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Design and Synthesis of 2-(3-Benzo[*b*]thienyl)-6,7-methylenedioxyquinolin-4-one Analogs as Potent Antitumor Agents that Inhibit Tubulin Assembly

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Abstract

As part of our continuing investigation of azo-flavonoid derivatives as potential anticancer drug candidates, a series of 2-aryl-6,7-methylenedioxyquinolin-4-one analogs was designed and synthesized. The design combined structural features of 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (**CHM-1**), a previously discovered compound with potent in vivo antitumor activity, and 2-arylquinolin-4-ones identified by CoMFA models. The newly synthesized analogs were evaluated for cytotoxicity against seven human cancer cell lines, and structure-activity relationship (SAR) correlations were established. Analogs **1**, **37**, and **39** showed potent cytotoxicity against different cancer cell lines. Compound **1** demonstrated selective cytotoxicity against Hep 3B (hepatoma) cells. Compound **37** was cytotoxic against HL-60 (leukemia), HCT-116 (colon cancer), Hep 3B (hepatoma), and SK-MEL-5 (melanoma) cells. Compound **39** exhibited broad cytotoxicity against all seven cancer cell lines, with IC_{50} values between 0.07–0.19 μM. Results from mechanism of action studies revealed that these new quinolone derivatives function as antitubulin agents.

Keywords

2-Arylquinolin-4-ones; CHM-1; Cytotoxicity; Tubulin inhibitor

Introduction

Microtubules of eukaryotic cells are known to play an important role in mitosis.[1] Therefore, compounds that target microtubules have long been investigated as anticancer drugs. Typically, such compounds arrest cells, at least transiently, in the mitotic phase of the cell cycle. Currently, two groups of antimitotic agents are used clinically for cancer treatment. Vinca alkaloids and

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estramustine inhibit tubulin polymerization into microtubules, while taxoids and epothilones stabilize microtubules and consequently interfere with their normal dynamic instability.[2, 3] Colchicine is another well-known antimitotic agent used for treating gout and inflammatory diseases. However, due to its high toxicity, colchicine is not used in cancer therapy. Combretastatin A-4, with a modified colchicine-like structure, exhibits a lower toxicity profile, while it still targets the same binding site as colchicine.[4, 5] The phosphate prodrug of combretastatin A-4 (CA-4) is currently in phase III clinical trials as an anticancer agent.[6, 7] Although several antimitotic agents are available, due to the structural complexity of vinca alkaloids and taxoids, there is still a need to identify novel anticancer drugs that target microtubules but have simplified structures, because such compounds can be readily prepared in a more cost-effective way.

In recent years, we have designed and synthesized three series of azo-flavonoids as new classes of antimitotic agents: 2-arylquinolone-4-ones,[8–15] 2-arylnaphthyridin-4-ones,[16–18] and 2-arylquinazolin-4-ones [19, 20] (Figure 1). Some of these compounds, especially analogs belonging to the 2-arylnaphthyridin-4-one family, demonstrated significant antitumor and anti-tubulin activities. In general, a good correlation was found between the cytotoxicity of these analogs and their inhibitory effects on tubulin polymerization. Additional mechanism of action studies revealed that the most active of these compounds are also potent inhibitors of colchicine binding to tubulin. Further modification of the 2-phenylquinolin-4-one series was directed by computer modeling studies combined with SAR results from previously synthesized compounds. In the current study, we report the design and syntheses of novel 2-arylquinolone-4-one analogs, which showed potent and selective inhibitory activities towards different human cancer cell lines.

Design

Both Conventional Comparative Molecular Field Analysis (CoMFA) and g^2 GRS CoMFA were used to identify structural requirements that may be essential for increasing the binding affinity of 2-phenylquinolin-4-one and 2-phenylnaphthyridin-4-one analogs for the colchicine site of tubulin. The training set of the models contained 51 compounds, and the cross-validated R^2 (q^2) values for conventional CoMFA and g^2 GRS CoMFA were 0.637 and 0.692, respectively. These QSAR models predicted that a larger heterocyclic aromatic ring at the C-2 position should enhance activity. The predictive power of the models was validated by the prediction for a test set of 53 compounds with known anti-tubulin potencies, and the predictive R^2 values were 0.546 and 0.426, respectively. Based on this study, new analogs were designed in both compound series (Figure 2).[21] As a proof of concept, the newly designed arylnaphthyridine analog 2-(3-benzo[*b*]thienyl)naphthyridin-4-one (**A**), which has a benzothienyl ring at the C-2 position of the naphthyridine, exhibited potent cytotoxicity in the low micromolar to nanomolar concentration range. Further, a mechanism of action study revealed that the compound also inhibited tubulin polymerization with an IC_{50} value of 0.37 μM. These results verified the success of our modeling design.[18] Thus, the same modifications were carried out at the C-2 position of the 2-arylquinolin-4-one series in the present study.

