, Me), 5.75 (s, 2H, NH_2), 7.51–7.61 (m, 3H, Ar-H), 7.79–8.00 (m, 4H, Ar-H), 8.53 (d, J = 8.0, 1H, Ar-H), 9.98 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.2, 107.2, 122.5, 123.4, 123.4, 124.2, 125.4, 127.6, 127.7, 128.2, 128.2, 131.3, 136.7, 147.9, 151.0, 152.7, 158.6, 160.4; EI MS: *m/z* 353 ([M + 2]$^+$, 30), 351 ([M]$^+$, 100), 322 (27), 320 (42), 212 (20), 155 (39), 128 (10); HREIMS *m/z* 351.0888 (calcd for $C_{18}H_{14}Cl\ N_5O$, 351.0888).

*6.1.4.3. 2-(phenylamino)-5-methyl-3-aminopyrimido[5,4-c]quinolin-4(3H)-one (**8c**).* White solid, yield 42.1%, m.p. 286.8–290.0 °C; IR(KBr): 1659 cm^{-1} (C=O), 3385, 3323 cm^{-1} (NH_2); ^{1}H NMR (400 MHz, DMSO-d_6): 2.98 (s, 3H, Me), 5.75 (s, 2H, NH_2), 7.19 (t, J = 8.0, 1H, Ar-H), 7.47 (t, J = 8.0, 2H, Ar-H), 7.59 (t, J = 8.0, 1H, Ar-H), 7.78–7.95 (m, 4H, Ar-H), 8.54 (d, J = 8.0, 1H, Ar-H), 9.81 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.9, 107.0, 121.5, 122.4, 123.6, 123.9, 125.1, 125.1, 127.6, 128.2, 128.2, 131.0, 137.6, 147.8, 150.9, 152.7, 158.4, 160.2; EI MS: *m/z* 317 ([M]$^+$, 100), 288 (16), 286 (49), 212 (16), 155 (26), 128 (7); HREIMS *m/z* 317.1274 (calcd for $C_{18}H_{15}N_5O$, 317.1274).

*6.1.5. Synthesis of 10-methyl-3-phenyl- [1,2,4]triazolo[1,5-a]pyrimidin[5,4-c]quinolin- 11(3H)-one (**9**)*

To a solution of 2-(phenylamino)-5-methyl-3-aminopyrimido [5,4-*c*]quinolin-4(3*H*)-one **8c** (0.99 g, 3mmol) prepared above, triethyl orthoformate (8 ml) was added under vacuum. The solution was stirred and refluxed for 4 h. At which time the reaction was completed, the solution was concentrated,the residue was recrystallized from petroleum ether/ethanol (8/2) to give pure compound **9**. Pale solid, yield 37.7%, m.p. 335.0–336.0 °C; IR(KBr): 1695 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): 3.08 (s, 3H, Me), 7.62 (d, J = 8.0, 2H, Ar-H), 7.74 (d, J = 8.0, 2H, Ar-H), 7.83–7.92 (m, 2H, Ar-H), 8.08 (d, J = 8.0, 2H, Ar-H), 8.66 (d, J = 8.0, 1H, Ar-H), 9.49 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 163.5, 155.8, 151.3, 144.2, 138.3, 136.3, 133.1, 131.0, 130.1, 127.0, 125.3, 124.8, 120.7, 108.5, 23.0; EI MS: *m/z* 328 ([M+1]$^+$, 20), 327 ([M]$^+$, 100), 301 (4), 300 (18), 271 (4), 128 (2), 77 (9); HREIMS *m/z* 327.1121 (calcd for $C_{19}H_{13}N_5O$, 327.1121).

6.2. Antiproliferative activity

6.2.1. Cell culture

All the cell lines except MCF-7 used in antiproliferative assay were cultured in RPMI 1640 medium containing 10% fetal calf serum. MCF-7 were cultured in DMEM medium containing 10% fetal calf serum.

6.2.2. Viability assay

The MTT assay was used to evaluate the in vitro antiproliferative activity of these synthesized compounds. This method is based on the reduction of the soluble 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells.

Cells were harvested during logarithmic growth phase and seeded in 96 well plates at a density of 2 × 10^4/ml cells/ml in a final volume of 190 ml/well and incubated at 37 °C in a 5% CO_2 incubator. After 24 h, 10 μL tested compounds was added to 96-well plates and cultured at 37 °C for 72 h. 20 μL of MTT (5 mg/ml stock solution of saline) was added to each well and incubated for 3 h at 37 °C. The supernatant was carefully removed from each well and 100 ml of DMSO was added to each well to dissolve the formazan crystals

which were formed by the cellular reduction of MTT. The absorbance of each well was detected in a microplate reader at 570 nm with 655 nm as reference. IC_{50} values were calculated according to the dose-dependent curves (Bliss's software).

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