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De novo design, synthesis and biological evaluation of 1,4-dihydroquinolin-4-ones and 1,2,3,4-tetrahydroquinazolin-4-ones as potent kinesin spindle protein (KSP) inhibitors

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ABSTRACT

Kinesin spindle protein (KSP) inhibitors are a promising class of anticancer agents that cause mitotic arrest in cells from a failure to form functional bipolar mitotic spindles. Here, we report the design, synthesis and biological evaluation of a novel series of 1,4-dihydroquinolin-4-ones and 1,2,3,4-tetrahydroquinazolin-4-ones using de novo design method. The synthesized compound was evaluated and proved to have potent inhibitory activities in the KSP ATPase. Compounds **15j** and **15p** show potent inhibitory activities in cell proliferation assays. Preferred compound **15j** markedly induced G2/M phase cell cycle arrest with characteristic monoastral spindles and subsequent cell death in A549 cells. In vivo evaluation of **15j** on the growth of transplantable S180 sarcoma in mice suggested its therapeutic potential for further development.

1. Introduction

Mitosis is a highly regulated process during cell division. Antimitotic agents, such as vinca alkaloids, taxol and epothilones, are a major class of cytotoxic drugs in the treatment of cancer. They interfere with the polymerization–depolymerization process of microtubules and lead to cell cycle arrest in mitosis.[1,2] However, these agents targeting microtubules displayed several mechanism-related undesirable side effects, such as neurotoxicity.[3] Thus, it is necessary to develop antimitotics which do not act on microtubule directly.

Members of the kinesin superfamily play important roles in cargo transport, spindle and chromosome movement, and regulation of microtubule dynamics.[4] Kinesin spindle protein (KSP, also known as Eg5), a member of the BimC kinesin subfamily, is responsible for the formation of the bipolar spindle,[5] which plays an important role in the early stage of mitosis and mediates centrosome separation.[6] Inhibition of KSP leads to a stable mitotic block without affecting microtubules, resulting in apoptotic cell death.[7] Therefore, KSP inhibitors have become attractive and promising anti-proliferative agents for cancer chemotherapy. Since the identification of the first KSP inhibitor, monastrol,[8] several inhibitors that exhibit great chemical diversity have also been reported including CK0106023 and many more (Fig. 1).[9–12]

In the literatures reported before, the lead compounds were usually gained by screen. Thus it is difficult to find novel inhibitors by compound screen. To design novel KSP inhibitors, we used the drug discovery program LUDI (Accelrys), an automated fragment-based suggestive program for the de novo design of protein ligands. LUDI uses a constrained docking approach that identifies optimal fragments fitting the binding cavity.

Here we describe the identification of a series of 1,4-dihydroquinolin-4-ones and 1,2,3,4-tetrahydroquinazolin-4-ones using the method of de novo design for the first time. Their in vitro and in vivo activities were also evaluated.

2. Results and discussion

Based on the 3-D structure of KSP, drug design was processed with the aid of LUDI produced by ACCERLRY. The 3D complex structure of monastrol with KSP (PDB ID: 1Q0B) was imported into the graphic modeling program InsightII 98.0 (Accelrys Inc.). We screened several small fragments (fragment database of Cambridge) which could interact with residues in the binding pocket of KSP by using the LUDI program. Twenty fragments with higher scores were

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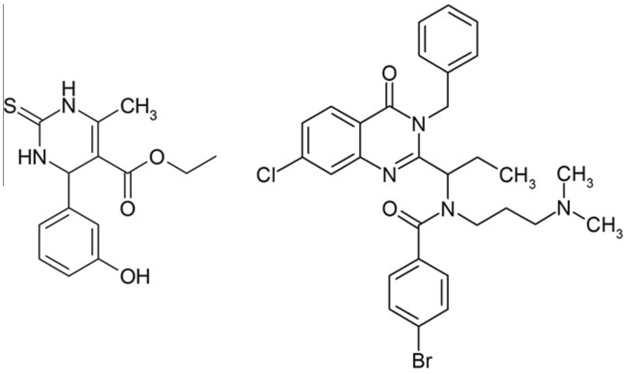


Figure 1. The KSP inhibitors monastrol and CK0106023.

selected to be grown further. Then, all of the fragments were put into the pocket of KSP and the common grounds were selected to design further. After several cycles, the structure which was shown as Figure 2 was gained with highest score. The compound was docked in to the KSP again by using the program GOLD (Fig. 2).

For output structures from de novo design programs are often not synthetically feasible, some modification was then done according to the docking result (Fig. 3). There is an H-bond between the ligand and the Glu116 of the receptor. There is also a side chain near the Glu118 of the receptor. Maybe an H-donor could be added here. We attached here a group of 3′ or 4′-OH phenyl. A benzene ring of the Tyr211 and an indole ring of the Trp127 were near the 3-CH_3, hydrophobic groups such as aryls could be attached here. The 2-hydroxyethyl at 6-position did not show any essential effect, and could be replaced with some aliphatic amino groups, such as piperazine, morphiline and piperidine. For easier synthesis, we changed the dihydroquinoline core into dihydroquinolinone. The modified structure was then validated by docking again (Fig. 4a).

The designed compounds were synthesized through the route outlined in Scheme 1. Nitration of 3′-chloro-acetophenone (**1**) gave 2′-nitro-5′-chloroacetophenone (**2**). Nucleophilic displacement of the 5′-chloro group by certain *sec*-amines such as *N*-methyl piperazine, morpholine or piperidine gave compound **3**. Condensation of **3** with benzaldehyde followed by hydrogenation and then treated with various acyl chlorides led to compound **5**. Cyclization of **5** in ethanolic sodium solution followed by deprotection through hydrogenation afforded 1,4-dihydroquinolin-4-ones **7i**–**7p** as target compounds.

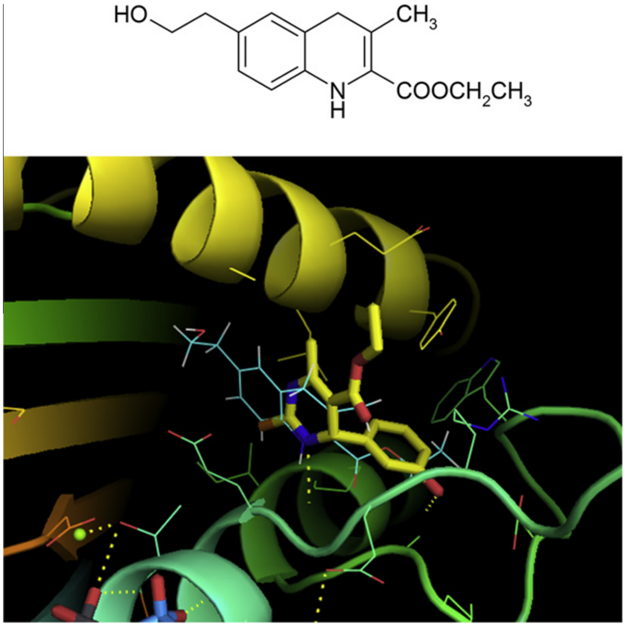


Figure 2. Structure with highest score (left) and the docking result with KSP (displayed by blue lines, right). Monastrol was used as a reference (displayed by yellow sticks).

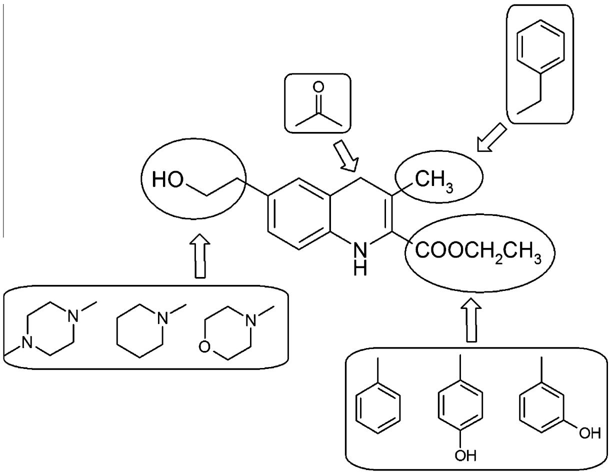


Figure 3. Modifications of the gained structure.

Compounds **7a**–**7h** were synthesized through the route very similar to that of **7i–7p** as shown in Scheme 2. Started from 3′-chloro-propiophenone, after nitration, nucleophilic displacement, hydrogenation, acylation, cyclization and then deprotection through hydrogenation afforded **7a**–**7h** as target compounds.

All synthesized compounds were tested for their inhibitory activities against KSP determined by measuring the MT-activated ATPase activity.[13] The anti-proliferative cytotoxic activity was also evaluated by using A549 and AGS cancer cell lines.[11] Monastrol[14] and CK0106023[15] were synthesized and used as moderate and potent positive control respectively.

The initial series tested were the 1,4-dihydroquinolin-4-ones (**7a–7p**) represented in Table 1. All the 16 tested compounds show more potent KSP inhibitory activities comparing to monastrol, 4 compounds (**7a**, **7m**, **7n**, **7p**) display lower KSP IC_{50} values than that of CK0106023.

Compounds with benzyl group attached to C3-position display more potent KSP inhibitory activities than compounds with methyl group at the same position, when the substitutes at C6-position are piperidin-1-yl or morphilin-4-yl. This result is consistent with our prediction. While it is contrary for the compounds with 4-CH_3-piperazin-1-yl group attached to C6-position. We consider that it may be due to the larger bulk of 4-CH_3-piperazin-1-yl group than that of piperidin-1-yl or morphilin-4-yl. The bulky substitute at C6-position hinders the larger benzyl group comparing to methyl at C3-position binding to the pocket. For compounds with benzyl group linked to C3-position, the hydroxyl group on the C2 phenyl ring improves the inhibitory activities against KSP, but the position of the hydroxyl group influence not markedly, whether at C3′- or C4′-position. While for compounds with methyl group at C3-position, the inhibitory activities were not improved but even decreased if there is a hydroxyl group attached to C3′- or C4′-position of the C2 phenyl ring. The reason led to this result is still studied.

The potent KSP inhibitory activities of the 1,4-dihydroquinolin-4-ones seem to prove a success in the design of novel KSP inhibitors, and the good result promoted us to test the in vitro anti-proliferative activities of the compounds. Unfortunately, all the 16 compounds show poor cytotoxicities against A549 and AGS, with

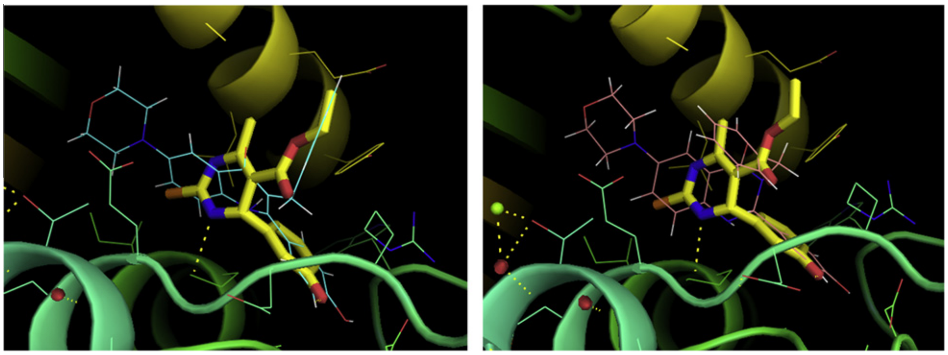
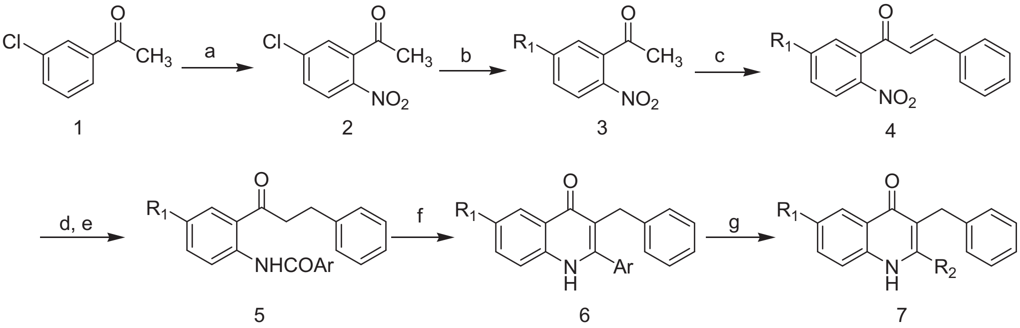
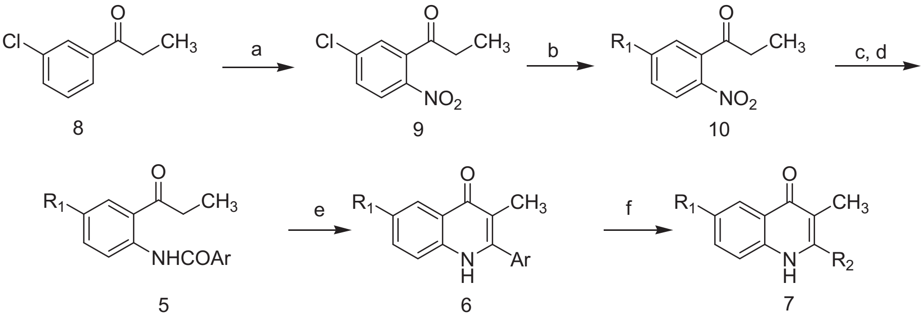


Figure 4. Docking results of the designed compounds 1,4-dihydroquinolin-4-one (displayed by blue lines, left) and 1,2,3,4-tetrahydroquinazolin-4-one (displayed by pink lines, right). Monastrol was used as a reference (displayed by yellow sticks).



Scheme 1. Reagents and conditions: (a) KNO_3, H_2SO_4, −10 to −5 °C, 30 min, 83.69%; (b) RH, DMF, 110 °C, 4.5 h, 81.22–92.20%; (c) benzaldehyde, NaOH, H_2O, room temperature, 2 h, 84.23–95.53%; (d) 10% Pd/C, H_2, methanol, ethyl acetate, room temperature, 4 h; (e) benzoyl chloride or 3-benzoxybenzoyl chloride or 4-benzoxybenzoyl chloride, TEA, THF, 0 °C, 2 h; (f) Na, EtOH, reflux, 12 h; (g) 10% Pd/C, H_2, EtOH, room temperature, 4 h.



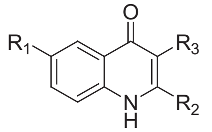
Scheme 2. Reagents and conditions: (a) HNO_3, −5 to −3 °C, 20 min, 88.30%; (b) R_1H, DMF, 110 °C, 4.5 h, 79.24–93.49%; (c) 10% Pd/C, H_2, methanol, ethyl acetate, room temperature, 4 h; (d) benzoyl chloride or 3-benzoxybenzoyl chloride or 4-benzoxybenzoyl chloride, TEA, THF, 0 °C, 2 h, 21.11–96.40% in two steps; (e) Na, EtOH, reflux, 12 h, 39.33–85.99%; (f) 10% Pd/C, H_2, EtOH, room temperature, 4 h, 17.07–97.21%.