In addition, when we re-analyzed results from our prior *in vivo* antitumor study of selected 2-phenylquinolin-4-one compounds, we found that analogs with a methylenedioxy functional group at the C-6 and -7 positions showed superior antitumor and safety profiles compared with C-6 mono-modified compounds. For instance, 2-(3-methoxyphenyl)-6-pyrrolinylquinolin-4-one (**B**, Figure 3) exhibited only moderate antitumor activity at the maximum tolerated dose (MTD, 25 mg/kg, ip, once weekly for 3 courses) against the OVCAR-3 xenograft model in nude mice. In contrast, 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (**CHM-1**, Figure 3) extended the life span of tumor-bearing mice by 124%, 133% and 79%, at dosages of 200, 134 and 79 mg/kg (ip, once weekly for 3 courses), respectively. Additionally, the

maximum tolerated dose of **CHM-1** was not reached at the highest tested dosage of 200 mg/kg.[22] Currently, the phosphate prodrug of **CHM-1** (**CHM-1-P-Na**, Figure 4) is under preclinical study.[22, 23] Therefore, the methylenedioxy ring of **CHM-1** was also incorporated into our newly designed 2-arylquinolone-4-one series, which led to the syntheses of 2-aryl-6,7-methylenedioxyquinolin-4-one analogs (**1**, **36–45**). Compound **56**, a ring isomer of analog **39**, was also synthesized, because ring isomerization is a common approach in structure-activity relationship studies of a compound series.

Chemistry

The synthetic procedure for target compounds (**1**, **36–45**) is illustrated in Scheme 1. The starting arylcarboxylic acids (**3–13**) were first treated with oxalyl chloride to form the corresponding acid chlorides (**14–24**), which were then reacted with 2-amino-4,5-methylenedioxyacetophenone (**2**) to give the corresponding amides (**25–35**). The intermediates (**25, 27–34**) were subjected to a cyclization reaction in *t*-BuOH, in the presence of *t*-BuOK, to afford the corresponding 2-arylquinolin-4-one analogs (**1**, **37–44**). Cyclization was also carried out with 1,4-dioxane as solvent, in the presence of NaOH, which yielded the target products (**1**, **36**, **39** and **45**) in yields of 40%–74%. When the starting compounds (**25–26**, **28–32** and **34**) were treated with *t*-BuOK in toluene at 90 °C for 72 h, the corresponding deacetylated products **46–53** were obtained unexpectedly.

Scheme 2 depicts the synthesis of compound **56**. The starting material **2** was first treated with 1-naphthylmethyl chloride (**54**) to give the corresponding amide (**55**). Compound **55** was then subjected to a cyclization reaction in 1,4-dioxane, in the presence of NaOH, which yielded 4-methyl-6,7-methylenedioxy-3-(1-naphthyl)quinolin-2-one (**56**) as the final product.

Results and Discussion

The newly synthesized 2-aryl-6,7-methylenedioxyquinolin-4-ones (**1**, **36–45**) and the ring isomer **56** were assayed for *in vitro* cytotoxicity against seven human cancer cell lines, including HL-60 (leukemia), HCT-116 (colon cancer), A549 (non-small cell lung carcinoma), Hep 3B (hepatoma), KB (epidermoid carcinoma of the nasopharynx), KB-VIN (p-glycoprotein- expressing epidermoid carcinoma of the nasopharynx), and DU145 (prostate cancer). The cytotoxicity results are summarized in Table 1. Compounds **46–53** were tested against the HL-60, HCT-116, H226, A549, Hep G2 and A498 cancer cell lines; however, none of the compounds showed significant activity (IC_{50} values were >10 μM).

Compound **1** exhibited significant cytotoxicity against Hep 3B cells, with an IC_{50} value of 0.06 μM, and moderate activity against HL-60 and HCT-116 cells, with IC_{50} values of 0.17 and 0.14 μM, respectively. This result, along with the prior cytotoxicity data obtained with compound **A**, confirmed the prediction generated by conventional and g^2 GRS CoMFA that a larger heterocyclic aromatic ring should be preferred at the C-2 position. In comparison, replacement of the 3-benzo[*b*]thienyl group (**1**) with a 2-benzo[*b*]thienyl group (**36**) resulted in significantly reduced cytotoxicity, demonstrating the importance of the exact linkage of the aromatic ring moiety.

Bioisosteric replacement of 3-benzo[*b*]thienyl (**1**) with 3-benzo[*b*]furanyl (**37**) led to increased potency against two cancer cell lines. Specifically, compared with **1**, the cytotoxic activity of **37** increased five-fold against HL-60 (IC_{50} 0.03 μM) and three-fold against HCT-116 (IC_{50} 0.05 μM) cells. The two compounds had similar potency against Hep 3B cells. Most importantly, **37** retained potency (IC_{50}: 0.59 μM) against KB-VIN cells, a vincristine-resistant epidermoid carcinoma of the nasopharynx cell line, compared with KB cells (IC_{50} 1.05 μM). As with **1** and **36**, moving the attachment of the benzofuran moiety from 3′ in **37** to 2′ in **38** was detrimental to cytotoxic activity.

Compound **39**, with a 1-naphthyl group rather than a 3-benzo[*b*]thienyl group (**1**) at the C-2 position, exhibited potent cytotoxicity towards all seven cancer cell lines, with IC_{50} values ranging from 0.07 to 0.19 μM. It should be noted that although **39** showed similar activity towards the HL-60, HCT-116, and Hep 3B cancer cell lines as compared with **1** and **37**, it showed significantly increased cytotoxicity towards A549, KB, KB-VIN and DU145 cells. Compound **40**, the ring positional isomer of **39**, was much less cytotoxic, as was the case with compounds **36** and **38**.

Surprisingly, cytotoxicity was reduced significantly when the C-2 aryl moiety was a quinoline group, no matter how it was attached (**41–44**). In addition, expanding the naphthalene ring of **39** to an anthracene (**45**) lowered the cytotoxicity remarkably. Thus, we speculate that the size of the C-2 substituted aromatic ring plays an important role in antitumor activity and that the binding pocket for this portion of the drug molecule is quite small.

