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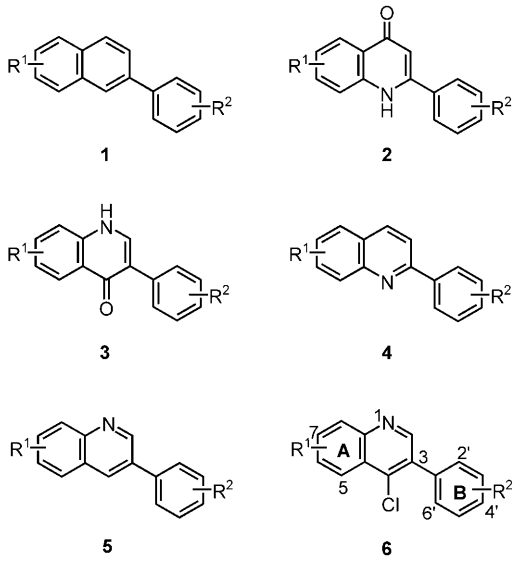
Synthesis, Antiproliferative Evaluation, and Structure–Activity Relationships of 3-Arylquinolines

Zhu-Ping Xiao, Peng-cheng Lv, Suo-Ping Xu, Tao-Tao Zhu, and Hai-Liang Zhu*[a]

Multidrug resistance and the neurological and hematological adverse side effects of the natural products in current clinical use, such as taxanes and vinca alkaloids, continue to drive the search for new synthetic antitumor agents.[1–3] A tricyclic structural pattern, such as a 2-phenyl-naphthalene scaffold (**1**), is



present in a large number of antineoplastic compounds.[4–8] While the simple scaffold alone may not be a potent pharmacophore, derivatives with appropriate substitution patterns on both ring systems could exhibit potent biological activities. A number of 2-phenyl-4-quinolones (**2**), 3-phenyl-4-quinolones (**3**), and their condensed heterocyclic derivatives (**4** and **5**) have been described in the literature.[9–14] Several of the compounds exhibited potent cytotoxicity in a number of systems.[7] More over, some aza analogues of the 2-phenyl-naphthalenes (**1**), 2-phenylquinolines (**4**), possessed interesting biological activities.[15,16] However, the 3-phenylquinoline (**5**) derivatives have yet to be investigated as potential anticancer agents. While no antitumor activity has been reported for the 3-phenylquinolines (**5**), the similarity in biological activities between the 2-phenyl-4-quinolones (**2**) and 3-phenyl-4-quinolones (**3**),[17,18] suggests that the 3-phenylquinolines (**5**) should exhibit similar biological activities to the 2-phenylquinolines (**4**), and be of interest as new chemotherapeutics.

Based on this hypothesis, a series of 3-phenylquinolines were designed and synthesized to mimic the "2-phenylnaphthalene-type" structural pattern (**1**).[19] The 3-phenylquinolines are expected to act through the same mechanism of action as the 2-phenylquinolines, which are known to intercalate DNA leading to inhibition of mammalian topoisomerase II.[15,16,20] In order to identify potent potential lead compounds, and probe the structure–activity relationships (SAR) within these compounds, both the phenyl and quinoline ring were substituted with groups of varying steric, electronic, and lipophilic properties.

Tricyclic pharmacophores are common in anticancer therapeutics,[5,8] for this reason, the basic tricyclic ring system (**6**) was retained and substitutions were made on the A-ring and B-ring. Thirty-four compounds substituted with various substituents (NO_2, F, Cl, Br, CH_3 and OMe), selected primarily for their wide range of electronic properties ($\sigma = +0.78$ to -0.36), using different substitution patterns were designed, synthesized and evaluated for their antiproliferative activity in vitro.