IC_{50} values more than 10 μM. This may be ascribed to poor solubilities of 1,4-dihydroquinolin-4-ones. Thus the solubility of the compounds should be increased in the further research.

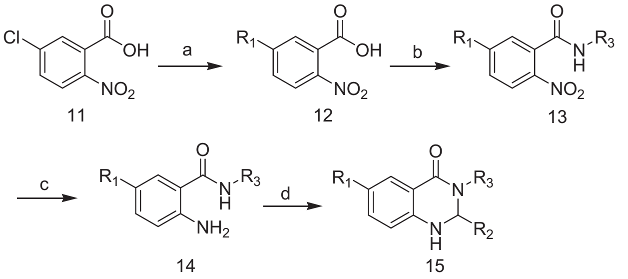
It could be clearly seen that the whole molecule of the 1,4-dihydroquinolin-4-ones share a large conjugated ring. Compounds containing this kind of structure usually possess poor solubility. In order to improve the solubility, the large conjugated ring should be changed. At the same time, the skeleton of the molecule should be kept in order to adapt to the binding cavity. Firstly, the double bond between C2 and C3 was replaced by single bond, and then the C3 atom was changed to N3 for easier synthesis. Thus, compounds with 1,2,3,4-tetrahydroquinolin-4-one core were designed. Then the newly designed molecule was docked into the binding pocket in order to check whether there is a great change in binding mode. It could be found in Fig. 4b, the newly designed molecule fits very well with a same binding mode comparing to 1,4-dihydroquinolin-4-ones. The large conjugated ring was disappeared and the solubility may be improved.

Compounds **15a**–**15p** were synthesized as displayed in Scheme 3. Nucleophilic displacement of the 5-chloro group of 5-chloro-2-nitrobenzoic acid (**11**) by certain second amines such as *N*-methyl piperazine, morpholine or pyrroline gave compound **12**. Condensation of **12** with benzylamine or methylamine hydrochloride using PyBOP as a coupling reagent followed by reduction of the nitro group led to compound **14**. In the step of reduction, hydrogenation was used when the *N*-substitute was methyl. While reduction by zinc powder was used on *N*-benzyl compounds to avoid the removing of benzyl group. Cyclization of **14** using the reported method gave 1,2,3,4-tetrahydroquinolin-4-ones **15a**–**15p** as target compounds.[16] Target compounds were all synthesized as racemic mixtures.

Table 1
Chemical structures of the 1,4-dihydroquinolin-4-ones and their KSP inhibitory activities



Compounds	R_1	R_2	R_3	KSP IC_{50} (μM)
7a	4-CH_3-piperazin-1-	Ph-	CH_3–	0.051
7b	4-CH_3-piperazin-1-	3-OH-Ph-	CH_3–	0.334
7c	4-CH_3-piperazin-1-	4-OH-Ph-	CH_3–	1.178
7d	Piperidin-1-	Ph-	CH_3–	0.360
7e	Piperidin-1-	3-OH-Ph-	CH_3–	0.232
7f	Piperidin-1-	4-OH-Ph-	CH_3–	1.943
7g	Morphilin-4-	Ph-	CH_3–	2.345
7h	Morphilin-4-	3-OH-Ph-	CH_3–	1.443
7i	4-CH_3-piperazin-1-	Ph-	$PhCH_2$–	3.959
7j	4-CH_3-piperazin-1-	3-OH-Ph-	$PhCH_2$–	1.307
7k	4-CH_3-piperazin-1-	4-OH-Ph-	$PhCH_2$–	1.485
7l	Piperidin-1-	Ph-	$PhCH_2$–	0.146
7m	Piperidin-1-	3-OH-Ph-	$PhCH_2$–	0.020
7n	Piperidin-1-	4-OH-Ph-	$PhCH_2$–	0.042
7o	Morphilin-4-	Ph-	$PhCH_2$–	0.828
7p	Morphilin-4-	3-OH-Ph-	$PhCH_2$–	0.023
Monastrol				7.452
CK0106023				0.053



Scheme 3. Reagents and conditions: (a) R_1H, 110 °C, 2 h, 95.04–100%; (b) benzylamine/methylamine hydrochloride, PyBOP, TEA, THF, room temperature, 2 h, 31.56–92.14%; (c) 10% Pd/C, H_2, EtOH, 4 h or Zn, CH_3COOH, CH_2Cl_2, room temperature, 2 h, 87.77–100%; (d) benzaldehyde or 3-hydroxybenzaldehyde or 4-hydroxybenzaldehyde, 4-toluene sulfonic acid, *N*,*N*-dimethylactamide, room temperature, 2 h, 14.55–84.73%.

The synthesized 1,2,3,4-tetrahydroquinolin-4-ones were tested as listed in Table 2. Among the 16 tested compounds, 14 compounds show more potent KSP inhibitory activities comparing to monastrol and 4 compounds (**15i**, **15j**, **15o** and **15p**) display lower KSP IC_{50} values than that of CK0106023. This result was comparable to 1,4-dihydroquinolin-4-ones, suggesting the binding abilities of the 1,2,3,4-tetrahydroquinolin-4-ones to target protein were kept. According to the SAR of 1,4-dihydroquinolin-4-ones, 4-CH_3-piperazin-1-yl group at C6-position was replaced by a smaller bulk group pyrrolidin-1-yl.

As listed in Table 2, compounds with pyrrolidin-1-yl group attached to C6-position show excellent KSP inhibitory activities whether the C3 substitute is methyl or benzyl group. Our former prediction was confirmed by this result. Compounds with benzyl group attached to C3-position display more potent KSP inhibitory activities than compounds with methyl group at the same position, when the substitutes at C6-position is piperidin-1-yl or pyrrolidin-1-yl. This observation was also reasonable according to our prediction. Surprisingly, the 6-(morpholin-4-yl)-3-benzyl compounds **15k**, **15l** and **15m** show weak enzyme inhibition comparing to 6-(morpholin-4-yl)-3-methyl compounds.

For compounds with benzyl group linked to C3-position, the hydroxyl group on the C2 phenyl ring improves the inhibitory activities against KSP, whether at C3′- or C4′-position. While for compounds with methyl group at C3-position, the inhibitory activities were not improved but even decreased if there is a hydroxyl group attached to C3′- or C4′-position of the C2 phenyl ring. This result is consistent with that of the 1,4-dihydroquinolin-4-ones.

The potent KSP inhibitory activities of most of the 1,2,3,4-tetrahydroquinolin-4-ones led us to test the in vitro anti-proliferative activities of the compounds. As shown in Table 2, although most compounds show IC_{50} values against A549 and AGS more than 10 μM, it was exciting that there are 2 compounds, **15j** and **15p**, show potent in vitro cytotoxicities, even compared to CK0106023, a reported potent cellular poison. Compound **15j** and **15p** displayed ~5- and ~2-fold more potent KSP inhibitory activity than CK0106023 respectively. Compound **15j** showed similar in vitro cytotoxicity against A549 with CK0106023 and ~8-fold more potent against AGS than CK0106023, while **15p** showed similar in vitro cytotoxicity against AGS with CK0106023. Compound **15j** was then selected for further in vitro and in vivo test due to its potent KSP inhibitory and anti-proliferative activities.

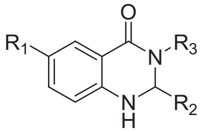
3. 15j Induced the G2/M phase cell cycle arrest

Suppression of cell growth by compound **15j** can be explained by arrest during the cell cycle.[17] As shown in Figure 5, DNA flow cytometric analysis indicated a dose-dependent accumulation of A549 cells at the G2/M phase which was seen as early as 24 h treatment (from 8.76% to 65.85%). For CK0106023-treated A549 cells, a dose-dependent accumulation from 8.76% to 75.50% at the G2/M phase was also seen in 24 h treatment (displayed in Fig. S1 as Supplementary data). G2/M accumulations were strengthened following 48 h treatment and induced a profound sub-G0 peak at the higher doses (12 and 16 μM). Thus, the anti-proliferative effect of **15j** was suggested to be attributable to the induction of cell cycle arrest at the G2/M phase of the cell cycle.

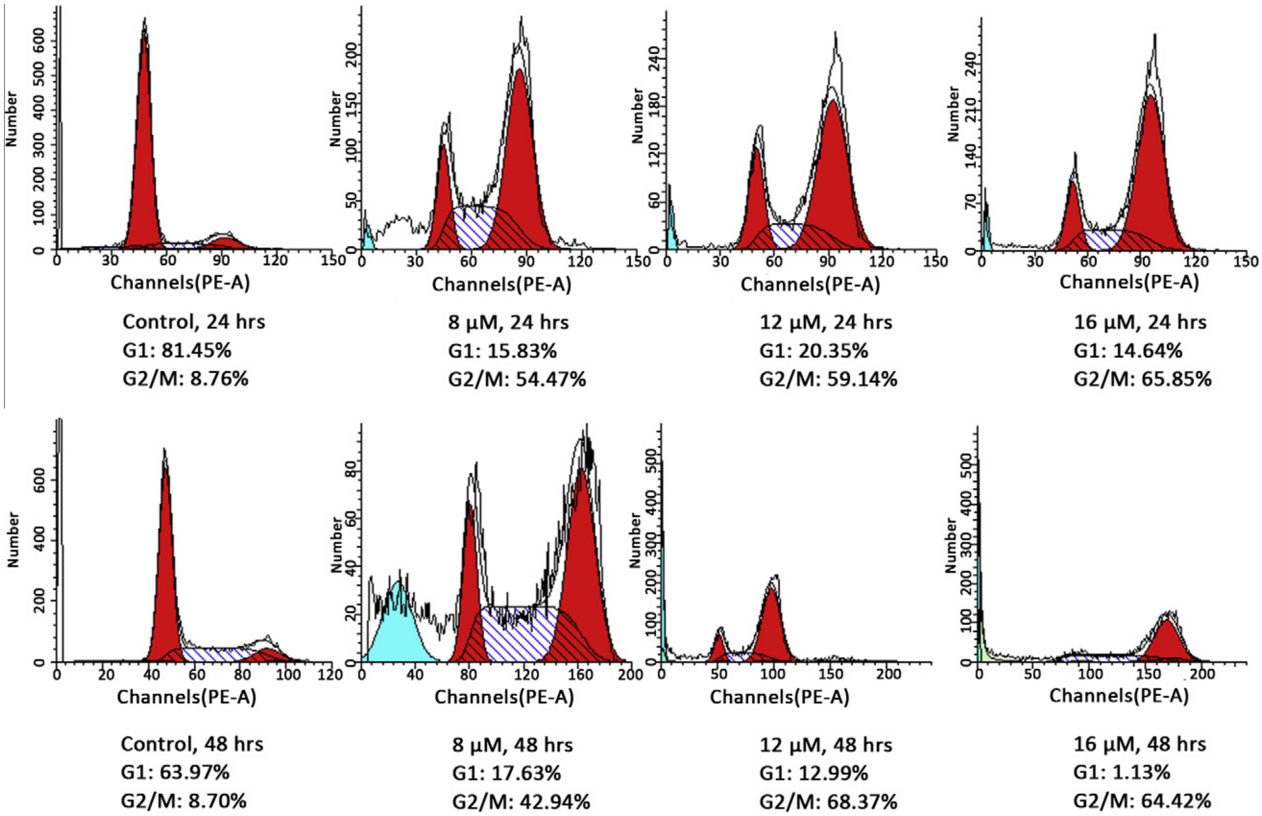
4. 15j Induced cell death via apoptosis

To further confirm the apoptosis induced by **15j**, flow cytometry with the fluorescein isothiocyanate Annexin V/propidium iodide (PI) double-staining assay was carried out to evaluate whether **15j**-induced cell death was due to apoptosis or necrosis.[17] As shown in Figure 6, after treated with 8, 12 and 16 μM **15j** for 24 h, the early and median apoptotic cells (right low section of fluorocytogram) were increased strikingly (from 0.4% to 48.0%, respectively) and the late apoptotic and necrotic cells (right upper section of fluorocytogram) were also increased (from 0.1% to 3.4%). Another apoptotic characteristic is the changes of mitochondrial membrane potential. As seen in Figure 7A, treatment of A549 cells with **15j** (8, 12 or 16 μM) for 24 h decreased the mitochondrial membrane potential in a dose-dependent manner. The signal represents apoptotic cells (region Q4) increased from 37.2% in control cells to 95.2% in treated cells (24 h, 16 μM). As the Bcl-2 family members are key regulators of apoptosis and are overexpressed in many malignancies. The up-regulation of Bcl-2 protects cells from apoptosis, whereas cells with increased Bax expression undergo apoptosis by suppressing Bcl-2 activity. The expression of Bcl-2 and Bax in **15j**-treated A549 cells were detected by Western blotting. It was shown that the expression of Bcl-2 was strikingly decreased and that of Bax was increased in **15j**-treated A549 cells, this led to an outstanding increase of the Bax/Bcl-2 ratio (Fig. 7B). Another apoptosis-related protein, p53, was also examined. The

Table 2
Chemical structures of the 1,2,3,4-tetrahydroquinolin-4-ones and their in vitro activities



Compounds	R^1	R^2	R^3	KSP IC_{50} (μM)	A549 IC_{50} (μM)	AGS IC_{50} (μM)
15a	Piperidin-1-	CH_3–	3-OH-Ph-	9.925	>10	>10
15b	Piperidin-1-	CH_3–	4-OH-Ph-	3.409	>10	>10
15c	Morpholin-4-	CH_3–	Ph-	0.162	>10	>10
15d	Morpholin-4-	CH_3–	4-OH-Ph-	1.047	>10	>10
15e	Pyrrolidin-1-	CH_3–	Ph-	0.169	>10	>10
15f	Pyrrolidin-1-	CH_3–	3-OH-Ph-	0.170	>10	>10
15g	Pyrrolidin-1-	CH_3–	4-OH-Ph-	0.149	>10	>10
15h	Piperidin-1-	$PhCH_2$–	Ph-	0.092	>10	>10
15i	Piperidin-1-	$PhCH_2$–	3-OH-Ph-	0.033	>10	>10
15j	Piperidin-1-	$PhCH_2$–	4-OH-Ph-	0.011	5.9	0.86
15k	Morpholin-4-	$PhCH_2$–	Ph-	37.130	>10	>10
15l	Morpholin-4-	$PhCH_2$–	3-OH-Ph-	2.942	>10	>10
15m	Morpholin-4-	$PhCH_2$–	4-OH-Ph-	2.767	>10	>10
15n	Pyrrolidin-1-	$PhCH_2$–	Ph-	0.073	>10	>10
15o	Pyrrolidin-1-	$PhCH_2$–	3-OH-Ph-	0.049	>10	>10
15p	Pyrrolidin-1-	$PhCH_2$–	4-OH-Ph-	0.025	>10	6.99
Monastrol				7.452	>10	>10
CK0106023				0.053	4.89	6.70



Control, 24 hrs
G1: 81.45%
G2/M: 8.76%

8 μM, 24 hrs
G1: 15.83%
G2/M: 54.47%

12 μM, 24 hrs
G1: 20.35%
G2/M: 59.14%

16 μM, 24 hrs
G1: 14.64%
G2/M: 65.85%

Control, 48 hrs
G1: 63.97%
G2/M: 8.70%

8 μM, 48 hrs
G1: 17.63%
G2/M: 42.94%

12 μM, 48 hrs
G1: 12.99%
G2/M: 68.37%

16 μM, 48 hrs
G1: 1.13%
G2/M: 64.42%

Figure 5. Treatment of A549 Cells with **15j** led to profound G2/M arrest. Cells were treated with **15j** (8, 12 or 16 μM) for 24 or 48 h. Control cells were treated with DMSO alone.

expression of p53 was increased markedly in **15j**-treated A549 cells (Fig. 7B). Taken together, these results demonstrated that **15j** induced cell death mainly via apoptosis in a dose-dependent manner.