Compound **56** was designed as a ring isomer of **39**. The *in vitro* bioassay data showed that moving the aromatic ring to C-3 and the carbonyl to C-2 to give a cyclic amide completely abolished cytotoxicity of the compound. Non-cyclic amide analogs **46–53** also exhibited poor *in vitro* activity.

In addition to the *in vitro* cytotoxicity study, we also performed initial mechanism of action studies with the newly synthesized 2-aryl-6,7-methylenedioxyquinolin-4-one analogs (**1**, **37–45**). As noted above, 2-arylquinolin-4-ones are azo-flavonoids, which were shown to inhibit tubulin assembly and the binding of colchicine to tubulin. Therefore, compounds **1** and **37–45** were tested for their *in vitro* activities in these assays in comparison with combretastatin A-4 (**CA-4**). The results are summarized in Table 2. The data showed that **1**, **37**, and **39**, the three compounds with the greatest *in vitro* cytotoxicity, were potent inhibitors of tubulin assembly, with IC_{50} values of 0.76, 0.58, and 0.64 μM, respectively. Although all three compounds were better assembly inhibitors than **CA-4** (IC_{50} 1.2 μM), they were less effective than CA-4 in inhibiting colchicine binding to tubulin.

Furthermore, results from a pharmacokinetic study in a mice model revealed that the 6,7-methylenedioxy moiety of **CHM-1-P-Na** is metabolized to an ortho-quinone (**D**, Figure 4, unpublished data). It is known that the para-quinone moiety in mitomycin C can be subjected to one-electron reduction by NADPH-cytochrome C (P450) reductase to form the corresponding semiquinone radical anion.[24] We postulate that similar reduction of the ortho-quinone moiety of **D** may take place to form the radical anion product **E**, which may be further metabolized or broken down into more cytotoxic metabolites in hypoxic cells. Because severe hypoxia is a common property of locally advanced solid tumors, this postulate may explain our finding that 6,7-methylenedioxyquinoline analogs (e.g. **CHM-1**) showed enhanced in vivo activity profiles compared with 6-monosubstituted analogs.

Among the active analogs, **37**, which showed potent cytotoxicity and reasonable solubility, was chosen for submission to the National Cancer Institute (NCI, USA) for further screening against 60 human tumor cell lines. In preliminary screening, **37** showed potent selective cytotoxicity against many leukemia cell lines (Figure 5). In addition, it also significantly inhibited the growth of several colon cancer (HCC-2998 and KM-12), CNS cancer (SF-539 and SNB-75), melanoma (SK-MEL-5), and ovarian cancer (IGROV1 and OVCAR-3) cell lines. Based upon these results, **37** appears to be an attractive candidate for further development as a potential anticancer agent in the clinic.

In conclusion, a series of 2-aryl-6,7-methylenedioxyquinolin-4-one analogs (**1**, **36–45**) were designed, synthesized, and evaluated for *in vitro* cytotoxicity. This design combined two structural features: larger heterocyclic aromatic rings at the C-2 position as predicted to be favorable by CoMFA models and a 6,7-methylenedioxy moiety rather than C-6 mono-

substituted analog based on the improved cytotoxicity of **CHM-1** and the metabolic pathway of **CHM-1-P-Na**. In our studies, **1** showed selective cytotoxicity against Hep 3B cells, **37** was active against HL-60, HCT-116, Hep 3B and KB-VIN cells, and **39** had potent cytotoxicity against all seven cancer cell lines with IC_{50} values between 0.07–0.19 μM. A mechanism of action study demonstrated that **1**, **37**, and **39** also function as antitubulin agents. Compound **37** was further selected for evaluation against 60 human cancer cell lines and was active against several types of cancer cells. The significant *in vitro* cytotoxicity of **37** and **39** suggested that they can be further developed as anticancer drugs.

Experimental Section

Chemistry

General Experimental Procedures—Reagents and solvents were obtained commercially and used without further purification. Reactions were monitored by thin-layer chromatography, using Merck plates with fluorescent indicator (TLC Silica gel 60 F_{254}). Flash column chromatography was performed on silica gel (Merck Slica gel 60, 40–63 μm) using a mixture of CH_2Cl_2 and EtOH as eluant. Melting points were determined on a Yanaco MP-500D melting point apparatus and were uncorrected. IR spectra were recorded on Shimadzu IRPrestige-21 spectrophotometers as KBr pellets. ^{1}H NMR spectra were obtained on a Bruker NMR AV 400 spectrometer in DMSO. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet; td, triple doublet; ddd, double double doublet. EI-MS spectra were measured with an HP 5995 GC–MS instrument. ESI-MS spectra were measured with a Bruker HCT ultra PTM Discovery system (Proteineer fc, UltiMate 3000). Elemental analyses (C, H, and N) were performed on a Perkin-Elmer 2400 Series II CHNS/O analyzer, and the results were within ±0.4% of the calculated values.

Preparation of arylcarbonyl chlorides (14–24)—Arylcarboxylic acids (**3**–**13**) were suspended in dry toluene (150 mL) at 20 ± 2 °C. Oxalyl chloride (2.2 eq) was added dropwise. The reaction mixtures were stirred for 30 min at 20 ± 2 °C, then DMF (2 drops) was added. The mixtures were stirred for 6 h, and then evaporated to dryness. The residues were washed with petroleum ether and used directly in the next step.