The general procedure for the preparation of 3-arylquinolines is described in Scheme 1. Generally, the condensation of an aldehyde with an anilines in EtOH gave the enamine (**7**) as a mixture of *Z*- and *E*-isomers.[21,22] Both regioisomers were able to cyclize to form the intermediate 3-aryl-4-quinolone (**8**), so separation of the *Z*- and *E*-isomers was not necessary. Heating the enamine (70–80 °C) in the presence of polyphosphoric acid (PPA) gave the 3-aryl-4-quinolone (**8**) in good to high yields. The 3-arylquinolone **8** scaffold was converted to the corresponding 3-arylquinoline structure to give derivatives **9**–**42**, using a method based on literature reports.[23,24] In the literature, 3-arylquinolone **8** was treated with refluxing $POCl_3$. The mixture was poured onto ice-cold water and basified with NaOH solution. The crude product was obtained after extraction with CH_2Cl_2 and purified by recrystallization or chromatography. Modification to this reported method circumvented the tedious work-up procedure; removal of excess $POCl_3$ followed by basification with $NaHCO_3$ gave the final product in high purity. Unfortunately, these conditions were not suitable for the synthesis of dimethoxy-3-arylquinolines ($R^2 = R^4 = OMe$).

3-Aryl-4-chloroquinolines (**9**–**42**) evaluated for their cytotoxicity against three human tumor cell lines: human hepatocellular liver carcinoma (Hep-G2), human erythromyeloblastoid leukemia (K562) and human oral epidermoid carcinoma (KB), and the normal human liver cell line (L02). The derivatives were tested over a range of concentrations (0.01–100 $\mu g\,mL^{-1}$) and the associated IC_{50} values were calculated (Table 1). Many of the 3-aryl-4-chloroquinolines significantly inhibited the growth of human cancer cell lines, while showing little toxicity against

[a] Z.-P. Xiao, P.-c. Lv, S.-P. Xu, T.-T. Zhu, H.-L. Zhu
State Key Laboratory of Pharmaceutical Biotechnology
Nanjing University, Nanjing 210093 (China)
Fax: (+86) 25-8359-2672
E-mail: zhuhl@nju.edu.cn

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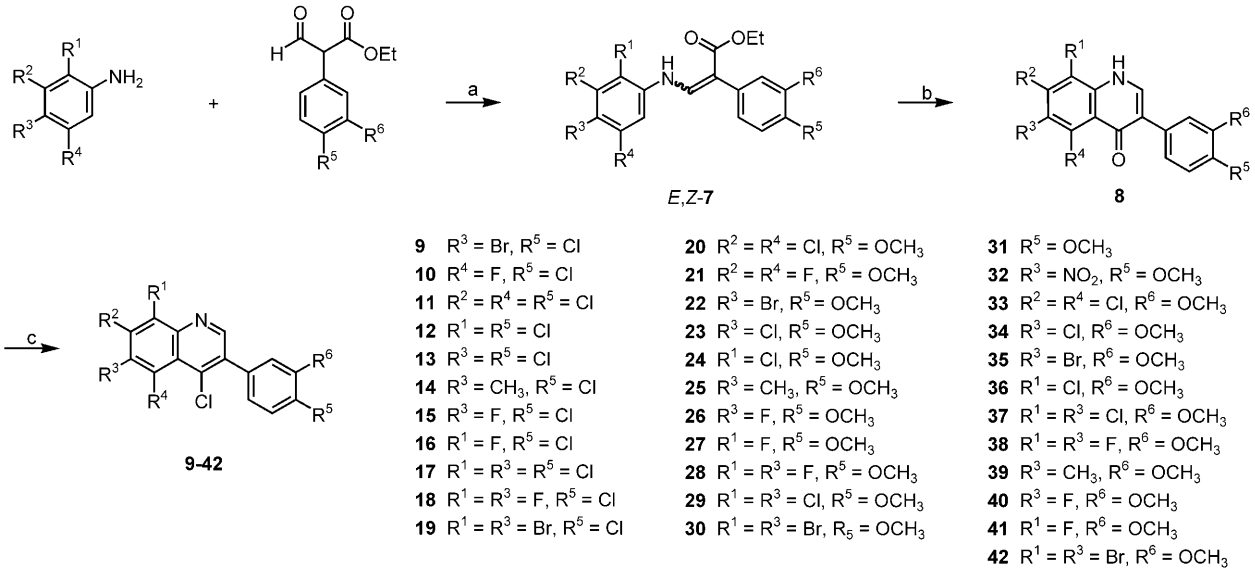