5. 15j Induced monopolar spindle morphology

To confirm that the cytotoxic activity was a consequence of KSP inhibition, we further characterized the cellular activity of

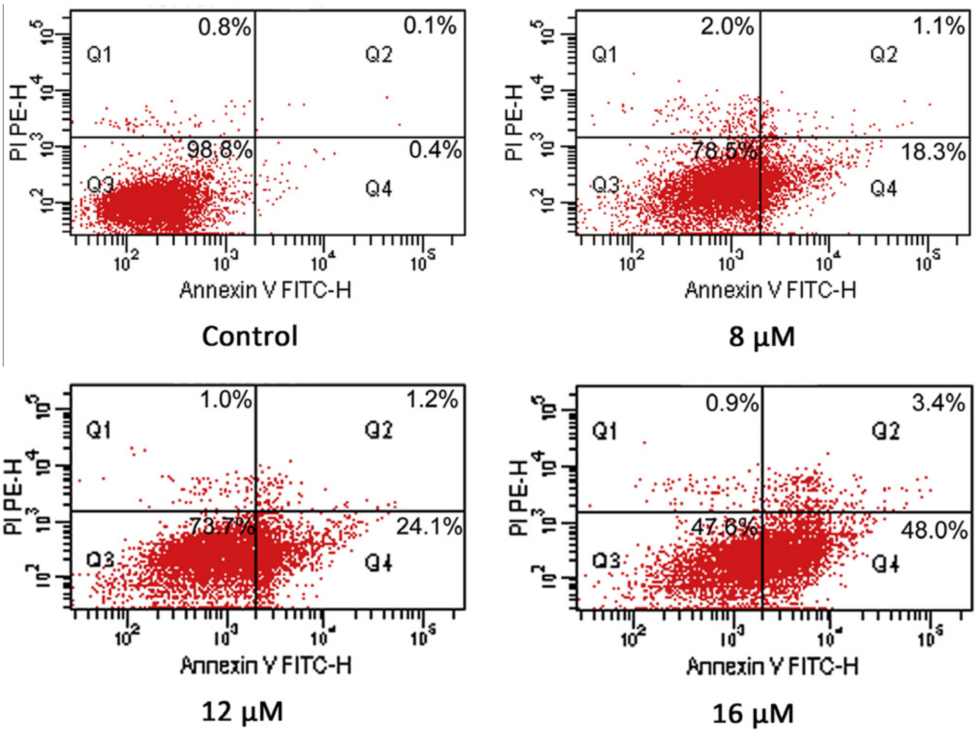


Figure 6. Induction of apoptosis, measured by Annexin V assay following treatment with **15j** (8, 12 or 16 μM) for 24 h. Control cells were treated with DMSO alone.

compounds **15j**.[17] The mitotic stage of the arrested cells by immunofluorescent staining of their DNA, as shown in Figure 8, was examined. Untreated or DMSO treated cells showed the normal morphology of nuclei (Fig. 8A and B), while **15j**-treated cells displayed monastral spindle phenotype (Fig. 8D), the same as CK0106023-treated cells (Fig. 8C). The monastral spindle phenotypes were indicated by arrowhead. Incubation with compound **15j** resulted in the accumulation of cells with the typical monopolar spindle morphology due to KSP inhibition.

6. 15j Inhibited the growth of transplantable tumors in mice

The potent antimitotic activity displayed by **15j** against cultured cells prompted us to test **15j** for antitumor activity in vivo. On the basis of tests of a range of doses of **15j** in healthy, nontumor-bearing mice on a daily 5×schedule, we estimated that the maximum tolerated dose of **15j** was ~50 mg/kg. Doses of 50, 25 and 12.5 mg/kg were administered by intraperitoneal (ip) injection every other day for 10 days to mice bearing transplantable S180 sarcoma. CK0106023 at its maximum tolerated dose (50 mg/kg) served as a positive control. As shown in Table 3, the growth of mouse-transplantable S180 sarcoma was significantly inhibited by **15j** compared with the tumor-bearing control (P <0.01), the inhibitory rate was 23.23%, 32.86%, and 47.74% for the doses of 12.5, 25, and 50 mg/kg, respectively. In addition, no signs of toxicity were observed in the **15j**-treated mice on the basis of body weight (Table 3). The inhibitory rate of positive control CK0106023 was 52.48% for the dose of 50 mg/kg, but a slight loss of body weight (P <0.01) was observed in CK0106023-treated mice due to the result.

7. Conclusions

In summary, a novel series of KSP inhibitors were constructed using de novo design strategy. The 1,4-dihydroquinolin-4-ones we initially designed displayed potent KSP inhibitory activities but showed poor cytotoxicities in vitro due to their poor solubility. Then a series of 1,2,3,4-tetrahydroquinolin-4-ones were identified as potent KSP inhibitors. The typical compound **15j** presented significant inhibitory effects on KSP and potent in vitro cytotoxicities. Moreover, **15j** was proved to induce cell death via apoptosis rather than necrosis. Microscopic studies showed that **15j** caused mitotic arrest in A549 cells by inducing the formation of monopolar spindle morphology. This fact indicated that cytotoxic activity of **15j** was a consequence of KSP inhibition during the onset of mitosis. Furthermore, the in vivo evaluation of **15j** on the growth of transplantable S180 sarcoma in mice suggested its therapeutic potential for further development. Further investigation on lead optimization and other molecular mechanisms are still under active investigation in our laboratories.

8. Experimental

8.1. Chemistry

All reagents were purchased from commercial sources and were used without further purification unless otherwise noted. Melting points (mp) were determined on Mel-Temp II apparatus and are uncorrected. Infrared spectra were run on Nicolet Impact 410 spectrophotometer using KBr film. Absorption band position is given in cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker ACF-300 spectrometer (300 MHz). Chemical shifts are expressed in ppm related to tetramethylsilane. Mass spectra were obtained on either a Mariner Mass Spectrum or a MAT-212 mass spectrometer. Elemental analyses were determined on Carlo Erba 1106 elementary analysis apparatus. Each of the target compounds was purified by silica gel (60 Å, 70–230 mesh) column chromatography. Concentration and evaporation of the solvent after reaction or extraction were carried out on a rotary evaporator (Buchi Rotavapor) operated at reduced pressure.

8.1.1. 5-Chloro-2-nitroacetophenone (2)

KNO_3 (13.50 g, 0.133 mol) was crushed and added with stirring into concentrate H_2SO_4. The mixture was cooled to below −10 °C

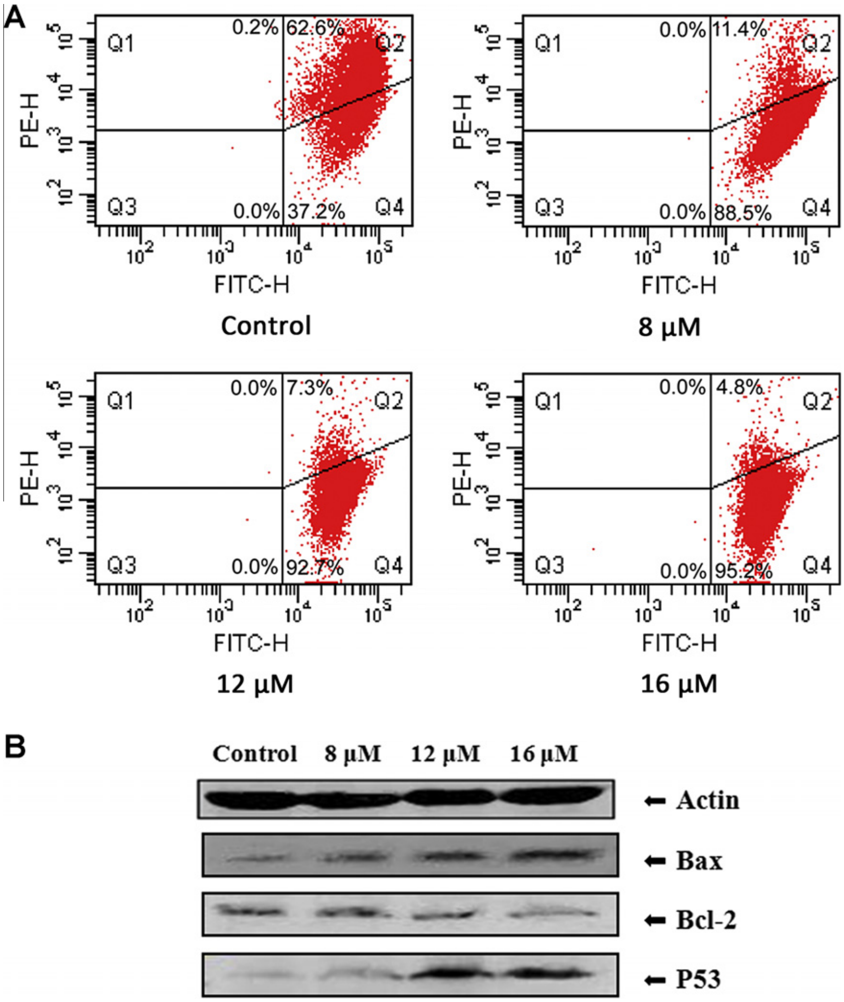


Figure 7. (A) Measurement of mitochondrial membrane potential, using JC-1 reagent. Cells were treated with **15j** (8, 12 or 16 μM) for 24 h. Control cells were treated with DMSO alone. Dot plots of red show live cells (region Q2) with intact mitochondrial membrane potential and apoptotic cells (region Q4) with lost mitochondrial potential, respectively. (B) Effect on Bcl-2 family and p53 in A549 cells after treatment with different concentrations of **15j** (8, 12 or 16 μM) for 24 h. Total cell lysates were prepared and immunoblotted. Western blots were detected with antibodies against Bax, Bcl-2, p53 and β-actin.

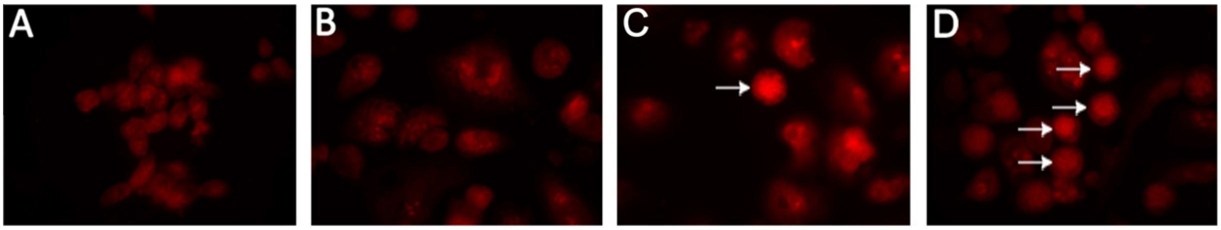


Figure 8. Compounds Induced Monastral Spindle (magnification 630×). A549 Cells were treated in the absence or presence of compound (8 μM) for 24 h, and DNA (red) of the cells was immunofluorescence stained with propidium iodide. (A) Cells without treatment. (B) Cells treated with DMSO alone. (C) CK0106023-treated cells. (D) **15j**-treated cells.

Table 3

Inhibitory effect of **1** on the growth of transplantable S180 sarcoma in mice[a]

Group	Dose (mg/kg)	Weight (g)		Tumor weight (g)	Inhibitory rate (%)
		Before treatment	After treatment		
Control	–	20.67 ± 1.50	26.22 ± 2.22	1.33 ± 0.38	
15j	50	20.1 ± 1.37	24.3 ± 2.71	0.70 ± 0.24**	47.74
	25	20.4 ± 1.50	25.2 ± 1.32	0.89 ± 0.45	32.86
	12.5	20.6 ± 2.07	26.2 ± 2.90	1.02 ± 0.21	23.23
CK0106023	50	20.8 ± 2.15	23.6 ± 2.95*	0.63 ± 0.19**	52.48

[a] The values are presented as means ± SD. ($n = 10$). Significant differences with control group were designated as *P <0.05 and **P <0.01. CK0106023 was used as positive drug.

and 3-chloroacetophenone (20 g/16.8 mL, 0.13 mol) was added at −10 to −5 °C. After a further 30 min at −10 to −5 °C, the mixture was poured into ice-water (500 g). The precipitate was collected by filteration and recrystallized in 95% ethanol to give (**2**) as a colorless crystal (21.60 g, 83.69%): mp 60–62 °C (lit[18] 63–65 °C).

8.1.2. 2-Nitro-5-(4-methylpiperazin-1-yl) acetophenone (3a)

The mixture of (**2**) (13.00 g, 0.065 mol), *N*-methylpiperazine (19.58 g/21.7 mL, 0.195 mol) and DMF (60 mL) was stirred at 110 °C for 4.5 h and then poured into water (200 mL). The precipitate was collected by filteration and washed using water and petroleum ether to give a yellow powder (13.92 g, 81.22%): mp 68–70 °C (lit[19] 67–69 °C).

8.1.3. 2-Nitro-5-(piperidin-1-yl) acetophenone (3b)

Following the synthetic procedure of compound (**3a**), the title compound was obtained from compound (**2**) (11.70 g, 0.059 mol), piperidine (14.98 g/17.4 mL, 0.176 mol), DMF (50 mL) as a yellow powder (13.41 g, 92.20%): mp 84.5–86 °C (lit[19] 100.5–101.5 °C).