Preparation of carboxamides (25–35, 55)—Into solutions of **14**–**24** (5.1 mmol) in 200 mL of dry toluene were added triethylamine (4 mL) and 2-amino-4,5-methylenedioxy acetophenone (**2**) (5 mmol). The mixtures were stirred at 20 ± 2 °C for 24 h, then evaporated. The residues were washed with acetone and EtOH, and then recrystallized from acetone or EtOH to form the pure carboxamides.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)-1-benzothiophene-3-carboxamide (25)—** Obtained as a pale yellow solid from 2-amino-4,5-methylenedioxyacetophenone (**2**) and benzo[*b*]thiophene-3-carbonyl chloride (**14**). mp 213–214 °C; MS (ESI) 340 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.63 (3H, s), 6.18 (2H, s), 7.43–7.53 (2H, m), 7.66 (1H, s), 8.10 (1H, dd, *J* = 1.2, 7.2 Hz), 8.30 (1H, s), 8.47 (1H, dd, *J* = 1.2, 7.2 Hz), 8.53 (1H, s), 12.75 (1H, s). IR (KBr): 1638, 1668 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)-1-benzothiophene-2-carboxamide (26)—** Obtained as a grayish white solid from **2** and benzo[*b*]thiophene-2-carbonyl chloride (**15**). mp 233–235 °C; MS (ESI) 340 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.69 (3H, s), 6.19 (2H, s), 7.46–7.57 (2H, m), 7.71 (1H, s), 8.09–8.13 (2H, m), 8.14 (1H, s), 8.25 (1H, s), 13.11 (1H, s). IR (KBr): 1640, 1655 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)-1-benzofuran-3-carboxamide (27)—**Obtained as a pale yellow solid from **2** and benzo[*b*]furan-3-carbonyl chloride (**16**). mp 144–145 °C; MS (ESI) 324 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.63 (3H, s), 6.19 (2H, s), 7.41–7.50 (2H, m), 7.68 (1H, s), 7.75 (1H, dd, *J* = 1.6, 6.8 Hz), 8.15 (1H, dd, *J* = 2.0, 8.8 Hz), 8.27 (1H, s), 8.71 (1H, s), 12.63 (1H,s). IR (KBr): 1635, 1677 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)-1-benzofuran-2-carboxamide (28)—**Obtained as a grayish white solid from **2** and benzo[*b*]thiophene-2-carbonyl chloride (**17**). mp 184–185 °C; MS (ESI) 324 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.69 (3H, s), 6.20 (2H, s), 7.40 (1H, t, *J* = 7.6 Hz), 7.55 (1H, td, *J* = 1.2, 7.8 Hz), 7.72–7.78 (3H, m), 7.84 (1H, d, *J* = 8.0 Hz), 8.36 (1H, s) 13.21 (1H, s). IR (KBr): 1667, 1682 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)naphthalene-1-carboxamide (29)—**Obtained as a grayish white solid from **2** and naphthalene-1-carbonyl chloride (**18**). mp 143–144 °C; MS (ESI) 334 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.59 (3H, s), 6.20 (2H, s), 7.60–7.68 (4H, m), 7.87 (1H, d, *J* = 7.2 Hz), 8.05–8.07 (1H, m), 8.15 (1H, d, *J* = 8.0 Hz), 8.33–8.38 (2H, m), 12.52 (1H, s). IR (KBr): 1647, 1672 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)naphthalene-2-carboxamide (30)—**Obtained as a pale yellow solid from **2** and naphthalene-2-carbonyl chloride (**19**). mp 172–173 °C; MS (ESI) 334 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.68 (3H, s), 6.19 (2H, s), 7.64–7.72 (3H, m), 7.99–8.06 (2H, m), 8.15 (2H, d, *J* = 8.8 Hz), 8.42 (1H, s), 8.58 (1H, s), 13.09 (1H, s). IR (KBr): 1636, 1670 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)quinoline-4-carboxamide (31)—**Obtained as a grayish white solid from **2** and quinoline-4-carbonyl chloride (**20**). mp 166–167 °C; MS (ESI) 335 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.59 (3H, s), 6.21 (2H, s), 7.69 (1H, s), 7.72 (1H, ddd, *J* = 1.2, 7.2 Hz), 7.81 (1H, d, *J* = 4.4 Hz), 7.87 (1H, ddd, *J* = 1.2, 7.4 Hz), 8.15 (1H, d, *J* = 8.4 Hz), 8.24 (1H, s), 8.31 (1H, d, *J* = 8.4 Hz), 9.09 (1H, d, *J* = 4.2 Hz), 12.48 (1H, s). IR (KBr): 1645, 1680 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)quinoline-3-carboxamide (32)—**Obtained as a pale yellow solid from **2** and quinoline-3-carbonyl chloride (**21**). mp 215–216 °C; MS (ESI) 335 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.66 (3H, s), 6.21 (2H, s), 7.73 (1H, s), 7.76 (1H, ddd, *J* = 1.2, 7.4 Hz), 7.94 (1H, ddd, *J* = 1.6, 6.8 Hz), 8.15 (1H, d, *J* = 8.4 Hz), 8.23 (1H, d, *J* = 7.2 Hz), 8.34 (1H, s), 8.92 (1H, s), 9.38 (1H, s), 12.05 (1H, s). IR (KBr): 1639, 1670 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)quinoline-2-carboxamide (33)—**Obtained as a yellow solid from **2** and quinoline-2-carbonyl chloride (**22**). mp 210–211 °C; MS (ESI) 335 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.69 (3H, s), 6.21 (2H, s), 7.73 (1H, s), 7.79 (1H, ddd, *J* = 1.2, 7.2 Hz), 7.95 (1H, ddd, *J* = 1.6, 7.6 Hz), 8.15 (1H, d, *J* = 7.2 Hz), 8.22 (1H, d, *J* = 8.0 Hz), 8.28 (1H, d, *J* = 8.8 Hz), 8.55 (1H, s), 8.65–8.68 (1H, d, *J* = 8.8). IR (KBr): 1647, 1670 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)quinoline-5-carboxamide (34)—**Obtained as a yellow solid from **2** and quinoline-5-carbonyl chloride (**23**). mp 210–211 °C; MS (ESI) 335 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.61 (3H, s), 6.21 (2H, s), 7.70 (1H, s), 7.82 (1H, dd, *J* = 4.4, 8.8 Hz), 8.03 (1H, t, *J* = 7.8 Hz), 8.11 (1H, d, *J* = 7.2 Hz), 8.30–8.35 (2H, m), 9.03 (1H, d, *J* = 8.4 Hz), 9.13 (1H, d, *J* = 4.4 Hz), 12.59 (1H, s). IR (KBr): 1636, 1672 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)anthracene-1-carboxamide (35)—**Obtained as a pale yellow solid from **2** and anthracene-1-carbonyl chloride (**24**). mp 206–207 °C; MS (ESI) 384 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.59 (3H, s), 6.21 (2H, s), 7.55–7.61 (2H, m), 7.62–7.67 (1H, m), 7.70 (1H, s), 7.90 (1H, d, *J* = 6.8 Hz), 8.13–8.16 (2H, m), 8.32 (1H, d, *J* = 8.4 Hz), 8.46 (1H, s), 8.73 (1H, s), 9.03 (1H, s), 12.60 (1H, s). IR (KBr): 1643, 1674 (C=O) cm^{-1}.