Scheme 1. Reagents and conditions: a) EtOH, 60-80 °C, 2–5 h; b) PPA, 70-80 °C, 2–5 h; c) $POCl_3$, reflux, 1–3 h.

Table 1. IC_{50} values [μM] of the 3-arylquinoline series.[a,b]

Compound	K562	Hep-G2	KB	L02
9	266±68	181±42	212±65	229±76
10	261±90	232±61	274±71	NA
11	NA	NA	NA	NA
12	61±3	18.2±5.5	36±13	143±42
13	227±87	90±29	175±58	223±65
14	NA	NA	NA	NA
15	68±14	19.5±6.2	72±41	130±48
16	16.1±3.6	9.9±4.2	78±26	208±88
17	140±41	44±20	102±38	NA
18	58±19	19±5.8	48±19	NA
19	132±39	72±21	113±37	NA
20	181±73	184±55	207±61	268±76
21	184±61	181±61	187±42	161±52
22	166±72	52±14	121±32	132±26
23	118±36	39±13	82±26	56±20
24	9.5±2.6	16.1±2.6	10.5±0.3	145±39
25	NA	197±42	NA	187±32
26	59±21	10.1±2.8	38.2±3.8	NA
27	6.3±2.4	1.7±0.2	4.2±0.3	NA
28	8.2±2.3	5.6±1.0	6.2±0.3	202±56
29	15.7±3.8	7.1±1.5	12.7±2.1	127±35
30	70±28	42±7	68±14	220±54
31	234±67	122±48	174±52	41±7
32	7.6±1.6	5.7±2.5	6.4±1.9	127±32
33	53±9	71±12	59±15	219±21
34	22.4±3.9	19.4±4.3	18.8±3.0	234±36
35	35.3±5.7	26±6	29±9	181±43
36	2.0±0.3	3.6±0.7	4.3±1.3	95±86
37	2.7±0.6	5.9±0.9	3.8±1.5	216±38
38	0.56±0.20	0.43±0.29	0.7±0.7	245±56
39	9.9±1.4	57±14	27.9±4.6	194±32
40	8.0±1.0	3.8±0.7	4.5±2.4	170±38
41	0.38±0.28	0.21±0.07	0.59±0.28	NA
42	16.1±2.3	37±7	33±5	199±42
5-FU	35±7	2.46±0.31	2.31±0.69	50±16

[a] NA = not active, that is, the IC_{50} value is greater than the maximum concentration tested (300 μM). [b] The IC_{50} value of a compound is defined as the concentration (μM) required to cause 50% cell death, compared with a control culture.

L02 hepatocytes. Conversely, fluorouracil (5-FU) inhibited the growth of all four cell lines equally. In general, cytotoxicity in one tumor cell line was often echoed in at least one other cell lines tested. Compounds **38**, **40** and **41** exhibited potent inhibitory activity in all tumor cell lines tested (Table 1).

Compounds substituted at the R^5 position (e.g., **20**, **22**, **24**) were uniformly less active than those substituted at the R^6 position (e.g., **33**, **35**, **36**), while compounds **11** and **14**, with a chlorine atom at R^5 position, were the least active. The position of substituents on the A-ring plays an important role with regard to biological activity. Derivative **36** bears a chlorine atom at R^1 on the A-ring, and exhibited very good inhibitory activity against all three human tumor cell lines. On the contrary, compound **34** containing a chlorine atom at the R^3 position is less active. Dihaloganated A-ring systems gave similar results, for example, compound **33** showed significant decrease in activity compared to compound **37**. Replacement of electron-donating groups with electron-withdrawing substituents at the R^3 result-

ed in a moderate to significant decrease in activity, in the order $NO_2 > F > Cl > Br > CH_3$. Of the 34 compounds tested, potent activities were noted for analogues **36**, **37**, **38** and **40**, but the most potent activity was found with compounds **41**, whose IC_{50} values against the three human tumor cell lines ranged from 0.59 to 0.21 μM, which is more potent than the known antitumor agent, 5-FU (Table 1).