8.1.4. 2-Nitro-5-(morphilin-4-yl) acetophenone (3c)

Following the synthetic procedure of compound (**3a**), the title compound was obtained from compound (**2**) (8.92 g, 45 mmol), morphiline (11.69 g/11.7 mL, 0.134 mol), DMF (45 mL) as a yellow powder (8.51 g, 76.13%): mp 140–141 °C (lit[18] 139–141 °C).

8.1.5. 1-[5-(4-Methylpiperazin-1-yl)-2-nitrophenyl]-3-phenylprop-2-ene-1-one (4a)

Compound (**3a**) (2.00 g, 7.60 mmol) and benzaldehyde (0.81 g/0.77 mL, 7.60 mmol) was added at room temperature to the solution of NaOH (0.40 g, 9.89 mmol) in water (4 mL) and 95% ethanol (2 mL). The mixture was stirred for further 2 h and then put into ice-box overnight. The precipitate was collected by filteration and washed using water to give a yellow powder (2.55 g, 95.53%): mp 169–172 °C; IR ($\mathrm{cm^{-1}}$): 3473, 3418, 2939, 2796, 1657, 1596, 1573, 1485, 1320, 1294, 1247, 1126, 1035, 862, 766, 703; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 2.36 (s, 3H), 2.55 (t, J = 5.16, 4H), 3.48 (t, J = 5.10, 4H), 6.69 (d, J = 2.85, 1H), 6.90 (m, 1H), 6.93–8.16 (m, 8H); EI-MS: 351 [M].

8.1.6. 1-[5-(Piperidin-1-yl)-2-nitrophenyl]-3-phenylprop-2-ene-1-one (4b)

Following the synthetic procedure of compound (**4a**), the title compound was obtained from compound (**3c**) (2.48 g, 10.00 mmol), benzaldehyde (1.06 g/1.01 mL, 10.00 mmol) as a yellow powder (2.83 g, 84.23%): mp 155–158 °C; IR ($\mathrm{cm^{-1}}$): 3462, 3419, 2936, 2911, 2850, 1656, 1595, 1568, 1472, 1449, 1312, 1259, 1233, 1127, 1051, 982, 863, 760, 700; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 1.59 (m, 2H), 1.69 (m, 4H), 3.48 (m, 4H), 6.64 (d, J = 2.85, 1H), 6.85 (m, 1H), 6.93–8.14 (m, 8H); EI-MS: 336 [M].

8.1.7. 1-[5-(Morphilin-4-yl)-2-nitrophenyl]-3-phenylprop-2-ene-1-one (4c)

Following the synthetic procedure of compound (**4a**), the title compound was obtained from compound (**3b**) (4.18 g, 16.72 mmol), benzaldehyde (1.77 g/1.69 mL, 16.72 mmol) as a yellow powder (4.98 g, 88.12%): mp 160–162 °C; IR ($\mathrm{cm^{-1}}$): 3449, 3420, 2963, 2855, 1651, 1596, 1478, 1448, 1318, 1238, 1124, 1030, 949, 866, 761; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 3.41 (t, J = 5.10, 4H), 3.86 (t, J = 4.86, 4H), 6.69 (d, J = 2.82, 1H), 6.90 (m, 1H), 6.98–8.19 (m, 8H); EI-MS: 338 [M].

8.1.8. 5-Chloro-2-nitropropiophenone (9)

$\mathrm{HNO_3}$ (d = 1.5, 120 mL) was cooled to −5 °C and 3-chloropropiophenone (**8**, 20.00 g, 0.12 mol) was added at −5 to −3 °C. After a further 20 min at −3 °C, the mixture was poured into ice-water (500 g). The precipitate was collected by filteration and recrystallized in ethanol to give (**9**) as a colorless crystal (22.36 g, 88.30%): mp77–78 °C (lit[20] 78–78.5 °C).

8.1.9. 2-Nitro-5-(4-methylpiperazin-1-yl) propiophenone (10a)

Following the synthetic procedure of compound (**3a**), the title compound was obtained from compound (**9**) (10.67 g, 0.05 mol), *N*-methylpiperazine (15.02 g/16.6 mL, 0.15 mol), DMF (50 mL) as a yellow powder (10.97 g, 79.24%): mp 100–102 °C; IR ($\mathrm{cm^{-1}}$): 3415, 2931, 1698, 1600, 1480, 1323, 1245, 1110, 1001, 929, 811, 751; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 1.25 (t, J = 7.26, 3H), 2.36 (s, 3H), 2.55 (t, J = 5.10, 4H), 2.74 (q, J = 7.26, 2H), 3.46 (t, J = 5.07, 4H), 6.52–8.09 (m, 3H); EI-MS: 277 [M].

8.1.10. 2-Nitro-5-(piperidin-1-yl)propiophenone (10b)

Following the synthetic procedure of compound (**3a**), the title compound was obtained from compound (**9**) (10.00 g, 0.047 mol), piperidine (11.95 g/13.9 mL, 0.14 mol), DMF (50 mL) as a yellow powder (10.50 g, 85.56%): mp 102–105 °C; IR ($\mathrm{cm^{-1}}$): 3395, 2936, 2846, 1707, 1594, 1474, 1314, 1240, 1109, 849, 821, 756; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 1.25 (t, J = 7.20, 3H), 1.69 (m, 6H), 2.71 (q, J = 7.26, 2H), 3.45 (m, 4H), 6.48–8.07 (m, 3H); EI-MS: 262 [M].

8.1.11. 2-Nitro-5-(morphilin-4-yl) propiophenone (10c)

Following the synthetic procedure of compound (**3a**), the title compound was obtained from compound (**9**) (10.00 g, 0.047 mol), morphiline (12.24 g/12.4 mL, 0.14 mol), DMF (50 mL) as a yellow powder (11.56 g, 93.49%): mp 115–117 °C; IR ($\mathrm{cm^{-1}}$): 3407, 2981, 1706, 1600, 1485, 1452, 1323, 1255, 1121, 1046, 929, 812, 755, 667; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 1.25 (t, J = 7.26, 3H), 2.72 (q, J = 7.26, 2H), 3.39 (t, J = 5.07, 4H), 3.85 (t, J = 4.83, 4H), 6.54–8.12 (m, 3H); EI-MS: 264 [M].

8.1.12. 1-[5-(4-Methylpiperazin-1-yl)-2-benzoylaminophenyl]-3-phenylpropyl-1-one (5a)

Compound (**4a**) (2.00 g, 5.70 mmol) was solved into methanol/ethyl acetate (50 mL/50 mL) and hydrogenized using Pd/C (10%) as catalyst. The mixture was filtered and the solution was dried in vacuum. The residue was solved in THF (30 mL), together with TEA (1.77 mL, 12.82 mmol), the solution of benzoyl chloride (0.66 g/0.54 mL, 4.70 mmol) in THF (5 mL) was added in drop at about 0 °C. After a further 2 h, the mixture was poured into cold water (100 mL). The precipitate was collected by filteration and washed using methanol to give a yellow powder (1.30 g, 71.24%): mp 156–157 °C; IR ($\mathrm{cm^{-1}}$): 3467, 3416, 2941, 2803, 2768, 1670, 1642, 1586, 1522, 1448, 1308, 1285, 1237, 1181, 1147, 986, 843, 749, 697; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 2.38 (s, 3H), 2.61 (t, J = 4.89, 4H), 3.09 (m, 2H), 3.18 (t, J = 4.86, 4H), 3.37 (m, 2H), 7.21–8.87 (m, 13H), 12.29 (s, 1H); EI-MS: 427 [M].

8.1.13. 1-[5-(4-Methylpiperazin-1-yl)-2-(3′-benzyloxybenzoylamino)phenyl]-3-phenylpropyl-1-one (5b)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4a**) (1.85 g, 5.70 mmol) and 3-benzyloxy benzoyl chloride (1.70 g, 6.84 mmol) as a yellow powder (2.35 g, 77.35%): mp 133.5–135 °C; IR ($\mathrm{cm^{-1}}$): 3408, 2936, 2805, 1640, 1604, 1507, 1453, 1306, 1230, 1179, 1009, 984, 842, 749, 695; ^{1}H NMR ($\mathrm{CDCl_3}$): δ 2.38 (s, 3H), 2.62 (m, 4H), 3.08 (t, J = 8.04, 2H), 3.18 (t, J = 4.68, 4H), 3.56 (t, J = 7.20, 2H), 5.16 (s, 2H), 7.03–8.85 (m, 17H), 12.23 (s, 1H); EI-MS: 533 [M].

8.1.14. 1-[5-(4-Methylpiperazin-1-yl)-2-(4′-benzyloxybenzoylamino)phenyl]-3-phenylpropyl-1-one (5c)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4a**) (1.00 g, 2.86 mmol) and 4-benzyloxy benzoyl chloride (0.92 g, 3.72 mmol) as a yellow powder (0.81 g, 49.09%): mp 159–162 °C; IR (cm^{-1}): 3416, 2934, 1666, 1609, 1586, 1527, 1452, 1305, 1241, 1186, 1021, 989, 838, 741, 698; ^{1}H NMR (CDCl$_3$): δ 2.39 (s, 3H), 2.63 (m, 4H), 3.08 (t, J = 8.01, 2H), 3.19 (t, J = 5.10, 4H), 3.36 (t, J = 8.04, 2H), 5.18 (s, 2H), 7.18–8.85 (m, 17H), 12.29 (s, 1H); EI-MS: 533 [M].

8.1.15. 1-[5-(Piperidin-1-yl)-2-benzoylaminophenyl]-3-phenylpropyl-1-one (5d)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4b**) (0.76 g, 2.47 mmol) and benzoyl chloride (0.38 g/0.31 mL, 2.71 mmol) as a yellow powder (0.98 g, 96.40%): mp 140–142 °C; IR (cm^{-1}): 3455, 3427, 2935, 2839, 1666, 1641, 1584, 1514, 1454, 1420, 1305, 1239, 1182, 985, 847, 702; ^{1}H NMR (CDCl$_3$): δ 1.25 (m, 2H), 1.58 (m, 4H), 3.07 (m, 2H), 3.10 (m, 4H), 3.37 (m, 2H), 7.22–8.85 (m, 13H), 12.29 (s, 1H); EI-MS: 412 [M].

8.1.16. 1-[5-(Piperidin-1-yl)-2-(3′-benzyloxybenzoylamino)phenyl]-3-phenylpropyl-1-one (5e)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4b**) (1.10 g, 3.57 mmol) and 3-benzyloxy benzoyl chloride (1.06 g, 4.29 mmol) as a yellow powder (1.40 g, 75.68%): mp 105–109 °C; IR (cm^{-1}): 3438, 2935, 2809, 1647, 1608, 1587, 1450, 1422, 1384, 1241, 1183, 1037, 986, 871, 741, 698; ^{1}H NMR (CDCl$_3$): δ 1.63 (m, 2H), 1.81 (m, 4H), 3.14 (m, 2H), 3.17 (m, 4H), 3.45 (t, J = 7.38, 2H), 5.26 (s, 2H), 7.34–8.91 (m, 17H), 12.37 (s, 1H); EI-MS: 518 [M].

8.1.17. 1-[5-(Piperidin-1-yl)-2-(4′-benzyloxybenzoylamino)phenyl]-3-phenylpropyl-1-one (5f)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4b**) (1.90 g, 6.17 mmol) and 4-benzyloxy benzoyl chloride (1.82 g, 7.40 mmol) as a yellow powder (1.98 g, 61.96%): mp 153–156 °C; IR (cm^{-1}): 3418, 2933, 1639, 1609, 1506, 1453, 1303, 1235, 1179, 984, 846, 749, 699; ^{1}H NMR (CDCl$_3$): δ 1.59 (m, 2H), 1.72 (m, 4H), 3.06 (m, 2H), 3.09 (m, 4H), 3.36 (t, J = 7.29, 2H), 5.15 (s, 2H), 7.07–8.83 (m, 17H), 12.22 (s, 1H); EI-MS: 518 [M].

8.1.18. 1-[5-(Morphilin-4-yl)-2-benzoylaminophenyl]-3-phenylpropyl-1-one (5g)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4c**) (1.00 g, 2.96 mmol) and benzoyl chloride (0.46 g/0.37 mL, 3.25 mmol) as a yellow powder (1.00 g, 81.64%): mp 138–141 °C; IR (cm^{-1}): 3458, 3421, 2965, 2849, 1645, 1521, 1425, 1305, 1236, 1117, 987, 705; ^{1}H NMR (CDCl$_3$): δ 3.00–3.11 (m, 2H), 3.12–3.14 (t, J = 4.89, 4H), 3.34–3.39 (m, 2H), 3.87–3.90 (t, J = 4.83, 4H), 7.20–8.90 (m, 13H), 12.31 (s, 1H); EI-MS: 414 [M].

8.1.19. 1-[5-(Morphilin-4-yl)-2-(3′-benzyloxybenzoylamino)phenyl]-3-phenylpropyl-1-one (5h)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**4c**) (1.37 g, 4.44 mmol) and 3-benzyloxy benzoyl chloride (1.31 g, 5.33 mmol) as a yellow powder (1.80 g, 78.00%): mp 147–150 °C; IR (cm^{-1}): 3417, 2852, 1646, 1588, 1523, 1451, 1306, 1267, 1181, 988, 955, 874, 742, 701; ^{1}H NMR (CDCl$_3$): δ 3.08 (m, 2H), 3.14 (m, 4H), 3.38 (m, 2H), 3.90 (t, J = 4.59, 4H), 5.20 (s, 2H), 7.20–8.89 (m, 17H), 12.32 (s, 1H); EI-MS: 520 [M].

8.1.20. 1-[5-(4-Methylpiperazin-1-yl)-2-benzoylaminophenyl]-3-propyl-1-one (5i)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10a**) (1.00 g, 4.05 mmol) and benzoyl chloride (0.68 g/0.56 mL, 4.86 mmol) as a yellow powder (0.30 g, 21.11%): mp 146–148 °C; IR (cm^{-1}): 3414, 2932, 2837, 1648, 1522, 1425, 1304, 1238, 1195, 985, 931, 699; ^{1}H NMR (CDCl$_3$): δ 1.19 (t, J = 7.20, 3H), 2.31 (s, 3H), 2.55 (t, J = 5.04, 4H), 3.03 (q, J = 7.23, 2H), 3.14 (t, J = 5.07, 4H), 7.16–8.81 (m, 8H), 12.33 (s, 1H); EI-MS: 351 [M].

8.1.21. 1-[5-(4-Methylpiperazin-1-yl)-2-(3′-benzyloxybenzoylamino)phenyl]-3-propyl-1-one (5j)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10a**) (1.00 g, 4.05 mmol) and 3-benzyloxy benzoyl chloride (1.20 g, 4.86 mmol) as a yellow powder (0.53 g, 28.65%): mp 135–138 °C; IR (cm^{-1}): 3429, 2931, 2779, 1669, 1648, 1588, 1521, 1454, 1310, 1191, 1012, 739, 699; ^{1}H NMR (CDCl$_3$): δ 1.18 (t, J = 7.23, 3H), 2.31 (s, 3H), 2.55 (t, J = 5.01, 4H), 3.02 (q, J = 7.20, 2H), 3.15 (t, J = 4.92, 4H), 5.10 (s, 2H), 7.07–8.79 (m, 12H), 12.32 (s, 1H); EI-MS: 457 [M].