***N*-(6-Acetyl-1,3-benzodioxol-5-yl)-2-(naphthalen-1-yl)acetamide (55)—**Obtained as a white solid from **2** and 1-naphthylmethyl chloride (**54**). mp 89–90 °C; MS (ESI) 384 m/z $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.53 (3H, s), 4.21 (2H, s) 6.13 (2H, s), 7.50–7.61 (5H, m), 7.90 (1H, d, *J* = 8.0 Hz), 7.95 (1H, dd, *J* = 2.0, 7.2 Hz), 8.03 (1H, dd, *J* = 1.2, 8.0 Hz), 8.15 (1H, s), 11.89 (1H, s). IR (KBr): 1641, 1684 (C=O) cm^{-1}.

Preparation of 2-aryl-6,7-methylenedioxyquinolin-4-ones (1, 37–44)

Method A: Into a suspension of **25**, **27–34** (2.95 mmol) in *t*-butyl alcohol (100 mL) was added potassium *t*-butoxide (1.66 g, 14.7 mmol). The mixture was refluxed under argon for 12 h, cooled and poured into a 10% ammonium chloride solution (100 mL). The solid precipitate was collected and washed with EtOH. The crude product was purified by flash chromatography (silica gel, CH_2Cl_2:EtOH 16:1–10:1).

2-(3-Benzo[*b*]thienyl)-6,7-methylenedioxyquinolin-4-one (1)—35% yield from **25** as a white solid. mp >330 °C; MS (ESI) 322 m/z $[M+H]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.15 (2H, s), 6.19 (1H, s), 7.11 (1H, s), 7.43 (1H, s), 7.47–7.54 (2H, m), 7.93 (1H, d, *J* = 7.6 Hz), 8.14 (1H, d, *J* = 7.6 Hz), 8.24 (1H, s), 11.80 (1H, s). IR (KBr): 1616 (C=O) cm^{-1}. Anal. Calcd for $C_{18}H_{11}NO_3S$: C, 67.00; H, 3.48; N, 4.25. Found: C, 67.28; H, 3.45; N, 4.36.

2-(3-Benzo[*b*]furyl)-6,7-methylenedioxyquinolin-4-one (37)—17% yield from **27** as a pale yellow solid. mp >315 °C; MS (ESI) m/z 306 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.12 (2H, s), 6.49 (1H, s), 7.13 (1H, s), 7.36–7.45 (3H, m), 7.69 (1H, d, *J* = 8.0 Hz), 8.14 (1H, s), 8.52 (1H, s). IR (KBr): 1626 (C=O) cm^{-1}. Anal. Calcd for $C_{18}H_{11}NO_4$: C, 70.82; H, 3.63; N, 4.59. Found: C, 70.52; H, 3.95; N, 4.21.

2-(2-Benzo[*b*]furyl)-6,7-methylenedioxyquinolin-4-one (38)—28% yield from **28** as a grayish white solid. mp >320 °C; MS (EI, 70 eV) m/z 305 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.19 (2H, s), 6.75 (1H, s), 7.30 (1H, s), 7.35 (1H, t, *J* = 7.6 Hz), 7.40 (1H, s), 7.45 (1H, t, *J* = 7.6 Hz), 7.71–7.79 (3H, m). IR (KBr): 1630 (C=O) cm^{-1}. Anal. Calcd for $C_{18}H_{11}NO_4$: C, 70.82; H, 3.63; N, 4.59. Found: C, 70.62; H, 3.84; N, 4.24.