To summarize the SAR, the replacement of R^5 for R^6 substituents on the B-ring leads to an increase cytotoxicity to the human tumor cell lines. Substitution at the R^3 or disubstitution at the R^2,R^4 positions shows a reduced level of activity against human tumor cell lines compared to that at the R^1 or R^1,R^3 positions. Finally, electron-donating groups at the R^3 position on theA-ring are detrimental to the cytotoxicity of the compound against the tumor cell lines tested.

The screening of 34 compounds has led to the discovery of two 3-aryl-4-chloroquinolines **38** and **41**, with high activity against human tumor cell lines, and low cytotoxicity in the normal human liver cell line L02. The high selectivity of these compounds for neoplastic over healthy tissue encourages us to further investigate these molecules as potential drugs. 3-Aryl-4-chloroquinolines **38** and **41** are suitable leads for future design and development of a focused chemical library to further evaluate and gain insight into the requirements for activity of this class of compounds.

Experimental Section

Chemistry

All chemicals (reagent grade) used were purchased from Sigma–Aldrich (US) and Sinopharm Chemical Reagent Co., Ltd. (China). Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). EI mass spectra were obtained on a Waters GCT mass spectrometer, and ^{1}H NMR spectra were recorded on a Bruker AV-300 or AV-500 spectrometer at 25 °C with TMS (tetramethylsilane) and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

General procedure for the preparation of 3-aryl-4-chloroquinolines: The synthesis of the enamine starting materials has been previously published.[21] A mixture of enamine (10.0 mmol) and PPA (20 g, 59 mmol) was stirred for 2–5 h (monitored using TLC) at 70–80 °C. The residue was cooled, poured onto ice/water (30 g). The precipitate was isolated by filtration and washed with H_2O to give 3-aryl-4-quinolones (**8**) with high purity in good to high yield (68–99%). A mixture of **8** (10 mmol) and $POCl_3$ (20 mL) was heated at reflux for 1–3 h. After the reaction reached completion, excess $POCl_3$ was removed under reduced pressure, and the crude residue was basified with saturated $NaHCO_3$. The precipitate was isolated by filtration and washed with H_2O to give 3-aryl-4-chloroquinolines with high purity. All compounds synthesized were fully characterized by spectroscopic methods and purity was confirmed by analytical methods. The ^{1}H NMR, EI mass spectra, elemental analyses data and physical properties of compounds **9**--**42** are available in the Supporting Information.

Biology

Human hepatocytes (L02) were purchased from Cell Bank of the Institute of Biochemistry and Cell Biology (Shanghai, China). Hepatocellular liver carcinoma (Hep-G2), Human erythromyeloblastoid leukemia (K562) and human oral epidermoid carcinoma (KB) were purchased from American Type Culture Collection. The optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader.

Cytotoxicity assays: The cytotoxic activity of 3-aryl-4-chloroquinolines **9**--**42** was determined using a MTT assay (Sigma–Aldrich), and 5-FU was used as the reference drug. Briefly, cell lines were seeded at a density of 7×10^3 cells/well in 96-well microtiter plates (Costar). After incubation for 24 h, cells were treated with the test compounds at final concentrations ranging from 0.01 to 100 μg mL^{-1}. After 48 h, cell survival was determined by the addition of an MTT solution (10 μL of 5 mg mL^{-1} MTT in PBS). After 4 h, 100 μL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 18 h, before the optical absorbance was measured. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of six wells from at least two independent experiments.

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