8.1.22. 1-[5-(4-Methylpiperazin-1-yl)-2-(4′-benzyloxybenzoylamino)phenyl]-3-propyl-1-one (5k)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10a**) (1.00 g, 4.05 mmol) and 4-benzyloxy benzoyl chloride (1.20 g, 4.86 mmol) as a yellow powder (1.15 g, 62.16%): mp 158–160 °C; IR (cm^{-1}): 3413, 2921, 1603, 1508, 1454, 1236, 1160, 1011, 837, 760, 702; ^{1}H NMR (CDCl$_3$): δ 1.25 (t, J = 7.17, 3H), 2.42 (s, 3H), 2.68 (m, 4H), 3.09 (q, J = 7.23, 2H), 3.25 (m, 4H), 5.16 (s, 2H), 7.04–8.87 (m, 12H), 12.33 (s, 1H); EI-MS: 457 [M].

8.1.23. 1-[5-(Piperidin-1-yl)-2-benzoylaminophenyl]-3-propyl-1-one (5l)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10b**) (1.00 g, 4.31 mmol) and benzoyl chloride (0.73 g/0.60 mL, 5.17 mmol) as a yellow powder (1.22 g, 84.24%): mp 168–170 °C; IR (cm^{-1}): 3429, 2938, 1645, 1520, 1457, 1300, 1241, 1195, 1127, 983, 929, 810, 699; ^{1}H NMR (CDCl$_3$): δ 1.26 (t, J = 7.20, 3H), 1.62 (m, 2H), 1.75 (m, 4H), 3.09 (m, 2H), 3.15 (m, 4H), 7.23–8.85 (m, 8H), 12.37 (s, 1H); EI-MS: 336 [M].

8.1.24. 1-[5-(Piperidin-1-yl)-2-(3′-benzyloxybenzoylamino)phenyl]-3-propyl-1-one (5m)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10b**) (1.00 g, 4.31 mmol) and 3-benzyloxy benzoyl chloride (1.27 g, 5.17 mmol) as a yellow powder (1.40 g, 73.48%): mp 140–141 °C; IR (cm^{-1}): 3422, 2931, 1670, 1649, 1594, 1524, 1450, 1311, 1195, 1049, 927, 848, 739; ^{1}H NMR (CDCl$_3$): δ 1.25 (t, J = 7.20, 3H), 1.62 (m, 2H), 1.75 (m, 4H), 3.06 (q, J = 7.17, 2H), 3.15 (m, 4H), 5.17 (s, 2H), 7.16–8.83 (m, 12H), 12.35 (s, 1H); EI-MS: 442 [M].

8.1.25. 1-[5-(Piperidin-1-yl)-2-(4′-benzyloxybenzoylamino)phenyl]-3-propyl-1-one (5n)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10b**) (1.00 g, 4.31 mmol) and 4-benzyloxy benzoyl chloride (1.27 g, 5.17 mmol) as a yellow powder (1.70 g, 89.23%): mp 155–157 °C; IR (cm^{-1}): 3422, 2931, 1642, 1603, 1507, 1454, 1237, 1196, 1160, 1000, 981, 850, 754, 699; ^{1}H NMR (CDCl$_3$): δ 1.25 (t, J = 7.20, 3H), 1.60 (m, 2H), 1.76 (m, 4H), 3.08 (m, 2H), 3.14 (m, 4H), 5.16 (s, 2H), 7.04–8.83 (m, 12H), 12.30 (s, 1H); EI-MS: 442 [M].

8.1.26. 1-[5-(Morphilin-4-yl)-2-benzoylaminophenyl]-3-propyl-1-one (5o)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10c**) (1.00 g, 4.27 mmol) and benzoyl chloride (0.72 g/0.59 mL, 5.13 mmol) as a yellow powder (0.83 g, 57.46%): mp 191–192 °C; IR (cm^{-1}): 3415, 3213, 2974, 2851, 1645, 1521, 1457, 1238, 1194, 1126, 985, 933, 707; ^{1}H NMR (CDCl$_3$): δ 1.26 (t, J = 7.23, 3H), 3.10 (q, J = 7.23, 2H), 3.17 (t, J = 4.86, 4H), 3.91 (t, J = 4.68, 4H), 7.21–8.90 (m, 8H), 12.39 (s, 1H); EI-MS: 338 [M].

8.1.27. 1-[5-(Morphilin-4-yl)-2-(3′-benzyloxybenzoylamino)phenyl]-3-propyl-1-one (5p)

Following the synthetic procedure of compound (**5a**), the title compound was obtained from compound (**10c**) (1.00 g, 4.27 mmol) and 3-benzyloxy benzoyl chloride (1.26 g, 5.13 mmol) as a yellow powder (1.81 g, 95.39%): mp 145–146 °C; IR (cm^{-1}): 3422, 2953, 1669, 1593, 1524, 1450, 1312, 1192, 1116, 1047, 985, 848, 740, 688; ^{1}H NMR (CDCl$_3$): δ 1.26 (t, J = 7.17, 3H), 3.09 (q, J = 7.23, 2H), 3.17 (t, J = 4.89, 4H), 3.91 (t, J = 4.68, 4H), 5.17 (s, 2H), 7.16–8.88 (m, 12H), 12.39 (s, 1H); EI-MS: 444 [M].

8.1.28. 2-(3′-Benzyloxyphenyl)-3-benzyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (6a)

Na (0.13 g, 5.63 mmol) was dissolved in ethanol (10 mL). Compound (**5b**) (1.00 g, 1.88 mmol) was then added into the solution. The mixture was refluxed for 12 h and the title compound was gained by column chromatography (ethyl acetate as eluant) as a yellow powder (0.38 g, 39.33%): mp 215–218 °C; IR (cm^{-1}): 3423, 2936, 1604, 1572, 1495, 1450, 1414, 1381, 1222, 1146, 1078, 1008, 932, 791, 734, 699; ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H), 2.61 (t, J = 5.07, 4H), 3.29 (t, J = 4.97, 4H), 3.89 (s, 2H), 4.82 (s, 2H), 6.86–8.19 (m, 17H); EI-MS: 515 [M].

8.1.29. 2-(4′-Benzyloxyphenyl)-3-benzyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (6b)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5c**) (1.06 g, 2.00 mmol) and Na (0.14 g, 6.00 mmol) as a yellow powder (0.49 g, 47.57%): mp 278–281 °C; IR (cm^{-1}): 3415, 2801, 1609, 1508, 1453, 1382, 1240, 1174, 1003, 925, 828, 698; ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H), 2.50 (m, 4H), 3.16 (t, J = 4.50, 4H), 3.75 (s, 2H), 5.19 (s, 2H), 6.94–7.51 (m, 17H), 11.48 (s, 1H); EI-MS: 515 [M].

8.1.30. 2-(3′-Benzyloxyphenyl)-3-benzyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (6c)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5e**) (1.30 g, 2.51 mmol) and Na (0.17 g, 7.53 mmol) as a yellow powder (0.89 g, 70.93%): mp 230–234 °C; IR (cm^{-1}): 3400, 3054, 1563, 1493, 1437, 1383, 1246, 1037, 929, 699; ^{1}H NMR (DMSO-d_6): δ 1.55–1.66 (m, 2H), 1.63–1.66 (m, 4H), 3.14–3.17 (m, 4H), 3.72 (s, 2H), 4.97 (s, 2H), 6.89–7.56 (m, 17H), 11.69 (s, 1H); EI-MS: 500 [M].

8.1.31. 2-(4′-Benzyloxyphenyl)-3-benzyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (6d)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5f**) (1.00 g, 1.93 mmol) and Na (0.13 g, 5.79 mmol) as a yellow powder (0.83 g, 85.99%): mp >300 °C; IR (cm^{-1}): 3416, 2930, 1616, 1507, 1418, 1237, 1172, 1117, 731, 697; ^{1}H NMR (DMSO-d_6): δ 1.66 (m, 2H), 1.71 (m, 4H), 3.15 (m, 4H), 3.75 (s, 2H), 5.19 (s, 2H), 6.94–7.50 (m, 17H); EI-MS: 500 [M].

8.1.32. 2-(3′-Benzyloxyphenyl)-3-benzyl-6-(morphilin-4-yl)-1,4-dihydroquinoline-4-one (6e)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5h**) (1.30 g, 2.50 mmol) and Na (0.17 g, 7.50 mmol) as a yellow powder (0.72 g, 57.37%): mp 216–220 °C; IR (cm^{-1}): 3458, 2957, 1661, 1571, 1412, 1384, 1260, 1121, 1081, 937, 790, 698; ^{1}H NMR (DMSO-d_6): δ 3.23 (t, J = 4.86, 4H), 3.88 (t, J = 4.89, 4H), 3.74 (s, 2H), 4.84 (s, 2H), 6.88–8.07 (m, 17H); EI-MS: 502 [M].

8.1.33. 2-(3′-Benzyloxyphenyl)-3-methyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (6f)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5j**) (0.50 g, 1.09 mmol) and Na (0.08 g, 3.28 mmol) as a yellow powder (0.28 g, 58.30%): mp 232–235 °C; IR (cm^{-1}): 3400, 3061, 2937, 2822, 1572, 1498, 1452, 1379, 1240, 1154, 1007, 949, 795, 749, 697; ^{1}H NMR (DMSO-d_6): δ 1.85 (s, 3H), 2.24 (s, 3H), 2.50 (m, 4H), 3.16 (t, J = 5.17, 4H), 5.19 (s, 2H), 7.15–7.50 (m, 12H); EI-MS: 439 [M].

8.1.34. 2-(4′-Benzyloxyphenyl)-3-methyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (6g)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5k**) (0.60 g, 1.31 mmol) and Na (0.09 g, 3.94 mmol) as a yellow powder (0.23 g, 39.91%): mp >300 °C; IR (cm^{-1}): 3069, 2787, 1609, 1540, 1494, 1451, 1379, 1238, 1010, 829, 748, 698; ^{1}H NMR (DMSO-d_6): δ 1.90 (s, 3H), 2.24 (s, 3H), 2.50 (m, 4H), 3.16 (t, J = 5.07, 4H), 5.22 (s, 2H), 6.88–7.80 (m, 12H), 11.36 (s, 1H); EI-MS: 439 [M].

8.1.35. 2-(3′-Benzyloxyphenyl)-3-methyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (6h)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5m**) (0.88 g, 2.03 mmol) and Na (0.14 g, 6.08 mmol) as a cream-colored powder (0.61 g, 71.93%): mp 253–255 °C; IR (cm^{-1}): 3054, 2933, 2854, 1574, 1545, 1493, 1451, 1383, 1235, 1007, 940, 864, 783, 745, 697; ^{1}H NMR (DMSO-d_6): δ 1.18 (m, 2H), 1.57 (m, 4H), 1.86 (s, 3H), 3.17 (m, 4H), 5.21 (s, 2H), 7.10–7.53 (m, 12H), 11.46 (s, 1H); EI-MS: 424 [M].

8.1.36. 2-(4′-Benzyloxyphenyl)-3-methyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (6i)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5n**) (0.60 g, 1.36 mmol) and Na (0.09 g, 4.07 mmol) as a yellow powder (0.36 g, 62.55%): mp 238–241 °C; IR (cm^{-1}): 3436, 2931, 1679, 1608, 1536, 1490, 1461, 1381, 1242, 1170, 993, 834, 697; ^{1}H NMR (DMSO-d_6): δ 1.55 (m, 2H), 1.66 (m, 4H), 1.89 (s, 3H), 3.15 (t, J = 4.83, 4H), 5.22 (s, 2H), 7.05–7.89 (m, 12H), 11.32 (s, 1H); EI-MS: 424 [M].

8.1.37. 2-(3′-Benzyloxyphenyl)-3-methyl-6-(morphilin-4-yl)-1,4-dihydroquinoline-4-one (6j)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5p**) (0.90 g, 2.03 mmol) and Na (0.14 g, 6.08 mmol) as a yellow powder (0.55 g, 63.69%): mp 237–239 °C; IR (cm^{-1}): 3436, 2953, 2859, 1573, 1536, 1496, 1451, 1379, 1236, 1120, 949, 785, 699; ^{1}H NMR (DMSO-d_6): δ 1.85 (s, 3H), 3.14 (t, J = 4.68, 4H), 3.79 (t, J = 4.92, 4H), 5.19 (s, 2H), 7.09–7.52 (m, 12H), 11.42 (s, 1H); EI-MS: 426 [M].

8.1.38. 2-Phenyl-3-methyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (7a)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5i**) (0.30 g, 0.85 mmol) and Na (0.06 g, 2.56 mmol) as a yellow powder (0.20 g, 70.27%): mp 299 °C; IR (cm^{-1}): 3242, 2939, 2807, 1582, 1555, 1497, 1450, 1379, 1234, 1146, 1003, 943, 882, 796, 703; ^{1}H NMR (DMSO-d_6): δ 1.88 (s, 3H), 2.24 (s, 3H), 2.50 (m, 4H), 3.17 (t, J = 4.62, 4H), 7.41–7.58 (m, 8H), 11.44 (s, 1H); EI-MS: 333 [M]. Anal. Calcd for $C_{21}H_{23}N_3O{\cdot}H_2O$: C, 71.79; H, 7.12; N, 11.97. Found: C, 71.37; H, 7.00; N, 11.50.

8.1.39. 2-(3′-Hydroxyphenyl)-3-methyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (7b)

Compound (**6f**) (0.20 g, 0.46 mmol) was dissolved in ethanol (50 mL). The solution was hydrogenated using Pd/C (10%) as catalyst. The title compound was gained as a pale yellow powder (0.06 g, 37.50%): mp >300 °C; IR (cm^{-1}): 3415, 3061, 2931, 2811, 1599, 1572, 1503, 1448, 1384, 1239, 1145, 947, 882, 793, 715; ^{1}H NMR (DMSO-d_6): δ 1.88 (s, 3H), 2.24 (s, 3H), 2.50 (m, 4H), 3.17 (m, 4H), 6.88–7.53 (m, 7H), 11.41 (s, 1H); EI-MS: 349 [M]. Anal. Calcd for $C_{21}H_{23}N_3O_2{\cdot}0.75H_2O$: C, 71.28; H, 6.65; N, 11.88. Found: C, 71.14; H, 6.87; C, 11.41.