2-(1-Naphthalenyl)-6,7-methylenedioxyquinolin-4-one (39)—52% yield from **29** as a grayish white solid. mp >350 °C; MS (ESI) m/z 316 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.08 (1H, s), 6.15 (2H, s), 7.03 (1H, s), 7.46 (1H, s), 7.56–7.63 (2H, m), 7.63–7.70 (2H, m), 7.83 (1H, d, *J* = 7.6 Hz), 8.06 (1H, d, *J* = 7.6 Hz), 8.11 (1H, d, *J* = 7.6 Hz), 11.90 (1H, s). IR (KBr): 1653 (C=O) cm^{-1}. Anal. Calcd for $C_{20}H_{13}NO_3$: C, 76.18; H, 4.16; N, 4.44. Found: C, 75.60; H, 3.94; N, 4.29.

2-(2-Naphthalenyl)-6,7-methylenedioxyquinolin-4-one (40)—48% yield from **30** as a white solid. mp >330 °C; MS (ESI) m/z 316 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.16 (2H, s), 6.49 (1H, s), 7.25 (1H, s), 7.42 (1H, s), 7.59–7.64 (2H, m), 7.88–7.95 (1H, m), 8.00–8.03 (1H, m), 8.06–8.12 (2H, m), 8.42 (1H, s). IR (KBr): 1616 (C=O) cm^{-1}. Anal. Calcd for $C_{20}H_{13}NO_3$: C, 76.18; H, 4.16; N, 4.44. Found: C, 76.04; H, 4.28; N, 4.28.

2-(4-Quinolinyl)-6,7-methylenedioxyquinolin-4-one (41)—54% yield from **31** as a pale yellow solid. mp >320 °C; MS (ESI) m/z 317 $[M+H]^+$. ^1H NMR (DMSO-d_6, δ): 6.12–6.29 (3H, m), 7.06 (1H, s), 7.47 (1H, s), 7.65–7.70 (2H, m), 7.86 (1H, t, *J* = 7.6 Hz), 7.93 (1H, d, *J* = 8.4 Hz), 8.15 (1H, d, *J* = 8.0 Hz), 9.03 (1H, s). IR (KBr): 1618 (C=O) cm^{-1}. Anal. Calcd for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86. Found: C, 72.35; H, 4.03; N, 8.60.

2-(3-Quinolinyl)-6,7-methylenedioxyquinolin-4-one (42)—65% yield from **32** as a grayish white solid, mp >320 °C; MS (ESI) m/z 317 $[M+H]^+$. ^1H NMR (DMSO-d_6, δ): 6.19 (2H, s), 6.62 (1H, s), 7.23 (1H, s), 7.44 (1H, s), 7.73 (1H, t, *J* = 7.6 Hz), 7.87 (1H, t, *J* = 7.6 Hz), 8.11–8.17 (2H, m), 8.88 (1H, s), 9.38 (1H, s). IR (KBr): 1618 (C=O) cm^{-1}. Anal. Calcd for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86. Found: C, 71.86; H, 3.76; N, 8.63.

2-(2-Quinolinyl)-6,7-methylenedioxyquinolin-4-one (43)—48% yield from **33** as a pale yellow solid. mp 345–347 °C; MS (EI, 70 eV) m/z 316 $[M]^+$. ^1H NMR (DMSO-d_6, δ): 6.18 (2H, s), 6.98 (1H, s), 7.43 (1H, s), 7.63 (1H, s), 7.73 (1H, t, *J* = 7.6 Hz), 7.91 (1H, t, *J* = 7.6 Hz), 8.11 (1H, d, *J* = 8.0 Hz), 8.27 (1H, d, *J* = 8.4 Hz), 8.32 (1H, d, *J* = 8.8 Hz), 8.60 (1H, d, *J* = 8.8 Hz), 11.85 (1H, s). IR (KBr): 1611 (C=O) cm^{-1}. Anal. Calcd for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86. Found: C, 71.76; H, 3.88; N, 8.46.

2-(5-Quinolinyl)-6,7-methylenedioxyquinolin-4-one (44)—47% yield from **34** as a pale yellow solid. mp >330 °C; MS (ESI) m/z 317 $[M+H]^+$. ^1H NMR (DMSO-d_6, δ): 6.03–6.19 (3H, m), 7.03 (1H, s), 7.47 (1H, s), 7.60 (1H, dd, *J* = 4.0, 8.4 Hz), 7.81 (1H, d, *J* = 6.8 Hz), 7.91 (1H, t, *J* = 7.8 Hz), 8.20 (1H, d, *J* = 8.4 Hz), 8.30 (1H, d, *J* = 6.4 Hz), 8.99 (1H, s), 11.91 (1H, s). IR (KBr): 1614 (C=O) cm^{-1}. Anal. Calcd for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86. Found: C, 72.08; H, 3.94; N, 8.66.

Preparation of 2-aryl-6,7-methylenedioxyquinolin-4-ones (1, 36, 39, 45) and 4-methyl-6,7-methylenedioxy-3-(1-naphthyl)quinolin-2-one (56)

Method B: Following the same procedure described in method A, except for the use of 1,4-dioxane as solvent in place of *t*-butylalcohol, and the use of NaOH in place of potassium *t*-butoxide. Compounds **1** and **39** were confirmed by comparison of mp and TLC with those of a sample obtained from method A.

2-(3-Benzo[*b*]thienyl)-6,7-methylenedioxyquinolin-4-one (1)—40% yield from **25** as a pale yellow solid.

2-(2-Benzo[*b*]thienyl)-6,7-methylenedioxyquinolin-4-one (36)—74% yield from **26** as a white solid. mp >350 °C; MS (ESI) m/z 322 $[M+H]^+$. ^1H NMR (DMSO-d_6, δ): 6.20 (2H, s), 7.21 (1H, s), 7.39–7.45 (3H, m), 7.96–8.16 (3H, m), 11.55 (1H, s). IR (KBr): 1616 (C=O) cm^{-1}. Anal. Calcd for $C_{18}H_{11}NO_3S$: C, 67.00; H, 3.48; N, 4.25. Found: C, 67.21; H, 3.47; N, 4.33.