8.1.40. 2-(4′-Hydroxyphenyl)-3-methyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (7c)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6g**) (0.20 g, 0.46 mmol) as an off-white powder (0.15 g, 94.36%): mp >300 °C; IR (cm^{-1}): 3478, 3247, 3067, 2808, 1612, 1573, 1500, 1481, 1382, 1230, 998, 944, 838, 791; ^{1}H NMR (DMSO-d_6): δ 1.90 (s, 3H), 2.24 (s, 3H), 2.50 (m, 4H), 3.16 (t, *J* = 4.59, 4H), 6.91–7.53 (m, 7H), 9.86 (s, 1H), 11.28 (s, 1H); EI-MS: 349 [M]. Anal. Calcd for $C_{21}H_{23}N_3O_2{\cdot}0.75H_2O$: C, 69.52; H, 6.76; N, 11.59. Found: C, 69.45; H, 6.63; N, 12.07.

8.1.41. 2-Phenyl-3-methyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (7d)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5l**) (0.60 g, 1.79 mmol) and Na (0.12 g, 5.36 mmol) as a cream-colored powder (0.36 g, 63.40%): mp 291–293 °C; IR (cm^{-1}): 3469, 2930, 1583, 1540, 1497, 1451, 1383, 1234, 934, 702; ^{1}H NMR (DMSO-d_6): δ 1.56 (m, 2H), 1.67 (m, 4H), 1.88 (s, 3H), 3.17 (t, *J* = 4.68, 4H), 7.44–7.58 (m, 8H); EI-MS: 318 [M]. Anal. Calcd for $C_{21}H_{22}N_2O$: C, 79.21; H, 6.96; N, 8.80. Found: C, 78.92; N, 7.13; N, 8.39.

8.1.42. 2-(3′-Hydroxyphenyl)-3-methyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (7e)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6h**) (0.30 g, 0.71 mmol) as a pale yellow powder (0.15 g, 63.48%): mp >300 °C; IR (cm^{-1}): 3400, 3066, 2933, 2853, 1599, 1572, 1448, 1368, 1234, 947, 872, 818, 778, 712; ^{1}H NMR (DMSO-d_6): δ 1.56 (m, 2H), 1.66 (m, 4H), 1.88 (s, 3H), 3.15 (t, *J* = 4.86, 4H), 6.88–7.52 (m, 7H), 9.78 (s, 1H), 11.38 (s, 1H); EI-MS: 334 [M]. Anal. Calcd for $C_{21}H_{22}N_2O_2{\cdot}0.25H_2O$: C, 74.45; H, 6.65; N, 8.27. Found: C, 74.48; H, 6.36; N, 8.34.

8.1.43. 2-(4′-Hydroxyphenyl)-3-methyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (7f)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6i**) (0.25 g, 0.59 mmol) as a pale yellow powder (0.12 g, 60.94%): mp >300 °C; IR (cm^{-1}): 3411, 2931, 2851, 1610, 1512, 1440, 1383, 1279, 1225, 1167, 839; ^{1}H NMR (DMSO-d_6): δ 1.55 (m, 2H), 1.66 (m, 4H), 1.90 (s, 3H), 3.15 (t, *J* = 4.68, 4H), 6.92–7.79 (m, 7H), 9.88 (s, 1H), 11.28 (s, 1H); EI-MS: 334 [M].

8.1.44. 2-Phenyl-3-methyl-6-(morphilin-4-yl)-1,4-dihydroquinoline-4-one (7g)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5o**) (0.50 g, 1.48 mmol) and Na (0.10 g, 4.44 mmol) as a yellow powder (0.28 g, 59.15%): mp 290–293 °C; IR (cm^{-1}): 3444, 2938, 2830, 1571, 1538, 1497, 1382, 1230, 1118, 944, 704; ^{1}H NMR (DMSO-d_6): δ 1.88 (s, 3H), 3.14 (t, *J* = 4.80, 4H), 3.79 (t, *J* = 4.56, 4H), 7.43–7.59 (m, 8H), 11.46 (s, 1H); EI-MS: 333 [M].

8.1.45. 2-(3′-Hydroxyphenyl)-3-methyl-6-(morphilin-4-yl)-1,4-dihydroquinoline-4-one (7h)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6j**) (0.30 g, 0.70 mmol) as a pale yellow powder (0.23 g, 97.21%): mp >300 °C; IR (cm^{-1}): 3400, 3061, 2956, 2850, 1601, 1572, 1449, 1365, 1267, 1232, 1118, 947, 825, 709; ^{1}H NMR (DMSO-d_6): δ 1.89 (s, 3H), 3.14 (t, *J* = 4.53, 4H), 3.78 (t, *J* = 4.35, 4H), 6.88–7.55 (m, 7H), 9.76 (s, 1H), 11.41 (s, 1H); EI-MS: 336 [M]. Anal. Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.10; H, 6.02; N, 8.05.

8.1.46. 2-Phenyl-3-benzyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (7i)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5a**) (0.50 g, 1.17 mmol) and Na (0.08 g, 3.51 mmol) as a yellow powder (0.36 g, 75.17%): mp 195 °C (dec.); IR (cm^{-1}): 3416, 3240, 2939, 2807, 1612, 1549, 1501, 1452, 1382, 1242, 1151, 1005, 925, 767, 699; ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H), 2.51 (m, 4H), 3.16 (m, 4H), 3.73 (s, 2H), 6.93–7.52 (m, 13H), 11.57 (s, 1H); EI-MS: 409 [M]. Anal. Calcd for $C_{27}H_{27}N_3O{\cdot}1.5H_2O$: C, 74.31; H, 6.88; N, 9.63. Found: C, 74.77; H, 7.07; N, 9.57.

8.1.47. 2-(3′-Hydroxyphenyl)-3-benzyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (7j)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6a**) (0.30 g, 0.70 mmol) as a pale yellow powder (0.22 g, 88.86%): mp >300 °C; IR (cm^{-1}): 3416, 3235, 2833, 1607, 1573, 1496, 1399, 1238, 979, 923, 825, 703; ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H), 2.50 (m, 4H), 3.16 (m, 4H), 3.74 (s, 2H), 6.78–7.55 (m, 12H), 9.84 (s, 1H), 11.57 (s, 1H); EI-MS: 425 [M].

8.1.48. 2-(4′-Hydroxyphenyl)-3-benzyl-6-(4-methylpiperazin-1-yl)-1,4-dihydroquinoline-4-one (7k)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6b**) (0.25 g, 0.49 mmol) as a pale yellow powder (0.19 g, 92.10%): mp297–299 °C; IR (cm $^{-1}$): 3415, 2931, 2838, 1580, 1526, 1490, 1407, 1387, 1245, 1173, 933, 827; ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H), 2.50 (m, 4H), 3.16 (m, 4H), 3.76 (s, 2H), 6.84–7.56 (m, 12H), 11.50 (s, 1H); EI-MS: 425 [M].

8.1.49. 2-Phenyl-3-benzyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (7l)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5l**) (1.00 g, 2.43 mmol) and Na (0.17 g, 7.28 mmol) as a yellow powder (0.51 g, 53.33%): mp >300 °C; IR (cm^{-1}): 3455, 2933, 2847, 1609, 1566, 1479, 1382, 1242, 1116, 920, 864, 823, 765, 698; ^{1}H NMR (DMSO-d_6): δ 1.64 (m, 2H), 1.78 (m, 4H), 3.25 (t, *J* = 5.13, 4H), 3.91 (s, 2H), 7.05–8.35 (m, 13H); EI-MS: 394 [M]. Anal. Calcd for $C_{27}H_{26}N_2O$: C, 82.20; H, 6.64; N, 7.10. Found: C, 81.97; H, 6.51; N, 7.02.

8.1.50. 2-(3′-Hydroxyphenyl)-3-benzyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (7m)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6c**) (0.40 g, 0.80 mmol) as a pale yellow powder (0.14 g, 42.68%): mp285–288 °C; IR (cm $^{-1}$): 3425, 3256, 2935, 1605, 1575, 1505, 1447, 1385, 1242, 1208, 929, 776, 721, 693; ^{1}H NMR (DMSO-d_6): δ 1.56 (m, 2H), 1.65 (m, 4H), 3.17 (t, *J* = 4.80, 4H), 3.75 (s, 2H), 6.80–7.52 (m, 12H); EI-MS: 410 [M]. Anal. Calcd for $C_{27}H_{26}N_2O_2$: C, 79.00; H, 6.38; N, 6.82. Found: C, 78.63; H, 6.29; N, 6.60.

8.1.51. 2-(4′-Hydroxyphenyl)-3-benzyl-6-(piperidin-1-yl)-1,4-dihydroquinoline-4-one (7n)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6d**) (0.50 g, 1.00 mmol) as a pale yellow powder (0.07 g, 17.07%): mp >300 °C; IR (cm^{-1}): 3409, 2935, 1612, 1519, 1473, 1385, 1260, 1241, 1170, 1029, 816, 700; ^{1}H NMR (DMSO-d_6): δ 1.56 (m, 2H), 1.67 (m, 4H), 3.18 (t, *J* = 4.71, 4H), 3.77 (s, 2H), 6.85–7.57 (m, 12H); EI-MS: 410 [M]. Anal. Calcd for $C_{27}H_{26}N_2O_2\cdot3.5H_2O$: C, 68.50; C, 6.98; N, 5.92. Found: C, 68.51; H, 7.08; N, 5.63.

8.1.52. 2-Phenyl-3-benzyl-6-(morphilin-4-yl)-1,4-dihydroquinoline-4-one (7o)

Following the synthetic procedure of compound (**6a**), the title compound was obtained from compound (**5o**) (0.50 g, 1.21 mmol) and Na (0.08 g, 3.62 mmol) as a yellow powder (0.30 g, 62.73%): mp 289–290 °C; IR (cm^{-1}): 3425, 2851, 2806, 1619, 1570, 1506, 1476, 1409, 1389, 1225, 1120, 935, 701; ^{1}H NMR (DMSO-d_6): δ 3.15 (t, *J* = 4.65, 4H), 3.75 (s, 2H), 3.79 (t, *J* = 4.17, 4H), 6.93–7.57 (m, 13H), 11.57 (s, 1H); EI-MS: 396 [M]. Anal. Calcd for $C_{26}H_{24}N_2O_2\cdot1.25H_2O$: C, 74.55; H, 6.33; N, 6.69. Found: C, 74.33; H, 6.25; N, 6.45.

8.1.53. 2-(3′-Hydroxyphenyl)-3-benzyl-6-(morphilin-4-yl)-1,4-dihydroquinoline-4-one (7p)

Following the synthetic procedure of compound (**7b**), the title compound was obtained from compound (**6e**) (0.40 g, 0.80 mmol) as a pale yellow powder (0.10 g, 30.46%): mp >300 °C; IR (cm^{-1}): 3480, 3132, 2807, 1614, 1574, 1490, 1446, 1236, 1112, 932, 817, 716, 696; ^{1}H NMR (DMSO-d_6): δ 3.14 (m, 4H), 3.75 (s, 2H), 3.77 (m, 4H), 6.78–7.58 (m, 12H), 10.06 (s, 1H), 11.58 (s, 1H); EI-MS: 412 [M]. Anal. Calcd for $C_{26}H_{24}N_2O_3\cdot5.5H_2O$: C, 61.06; H, 5.85; N, 5.48. Found: C, 61.00; H, 5.72; N, 5.91.

8.1.54. 2-Nitro-5-(piperidin-1-yl) benzoic acid (12a)

The mixture of 5-chloro-2-nitrobenzoic acid (10 g, 0.05 mol) and piperidine (21.10 g/24.5 mL, 0.25 mol) was stirred at 110 °C for 2 h. The mixture was then cooled, pH of which was adjusted to about 3. The precipitate was collected by filteration and washed by water to gain (**12a**) as a yellow powder (11.88 g, 95.04%): mp 151–154 °C. The product was used directly in next step without further purification.

8.1.55. 2-Nitro-5-(morphilin-4-yl) benzoic acid (12b)

Following the synthetic procedure of compound (**12a**), the title compound was obtained from 5-chloro-2-nitrobenzoic acid (10 g, 0.05 mol) and morphiline (21.61 g/21.6 mL, 0.25 mol) as a yellow powder (13.01 g, 100%): mp 187–190 °C. The product was used directly in next step without further purification.

8.1.56. 2-Nitro-5-(pyrrolidin-1-yl) benzoic acid (12c)

Following the synthetic procedure of compound (**12a**), the title compound was obtained from 5-chloro-2-nitrobenzoic acid (6 g, 0.03 mol), pyrrolidine (10.58 g/12.45 mL, 0.15 mol) as a yellow powder (6.72 g, 95.66%): mp 192–194 °C. The product was used directly in next step without further purification.

8.1.57. *N*-Methyl-2-nitro-5-(piperidin-1-yl)benzamide (13a)

The mixture of (**12a**) (2.50 g, 10 mmol), PyBOP (5.20 g, 10 mmol), TEA (5 mL) and dry THF (100 mL) was stirred at room temperature for 10 min, then methylamine hydrochloride (0.68 g, 10 mmol) was added. The mixture was stirred for further 2 h and filtered. The filterate was purified by column chromatography (Petroleum ether/Ethyl acetate = 1:1). The title compound was gained as a yellow powder (1.40 g, 53.23%): mp186–187 °C; IR (cm^{-1}): 3466, 3271, 2859, 1651, 1575, 1494, 1329, 1250, 1086, 868, 825; ^{1}H NMR (CDCl$_3$): δ 1.68 (m, 6H), 3.02 (d, *J* = 4.80, 3H), 3.44 (m, 4H), 5.65 (br, 1H), 6.72–8.06 (m, 3H); EI-MS: 263 [M].

8.1.58. *N*-Methyl-2-nitro-5-(morphilin-4-yl)benzamide (13b)

Following the synthetic procedure of compound (**13a**), the title compound was obtained from compound (**12b**) (2.00 g, 8 mmol), methylamine hydrochloride (0.54 g, 8 mmol) as a yellow powder (2.18 g, 92.14%): mp186–189 °C; IR (cm^{-1}): 3487, 3329, 2891, 1669, 1615, 1574, 1553, 1493, 1325, 1236, 1034, 826, 631; ^{1}H NMR (CDCl$_3$): δ 3.03 (d, *J* = 5.52, 3H), 3.38 (t, *J* = 5.10, 4H), 3.85 (t, *J* = 4.80, 4H), 5.66 (br, 1H), 6.77–8.11 (m, 3H); EI-MS: 265 [M].

8.1.59. *N*-Methyl-2-nitro-5-(pyrrolidin-1-yl)benzamide (13c)

Following the synthetic procedure of compound (**13a**), the title compound was obtained from compound (**12c**) (2.36 g, 10 mmol), methylamine hydrochloride (0.68 g, 10 mmol) as a yellow powder (1.07 g, 42.97%): mp 207–209 °C; IR (cm^{-1}): 3426, 2959, 1640, 1555, 1482, 1318, 1271, 1088, 818, 752; ^{1}H NMR (CDCl$_3$): δ 2.05 (m, 4H), 3.00 (d, *J* = 4.63, 3H), 3.38 (t, *J* = 6.73, 4H), 5.84 (br, 1H), 6.40–8.04 (m, 3H); EI-MS: 249 [M].