2-(1-Naphthalenyl)-6,7-methylenedioxyquinolin-4-one (39)—58% yield from **29** as a grayish white solid.

2-(1-Anthracenyl)-6,7-methylenedioxyquinolin-4-one (45)—48% yield from **35** as a yellow solid. mp >320 °C; MS (ESI) m/z 366 $[M+H]^+$. ^1H NMR (DMSO-d_6, δ): 6.15 (3H, s), 7.03 (1H, s), 7.43–7.70 (5H, m), 8.08 (2H, t, *J* = 7.6 Hz), 8.25 (1H, d, *J* = 8.4 Hz), 8.47 (1H, s), 8.70 (1H, s), 11.98 (1H, s). IR (KBr): 1632 (C=O) cm^{-1}. Anal. Calcd for $C_{24}H_{15}NO_3$: C, 78.89; H, 4.14; N, 3.83. Found: C, 78.24; H, 4.40; N, 3.37.

4-Methyl-6,7-methylenedioxy-3-(1-naphthyl)quinolin-2-one (56)—61% yield from **55** as a white solid. mp >260 °C; MS (EI, 70 eV) m/z 329 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 2.02 (3H, s), 6.13 (2H, s), 6.91 (1H, s), 7.29–7.32 (2H, m), 7.39–7.47 (2H, m), 7.52 (1H, ddd, *J* = 1.6, 6.4 Hz), 7.58 (1H, t, *J* = 8.0 Hz), 7.93–8.01 (2H, m), 11.84 (1H, s). IR (KBr): 1632 (C=O) cm^{-1}. Anal. Calcd for $C_{21}H_{15}NO_3$: C, 76.58; H, 4.59; N, 4.25. Found: C, 75.88; H, 5.07; N, 4.06.

Deacetylation of compounds 25, 26, 28–32 and 34—Into a suspension of compound (2.95 mol) in dry toluene (150 mL) was added potassium *t*-butoxide (1.66 g, 14.75 mol). The mixture was heated under argon at 90 °C for 72 h, and then concentrated and neutralized with 20% HOAc The resulting solid precipitate was collected and purified by flash chromatography (silica gel, CH_2Cl_2-EtOH) to afford **46–53**.

***N*-(1,3-Benzodioxol-5-yl)-1-benzothiophene-3-carboxamide (46)—**28% yield from **25** as a white solid. mp 188−189 °C; MS (EI, 70 eV) m/z 297 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.01 (2H, s), 6.90 (1H, d, *J* = 8.4 Hz), 7.17 (1H, dd, *J* = 2.0, 8.4 Hz), 7.40−7.51 (3H, m), 8.06 (1H, dd, *J* = 1.2, 7.2 Hz), 8.37 (1H, dd, *J* = 0.8, 8.0 Hz), 8.46 (1H, s), 10.29 (1H, s). IR (KBr): 1647 (C=O) cm^{-1}. Anal. Calcd for $C_{16}H_{11}NO_3S$: C, 64.63; H, 3.73; N, 4.71. Found: C, 64.32; H, 3.94; N, 4.50.

***N*-(1,3-Benzodioxol-5-yl)-1-benzothiophene-2-carboxamide (47)—**36% yield from **26** as a grayish white solid. mp 171−173 °C; MS (EI, 70 eV) m/z 297 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.01 (2H, s), 6.91 (1H, d, *J* = 8.4 Hz), 7.15 (1H, dd, *J* = 2.0, 8.4 Hz), 7.38 (1H, d, *J* = 2.4 Hz), 7.41−7.54 (2H, m), 7.96–8.05 (2H, m), 8.28 (1H, s), 10.41 (1H, s). IR (KBr): 1634 (C=O) cm^{-1}. Anal. Calcd for $C_{16}H_{11}NO_3S$: C, 64.63; H, 3.73; N, 4.71. Found: C, 64.28; H, 3.98; N, 4.46.

***N*-(1,3-Benzodioxol-5-yl)-1-benzofuran-2-carboxamide (48)—**28% yield from **28** as a yellow solid. mp 156-158 °C; MS (EI, 70 eV) m/z 281 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.03 (2H, s), 6.93 (1H, d, *J* = 8.4 Hz), 7.27 (1H, dd, *J* = 2.0, 8.4 Hz), 7.37 (1H, t, *J* = 7.6 Hz), 7.47 (1H, d, *J* = 2.0 Hz), 7.51 (1H, ddd, *J* = 2.0, 8.4 Hz), 7.71–7.75 (2H, m), 7.82 (1H, d, *J* = 8.0 Hz), 10.49 (1H, s). IR (KBr): 1668 (C=O) cm^{-1}. Anal. Calcd for $C_{16}H_{11}NO_4$: C, 68.32; H, 3.94; N, 4.98. Found: C, 68.22; H, 4.14; N, 4.22.