8.1.60. *N*-Benzyl-2-nitro-5-(piperidin-1-yl)benzamide (13d)

Following the synthetic procedure of compound (**13a**), the title compound was obtained from compound (**12a**) (2.50 g, 10 mmol), benzylamine (1.07 g, 10 mmol) as a yellow powder (1.07 g, 31.56%): mp176–178 °C; IR (cm^{-1}): 3453, 2938, 2837, 1639, 1578, 1488, 1319, 1239, 1077, 850, 739, 692; ^{1}H NMR (CDCl$_3$): δ 1.57–1.60 (m, 6H), 3.48 (t, *J* = 4.20, 4H), 4.45 (d, *J* = 5.91, 2H), 6.82 –7.97 (m, 8H), 8.84 (t, *J* = 5.91, 1H); EI-MS: 339 [M].

8.1.61. *N*-Benzyl-2-nitro-5-(morphilin-4-yl)benzamide (13e)

Following the synthetic procedure of compound (**13a**), the title compound was obtained from compound (**12b**) (5.04 g, 20 mmol), benzyl amine (2.14 g, 20 mmol) as a yellow powder (4.12 g, 69.21%): mp151–152 °C; IR (cm^{-1}): 3473, 3242, 3069, 2845, 2397, 1641, 1499, 1331, 1243, 1118, 1035, 825, 728, 696; ^{1}H NMR (CDCl$_3$): δ 3.36 (t, *J* = 4.89, 4H), 3.84 (t, *J* = 5.07, 4H), 4.64 (d, *J* = 5.85, 2H), 6.78–7.41 (m, 8H), 8.07 (t, *J* = 5.58,1H); EI-MS: 341 [M].

8.1.62. *N*-Benzyl-2-nitro-5-(pyrrolidin-1-yl)benzamide (13f)

Following the synthetic procedure of compound (**13a**), the title compound was obtained from compound (**12c**) (2.36 g, 10 mmol), benzylamine (1.07 g, 10 mmol) as a yellow powder (1.75 g, 53.85%): mp160–163 °C; IR (cm^{-1}): 3422, 3247, 2961, 2868, 1598, 1507, 1482, 1318, 1299, 1088, 848, 818, 752; ^{1}H NMR (CDCl$_3$): δ 2.07 (m, 4H), 3.39 (m, 4H), 4.66 (d, *J* = 5.52, 2H), 5.89 (br, 1H), 6.44–8.08 (m, 8H); EI-MS: 325 [M].

8.1.63. *N*-Methyl-2-amino-5-(piperidin-1-yl)benzamide (14a)

Compound (**13a**) (0.70 g, 2.66 mmol) was dissolved in ethanol (30 mL) and hydrogenated using Pd/C (10%) as catalyst. The title compound was gained as a pale yellow powder (0.62 g, 100%): mp113–115 °C; IR (cm^{-1}): 3444, 3314, 2937, 2801, 1638, 1581, 1499, 1300, 1255, 1163, 944, 829, 619; ^{1}H NMR (CDCl$_3$): δ 1.24 (t, *J* = 4.20, 2H), 1.55 (m, 4H), 2.92 (m, 3H), 2.96 (m, 4H), 4.90 (br, 2H), 6.11 (br, 1H), 6.63–6.96 (m, 3H); EI-MS: 233 [M].

8.1.64. *N*-Mehtyl-2-amino-5-(morphilin-4-yl)benzamide (14b)

Following the synthetic procedure of compound (**14a**), the title compound was obtained from compound (**13b**) (0.50 g, 1.89 mmol) as a pale yellow powder (0.43 g, 96.98%): mp146–149 °C; IR (cm^{-1}): 3431, 2816, 1634, 1531, 1496, 1448, 1256, 1110, 953, 876, 829; ^{1}H NMR (CDCl$_3$): δ 2.97 (m, 4H), 3.07 (m, 3H), 3.86 (m, 4H), 4.60 (br, 2H), 6.11 (s, 1H), 6.67–6.93 (m, 3H); EI-MS: 235 [M].

8.1.65. *N*-Methyl-2-amino-5-(pyrrolidin-1-yl)benzamide (14c)

Following the synthetic procedure of compound (**14a**), the title compound was obtained from compound (**13c**) (0.50 g, 2.00 mmol) as a pale yellow powder (0.40 g, 91.32%): mp149–152 °C; IR (cm^{-1}): 3476, 3419, 2955, 2833, 1641, 1583, 1504, 1484, 1371, 1166, 810, 612; ^1H NMR ($CDCl_3$): δ 1.98 (m, 4H), 2.97 (d, *J* = 5.10, 3H), 3.24 (m, 4H), 6.30 (br, 1H), 6.56–6.69 (m, 3H); EI-MS: 219 [M].

8.1.66. *N*-Benzyl-2-amino-5-(piperidin-1-yl)benzamide (14d)

Compound (**13d**) (1.00 g, 2.94 mmol) was dissolved in CH_2Cl_2 (50 mL), Zn powder (0.96 g, 14.70 mmol) was added followed by the adding of acetic acid (1.50 mL). The mixture was stirred for further 2 h and filtered. The filterate was washed by water, dried and evaporated to gain (**14d**) as a yellow powder (0.80 g, 87.77%): mp163–165 °C; IR (cm^{-1}): 3473, 3309, 2934, 2354, 1801, 1636, 1537, 1239, 735, 696, 616; ^1H NMR ($CDCl_3$): δ 1.47 (m, 2H), 1.62 (m, 4H), 1.62 (t, *J* = 5.13, 4H), 4.42 (d, *J* = 5.97, 2H), 5.94 (s, 2H), 6.61–7.33 (m, 8H), 8.74 (t, *J* = 5.97, 1H); EI-MS: 309 [M].

8.1.67. *N*-Benzyl-2-amino-5-(morphilin-4-yl)benzamide (14e)

Following the synthetic procedure of compound (**14d**), the title compound was obtained from compound (**13e**) (1.50 g, 4.40 mmol) as a yellow to brown powder (1.31 g, 95.76%): mp149–151 °C; IR (cm^{-1}): 3463, 3396, 3345, 2953, 2823, 1534, 1639, 1258, 1106, 940, 868, 825, 605; ^1H NMR ($CDCl_3$): δ 2.99 (t, *J* = 4.65, 4H), 3.83 (t, *J* = 4.65, 4H), 4.62 (d, *J* = 5.67, 2H), 6.40–7.37 (m, 8H); EI-MS: 311 [M].

8.1.68. *N*-Benzyl-2-amino-5-(pyrrolidin-1-yl)benzamide (14f)

Following the synthetic procedure of compound (**14d**), the title compound was obtained from compound (**13f**) (1.30 g, 4.00 mmol) as a pale green powder (1.20 g, 100%): mp146–148 °C; IR (cm^{-1}): 3421, 3312, 2956, 1642, 1583, 1537, 1427, 1372, 1290, 1165, 987, 808; ^1H NMR ($CDCl_3$): δ 2.00 (m, 4H), 3.25 (m, 4H), 4.60–4.62 (d, *J* = 4.80, 2H), 6.78–7.36 (m, 8H); EI-MS: 295 [M].

8.1.69. 2-(3′-Hydroxyphenyl)-3-methyl-6-(piperidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15a)

The mixture of (**14a**) (0.17 g, 0.73 mmol), 3-hydroxybenzaldehyde (0.18 g, 1.46 mmol) and *N*,*N*-dimethylacetamide (DMA) (7 mL) was stirred at room temperature for 2 h, using *p*-toluene sulfonic acid as catalyst and then poured into ice-water (20 mL). The mixture was extracted by ethyl acetate (20 mL × 3). The organic layer was purified by column chromatography (Petroleum ether/Ethyl acetate = 2:1). The title compound was gained as a pale yellow powder (0.16 g, 65.07%): mp 222–224 °C; IR (cm^{-1}): 3474, 3415, 3306, 2955, 2823, 1638, 1617, 1518, 1484, 1393, 1200, 1143, 890, 818, 767; ^1H NMR (DMSO-d_6): δ 1.48 (m, 2H), 1.61 (m, 4H), 2.84 (s, 3H), 2.92 (t, *J* = 5.40, 4H), 5.65 (d, *J* = 2.40, 1H), 6.81 (d, *J* = 2.10, 1H), 6.55–6.75, 6.93–7.20 (m, 7H), 9.47 (s, 1H); EI-MS: 337 [M]. Anal. Calcd for $C_{20}H_{23}N_3O_2$: C, 71.19; H, 6.87; N, 12.45. Found: C, 71.01; H, 6.86; N, 12.45.

8.1.70. 2-(4′-Hydroxyphenyl)-3-methyl-6-(piperidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15b)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14a**) (0.20 g, 0.86 mmol), 4-hydroxybenzaldehyde (0.21 g, 1.72 mmol)as a pale yellow powder (0.21 g, 72.60%): mp 231–233 °C; IR (cm^{-1}): 3422, 2941, 2809, 1632, 1616, 1505, 1386, 1227, 1160, 1115, 835, 722, 684; ^1H NMR (DMSO-d_6): δ 1.53 (m, 2H), 1.68 (m, 4H), 2.83 (s, 3H), 2.98 (m, 4H), 5.67 (d, *J* = 2.10, 1H), 6.79 (d, *J* = 2.10, 1H), 6.62–6.77, 6.99–7.25 (m, 7H), 9.57 (s, 1H); EI-MS: 337 [M]. Anal. Calcd for $C_{20}H_{23}N_3O_2$: C, 71.19; H, 6.87; N, 12.45. Found: C, 71.14; H, 7.11; N, 12.84.

8.1.71. 2-Phenyl-3-methyl-6-(morphilin-4-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15c)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14b**) (0.35 g, 1.49 mmol), benzaldehyde (0.32 g, 2.98 mmol)as a pale yellow powder (0.07 g, 14.55%): mp 227–229 °C; IR (cm^{-1}): 3471, 3285, 2848, 2803, 1639, 1619, 1513, 1444, 1391, 1120, 820, 703; ^1H NMR (DMSO-d_6): δ 2.85 (s, 3H), 2.93 (t, *J* = 4.73, 4H), 3.71 (t, *J* = 4.56, 4H), 5.75 (d, *J* = 2.33, 1H), 6.88 (d, *J* = 2.22, 1H), 6.59–6.62, 6.95–7.35 (m, 8H); EI-MS: 323 [M]. Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.55; N, 12.99. Found: C, 70.38; H, 6.54; N, 13.45.

8.1.72. 2-(4′-Hydroxyphenyl)-3-methyl-6-(morphilin-4-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15d)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14b**) (0.15 g, 0.64 mmol), 4-hydroxybenzaldehyde (0.16 g, 1.28 mmol)as a pale yellow powder (0.13 g, 60.09%): mp 246–248 °C; IR (cm^{-1}): 3437, 2853, 2813, 1636, 1516, 1453, 1343, 1234, 1109, 977, 841, 777, 687; ^1H NMR (DMSO-d_6): δ 2.78 (s, 3H), 2.94 (m, 4H), 3.71 (t, *J* = 4.50, 4H), 5.63 (d, *J* = 2.10, 1H), 6.76 (d, *J* = 1.80, 1H), 6.59–6.74, 6.96–7.20 (m, 7H), 9.50 (s, 1H); EI-MS: 339 [M]. Anal. Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.27; H, 6.53; N, 12.63.

8.1.73. 2-Phenyl-3-methyl-6-(pyrrolidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15e)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14c**) (0.20 g, 0.91 mmol), benzaldehyde (0.19 g, 1.83 mmol)as a yellow powder (0.20 g, 71.34%): mp 233–235 °C; IR (cm^{-1}): 3437, 2838, 1638, 1619, 1515, 1485, 1385, 1311, 1160, 995, 810, 775, 700; ^1H NMR (DMSO-d_6): δ 1.91 (m, 4H), 2.87 (s, 3H), 3.13 (m, 4H), 5.69 (s, 1H), 6.86 (s, 1H), 6.58, 7.31 (m, 8H); EI-MS: 307 [M]. Anal. Calcd for $C_{19}H_{21}N_3O$: C, 74.24; H, 6.89; N, 13.67. Found: C, 74.05; H, 6.90; N, 14.04.

8.1.74. 2-(3′-Hydroxyphenyl)-3-methyl-6-(pyrrolidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15f)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14c**) (0.17 g, 0.78 mmol), 4-hydroxybenzaldehyde (0.19 g, 1.55 mmol)as a brown powder (0.17 g, 67.81%): mp 224–226 °C; IR (cm^{-1}): 3415, 2955, 2838, 1637, 1620, 1514, 1484, 1390, 1238, 1158, 995, 810, 769; ^1H NMR (DMSO-d_6): δ 1.89 (m, 4H), 2.79 (s, 3H), 3.07 (m, 4H), 5.53 (s, 1H), 6.80 (s, 1H), 6.44–6.67, 7.02–7.07 (m, 7H), 9.37 (s, 1H); EI-MS: 323 [M]. Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.55; N, 12.99. Found: C, 70.27; H, 6.61; N, 13.03.

8.1.75. 2-(4′-Hydroxyphenyl)-3-methyl-6-(pyrrolidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15g)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14c**) (0.17 g, 0.78 mmol), 4-hydroxybenzaldehyde (0.19 g, 1.55 mmol)as a brown powder (0.12 g, 47.86%): mp 235–240 °C; IR (cm^{-1}): 3492, 3411, 2877, 2809, 1614, 1592, 1513, 1483, 1237, 1170, 838, 810; ^1H NMR (DMSO-d_6): δ 1.92 (m, 4H), 2.79 (s, 3H), 3.14 (m, 4H), 5.56 (s, 1H), 6.86 (s, 1H), 6.40–6.72, 7.11–7.13 (m, 7H), 9.49 (s, 1H); EI-MS: 323 [M]. Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.55; N, 12.99. Found: C, 70.24; H, 6.67; C, 13.07.

8.1.76. 2-Phenyl-3-benzyl-6-(piperidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15h)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14d**) (0.20 g, 0.65 mmol), benzaldehyde (0.14 g, 1.29 mmol)as a pale yellow powder (0.05 g, 19.46%): mp 140–142 °C; IR (cm^{-1}): 3480, 3299, 3025, 2931, 1621, 1506, 1311, 1209, 1117, 937, 765, 696; ^{1}H NMR (DMSO-d$_6$): δ 1.63 (m, 2H), 1.85 (m, 4H), 3.44 (m, 4H), 3.84–3.89&5.29–5.34 (dd, *J* = 434.31, 15.36 Hz, 2H), 5.83 (s, 1H), 6.90 (br, 1H), 6.77–7.59 (m, 13H); EI-MS: 397 [M]. Anal. Calcd for $C_{26}H_{27}N_3O$: C, 78.56; H, 6.85; N, 10.57. Found: C, 78.26; H, 6.73; N, 10.40.

8.1.77. 2-(3′-Hydroxyphenyl)-3-benzyl-6-(piperidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15i)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14d**) (0.20 g, 0.65 mmol), 3-hydroxybenzaldehyde (0.16 g, 1.29 mmol)as a pale yellow powder (0.08 g, 29.93%): mp 145–147 °C; IR (cm^{-1}): 3458, 3328, 2931, 2801, 1620, 1509, 1286, 1218, 1023, 818, 778, 696; ^{1}H NMR (DMSO-d$_6$): δ 1.48 (m, 2H), 1.58 (m, 4H), 2.93 (m, 4H), 3.74–.79&5.32–5.37 (dd, *J* = 474.87, 15.36 Hz, 2H), 5.56 (d, *J* = 2.46, 1H), 6.86 (d, *J* = 2.19, 1H), 6.55–7.36 (m, 12H), 9.43 (s, 1H); EI-MS: 413 [M]. Anal. Calcd for $C_{26}H_{27}N_3O_2$: C, 75.52; H, 6.58; N, 10.16. Found: C, 75.19; H, 6.59; N, 9.96.

8.1.78. 2-(4′-Hydroxyphenyl)-3-benzyl-6-(piperidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15j)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14d**) (0.20 g, 0.65 mmol), 4-hydroxybenzaldehyde (0.16 g, 1.29 mmol)as a pale yellow powder (0.12 g, 44.89%): mp 249 °C; IR (cm^{-1}): 3415, 3172, 2936, 2606, 1616, 1503, 1263, 1235, 940, 832, 695; ^{1}H NMR (DMSO-d$_6$): δ 1.48 (m, 2H), 1.61 (m, 4H), 2.93 (m, 4H), 3.75–3.80&5.21–5.26 (dd, *J* = 437.04, 14.64 Hz, 2H), 5.55 (s, 1H), 6.80 (s, 1H), 6.56–7.33 (m, 12H), 9.44 (s, 1H); EI-MS:413 [M]. Anal. Calcd for $C_{26}H_{27}N_3O_2$: C, 75.52; H, 6.58; N, 10.16. Found: C, 75.25; H, 6.62; N, 9.89.

8.1.79. 2-Phenyl-3-benzyl-6-(morphilin-4-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15k)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14e**) (0.20 g, 0.64 mmol), benzaldehyde (0.14 g, 1.29 mmol) as a pale yellow powder (0.11 g, 42.88%): mp 188–190 °C; IR (cm^{-1}): 3480, 3290, 3025, 2931, 1621, 1506, 1311, 1209, 1117, 942, 699; ^{1}H NMR (DMSO-d$_6$): δ 2.94 (t, *J* = 4.74, 4H), 3.71 (t, *J* = 4.82, 4H), 3.81–5.34 (dd, *J* = 445.43, 15.36 Hz, 2H), 5.69 (d, *J* = 2.61, 1H), 6.96 (d, *J* = 2.56, 1H), 6.59–7.35 (m, 13H); EI-MS: 399 [M]. Anal. Calcd for $C_{25}H_{25}N_3O_2$: C, 75.16; H, 6.31; N, 10.52. Found: C, 75.12; H, 6.24; N, 10.58.

8.1.80. 2-(3′-Hydroxyphenyl)-3-benzyl-6-(morphilin-4-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15l)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14e**) (0.31 g, 1.00 mmol), 3-hydroxybenzaldehyde (0.24 g, 2.00 mmol)as a pale yellow powder (0.23 g, 55.61%): mp 226–228 °C; IR (cm^{-1}): 3360, 3215, 2952, 1637, 1506, 1451, 1415, 1266, 1238, 1170, 1125, 948, 881, 815, 732; ^{1}H NMR (DMSO-d$_6$): δ 3.03 (t, *J* = 4.71, 4H), 3.75 (t, *J* = 4.79, 4H), 3.76–5.37 (dd, *J* = 470.88, 15.39 Hz, 2H), 5.59 (s, 1H), 6.71 (d, *J* = 2.16, 1H), 6.61–7.36 (m, 12H), 9.45 (s, 1H); EI-MS: 415 [M]. Anal. Calcd for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.06; N, 10.11. Found: C, 71.92; H, 6.20; N, 9.95.

8.1.81. 2-(4′-Hydroxyphenyl)-3-benzyl-6-(morphilin-4-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15m)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14e**) (0.10 g, 0.32 mmol), 4-hydroxybenzaldehyde (0.08 g, 0.64 mmol)as a pale yellow powder (0.10 g, 74.59%): mp 253–255 °C; IR (cm^{-1}): 3418, 3290, 2823, 1632, 1513, 1228, 1109, 944, 836, 738, 703; ^{1}H NMR (DMSO-d$_6$): δ 2.94 (t, 4H, *J* = 4.66), 3.71 (t, 4H, *J* = 4.87), 3.76–5.27 (dd, *J* = 437.79, 2H, 15.35 Hz), 5.56 (d, *J* = 2.13, 1H), 6.81 (d, *J* = 2.14, 1H), 6.59–7.31 (m, 12H), 9.45 (s, 1H); EI-MS: 415 [M]. Anal. Calcd for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.06; N, 10.11. Found: C, 71.88; H, 6.03; N, 9.88.

8.1.82. 2-Phenyl-3-benzyl-6-(pyrrolidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15n)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14f**) (0.20 g, 0.68 mmol), benzaldehyde (0.14 g, 1.36 mmol)as a pale yellow powder (0.22 g, 84.73%): mp 193–195 °C; IR (cm^{-1}): 3454, 3307, 2944, 1616, 1522, 1487, 1302, 1160, 968, 802, 695; ^{1}H NMR (DMSO-*d*$_6$): δ 1.92 (m, 4H), 3.14 (m, 4H), 3.81–5.34 (dd, J_1 = 445.2, $J_{2\ =\ 15.36,}$ 2H), 5.64 (d, *J* = 2.88, 1H), 6.56–6.61, 7.24–7.35 (m, 13H), 6.91 (d, *J* = 2.40, 1H); EI-MS: 383 [M]. Anal. Calcd for $C_{25}H_{25}N_3O$: C, 78.30; H, 6.57; N, 10.96. Found: C, 77.98; H, 6.48; N, 10.73.

8.1.83. 2-(3′-Hydroxyphenyl)-3-benzyl-6-(pyrrolidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15o)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14f**) (0.20 g, 0.68 mmol), 3-hydroxybenzaldehyde (0.17 g, 1.36 mmol)as a pale yellow powder (0.28 g, 100%): mp 159–161 °C; IR (cm^{-1}): 3490, 3325, 2828, 1617, 1582, 1515, 1481, 1290, 1232, 1160, 972, 808, 741, 695; ^{1}H NMR (DMSO-*d*$_6$): δ 1.92 (m, 4H), 3.15 (m, 4H), 3.75–5.37 (dd, J_1 = 471.1, J_2 = 15.31, 2H), 5.52 (d, *J* = 2.28, 1H), 6.50–6.72, 7.06–7.33 (m, 12H), 6.92 (d, *J* = 2.31, 1H), 9.34 (s, 1H); EI-MS: 399 [M]. Anal. Calcd for $C_{25}H_{25}N_3O_2$: C, 75.16; H, 6.31; N, 10.52. Found: C, 75.06; H, 6.34; N, 10.33.

8.1.84. 2-(4′-Hydroxyphenyl)-3-benzyl-6-(pyrrolidin-1-yl)-1,2,3,4-tetrahydro-1*H*-quinazolin-4-one (15p)

Following the synthetic procedure of compound (**15a**), the title compound was obtained from compound (**14f**) (0.20 g, 0.68 mmol), 4-hydroxybenzaldehyde (0.17 g, 1.36 mmol)as a pale yellow powder (0.22 g, 81.34%): mp 242–245 °C; IR (cm^{-1}): 3387, 3363, 2952, 1614, 1590, 1516, 1482, 1235, 1172, 803, 736, 694; ^{1}H NMR (DMSO-*d*$_6$): δ 1.91 (m, 4H), 3.15 (m, 4H), 3.77–5.26 (dd, J_1 = 423.9, J_2 = 15.31, 2H), 5.52 (d, *J* = 2.64, 1H), 6.38–6.68, 7.07–7.30 (m, 12H), 6.92 (d, *J* = 2.58, 1H), 9.35 (s, 1H); EI-MS: 399 [M]. Anal. Calcd for $C_{25}H_{25}N_3O_2$: C, 75.16; H, 6.31; N, 10.52. Found: C, 75.19; H, 6.31; N, 10.36.

8.2. Purification of KSP and ATPase activity assay

These studies were made following the procedure reported by our group.[17]

8.3. SRB proliferation assays

Sulforhodamine B, trichloroacetic acid, trizma base, and acetic acid were purchased from Sigma Chemical Co. (St. Louis, USA). Cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum at 37 °C in humidified air under 5% CO_2. A pilot-screening operation was initiated in which the panel lines were inoculated onto a series of standard 96-well plates on day 0, in the majority of cases at 5×10^3 cells/well, and then preincubated in the absence of the test compounds for 24 h. Then the medium was discarded and replaced with serum-free medium containing the desired concentrations (0, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5, 5, and 10 μM) of test compounds dispensed from serial dilutions, and the plates were incubated for another 48 h at 37 °C. Following this, the cells were fixed in situ, washed, and dried. SRB was added, followed by further washing and drying of the stained, adherent cell mass. The inhibition of cell proliferation was assessed by measuring changes in total optical density after a culture of each cell line that was subjected to 48 h of drug treatment. The results were obtained in one independent experiment run in triplicate.

8.4. Flow cytometry

Briefly, A549 cells were cultured with **15j** or CK0106023 (8, 12, 16 μM) for 24 h or 48 h at 37 °C. 1×10^6 cells were then collected, washed twice with ice-cold phosphate-buffered saline (PBS), and fixed in 70% ethanol at 4 °C until further procession. Cells were washed again with PBS and incubated with propidium iodide (PI, 20 μg/mL)/RNase A (20 μg/mL) in PBS for 30 min in the dark at room temperature. Data acquisition and analysis were performed with FACSCalibur and CellQuest software (Becton Dickinson, CA). The percentage of apoptotic cells was calculated from the number of cells in sub-G1 phase, representing fragmented cell vesicles.

8.5. Annexin V/PI double-staining assay

A549 cells (1×10^6 cells/mL) were treated with **15j** for 24 h, harvested, and then washed and resuspended with PBS. Apoptotic cells were quantified by annexin V-FITC-PI double staining, using a kit purchased from Bipec, according to the manufacturer's instructions. Flow cytometric analysis was performed immediately after supravital staining. Data acquisition and analysis were performed with FACSCalibur and CellQuest software.

8.6. JC-1 mitochondrial membrane potential detection assay

The fluorescent cationic dye JC-1 (Bipec) was used for detection of mitochondrial membrane transition events in live cells, to provide an early indication of the initiation of cellular apoptosis. For this assay, A549 treated with different concentrations of **15j** (8, 12 and 16 μM) for 24 h were harvested with ice-cold PBS and resuspended in RPMI-1640 medium at a density of 1×10^6 cells/ml. The pellet was resuspended in incubation buffer containing JC-1 at 37 °C in a 5% CO_2 incubator for 15–20 min. Cells were washed and analyzed for apoptosis using a flow cytometer (FACSCalibur, Becton Dickinson), and analyzed by the software Modfit and Cell-Quest (BD Biosciences, Franklin Lakes, NJ).

8.7. Western blot analysis

The effect of **15j** treatment on the expression of the Bcl-2 family of anti- (Bcl-2) and pro-apoptotic (Bax) proteins and the p53 protein was determined to gain insight into the mechanism for **15j**-induced cell death. A549 cells were incubated with **15j** at various concentrations (8, 12 and 16 μM) for 24 h. Cells were collected and lysed in lysis buffer (50 mM Tris–Cl, pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% (m/v) NP-40, 0.2 mM PMSF, 0.1 mM NaF and 1.0 mM DTT). The lysates were clarified by centrifugation at 4 °C for 15 min at 13,000*g*. The protein concentration was determined with the BCA reagent. Equal amounts of protein were subjected to electrophoresis on 10 % SDS–polyacrylamide gels and transferred onto the PVDF membranes (Millipore, Boston, MA). The blots were incubated with appropriate primary antibodies overnight at 4 °C followed by IRDye^{TM}800 conjugated secondary antibody for 1 h at 37 °C. Detection was performed by the Odyssey Infrared Imaging System (LI-COR Inc., USA). All blots were stripped and reprobed with polyclonal anti-β-actin antibody to ascertain equal loading of proteins.

8.8. Immunofluorescence microscopy

The A549 cells were left to adhere for at least 48 h on poly-(D-lysine)-coated glass coverslips before the addition of test compound. Following incubation with **15j** (8 μM) for 24 h, cells were fixed with 4% paraformaldehyde–PBS at 37 °C for 45 min, washed with PBS for 5 min twice, permeabilized with 0.2% TX100 in PBS for 30 min at 4 °C, and washed with PBS for 5 min. DNA/chromatin was stained with propidium iodide. Images were collected with Leica TCS SP2 (Leica, German) fluorescence microscope using Leica confocal software.

8.9. In vivo activity of 15j against transplantable tumors in mice

Mouse sarcoma S180 cell lines were provided by Jiangsu Institution of Anticancer Agents and reproduced in our laboratory. ICR Mice (Grade II, 5-weeks-old) weighing 18–22 g were provided by China Pharmaceutical University (Certificate No. SCXK-2002-0011, Nanjing, China). Half of them were male, and the others were female. Rodent laboratory chow and tap water were provided ad libitum, and maintained under controlled conditions with a temperature of 18–24 °C, humidity of 70%, and a 12/12 h light/dark cycle.

All samples were formulated in 10% ethanol–10% cremaphor in water and administered by intraperitoneal (ip) injection. Ascites of the S180-bearing mice were drawn out under aseptic conditions and then diluted three fold with aseptic saline. The diluted S180 cell suspension was subcutaneously (sc) inoculated into the armpit of ICR mice for 0.2 ml per mouse. After 24 h, mice were divided into five groups, each consisting of ten mice. The inoculated mice were administered with **15j** at the doses of 50, 25 and 12.5 mg/kg or CK0106023 at a dose of 50 mg/kg for 10 days every other day. The control groups received the same volume of saline. The dose volume was 0.2 ml/10 g body weight. On day 11, mice were weighed and sacrificed by cervical dislocation. The solid tumors were collected and weighed. Then, the inhibitory rate against the growth of tumor was calculated. The inhibitory rate (%) = $[(C - T)/C] \times 100$, where C is the average tumor weight of the control group; T is the average tumor weight of sample-treated groups.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.029.

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