***N*-(1,3-Benzodioxol-5-yl)naphthalene-1-carboxamide (49)—**32% yield from **29** as a white solid. mp 204–206 °C; MS (EI, 70 eV) m/z 291 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.02 (2H, s), 6.91 (1H, d, *J* = 8.4 Hz), 7.22 (1H, dd, *J* = 2.0, 8.4 Hz), 7.51 (1H, d, *J* = 2.0 Hz), 7.56–7.63 (3H, m), 7.72 (1H, d, *J* = 6.4 Hz), 7.99–8.03 (1H, m), 8.06 (1H, d, *J* = 8.4 Hz), 8.16–8.20 (1H, m), 10.46 (1H, s). IR (KBr): 1643 (C=O) cm^{-1}. Anal. Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 73.83; H, 4.82; N, 4.26.

***N*-(1,3-Benzodioxol-5-yl)naphthalene-2-carboxamide (50)—**21% yield from **30** as a pale yellow solid. mp 184–185 °C; MS (EI, 70 eV) m/z 291 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.02 (2H, s), 6.94 (1H, d, *J* = 8.4 Hz), 7.24 (1H, dd, *J* = 2.0, 8.4 Hz), 7.50 (1H, d, *J* = 2.0 Hz), 7.60–7.68 (2H, m), 7.98–8.15 (4H, m), 8.56 (1H, s), 10.38 (1H, s). IR (KBr): 1636 (C=O) cm^{-1}. Anal. Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.19; H, 4.62; N, 4.56.

***N*-(1,3-Benzodioxol-5-yl)quinoline-4-carboxamide (51)—**31% yield from **31** as a white solid. mp 167–168 °C; MS (EI, 70 eV) m/z 292 $[M]^+$. ^{1}H NMR (DMSO-d_6, δ): 6.02 (2H, s), 6.93 (1H, d, *J* = 8.4 Hz), 7.16 (1H, dd, *J* = 1.6, 8.4 Hz), 7.43 (1H, d, *J* = 2.0 Hz), 7.66–7.72 (2H, m), 7.84 (1H, t, *J* = 8.0 Hz), 8.11 (2H, d, *J* = 8.8 Hz), 9.01 (1H, s), 10.65 (1H, s). IR

(KBr): 1636 (C=O) cm^{-1}. Anal. Calcd for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58. Found: C, 69.65; H, 4.32; N, 9.34.

***N*-(1,3-Benzodioxol-5-yl)quinoline-3-carboxamide (52)—**23% yield from **32** as a yellow solid. mp 254–256 °C; MS (EI, 70 eV) m/z 292 $[M]^+$. ^1H NMR (DMSO-d_6, δ): 6.05 (2H, s), 6.95 (1H, d, *J* = 8.4 Hz), 7.23 (1H, dd, *J* = 2.0, 8.4 Hz), 7.49 (1H, d, *J* = 2.0 Hz), 7.74 (1H, ddd, *J* = 1.2, 8.0 Hz), 7.90 (1H, ddd, *J* = 1.6, 8.4 Hz), 8.10–8.18 (2H, m), 8.92 (1H, s), 9.33 (1H, s), 10.56 (1H, s). IR (KBr): 1659 (C=O) cm^{-1}. Anal. Calcd for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58. Found: C, 69.73; H, 4.35; N, 9.42.

***N*-(1,3-Benzodioxol-5-yl)quinoline-5-carboxamide (53)—**20% yield from **34** as a white solid. mp 202–203 °C; MS (EI, 70 eV) m/z 292 $[M]^+$. ^1H NMR (DMSO-d_6, δ): 6.02 (2H, s), 6.95 (1H, d, *J* = 8.4 Hz), 7.21 (1H, dd, *J* = 2.0, 8.0 Hz), 7.50 (1H, d, *J* = 2.0 Hz), 7.63 (1H, dd, *J* = 4.0, 8.4 Hz), 7.83–7.90 (2H, m), 8.16–8.20 (1H, m), 8.63 (1H, d, *J* = 8.0 Hz), 8.98 (1H, dd, *J* = 1.6, 4.0 Hz), 10.58 (1H, s). IR (KBr): 1643 (C=O) cm^{-1}. Anal. Calcd for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58. Found: C, 69.68; H, 4.33; N, 9.52.

Biological Evaluations

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays [25,26] —HL-60, HCT-116 and Hep 3B cells were treated with tested compounds for the indicated periods. After treatment, cells were washed once with PBS and incubated with MTT (Sigma, St. Louis, MO, USA) for 2 h. The formazan precipitate was dissolved in 150 μL of DMSO, and the absorbance was measured with an ELISA reader at 570 nm.

SRB assays [27]—A549, KB, KB-VIN and DU145 cells were treated with tested compounds for the indicated periods. After additional incubation with DMSO or compounds, cells were fixed with 10% TCA, and SRB at 0.4% (w/v) in 1% acetic acid was added to stain cells. Unbound SRB was washed out with 1% acetic acid, and SRB bound to the cells was solubilized with 10 mM Trizma base. Absorbance was read at 515 nm.

Tubulin assays—Tubulin assembly was measured by turbidimetry as described in detail previously.[28] Assay mixtures contained 1.0 mg/mL (10 μM) tubulin and varying drug concentrations and were preincubated 15 min at 30 °C in the absence of GTP. The samples were placed on ice, and 0.4 mM GTP was added. Reaction mixtures were transferred to cuvettes held at 0 °C, and turbidity development was followed for 20 min at 30 °C after a rapid temperature jump. Drug concentrations that inhibited increase in turbidity by 50% relative to a control sample were determined.

Inhibition of the binding of [^3H]colchicine to tubulin was measured as described in detail previously.[29] Incubation of 1.0 μM tubulin with 5.0 μM [^3H]colchicine and either 1.0 or 5.0 μM inhibitor was for 10 min at 37 °C. At this time point, approximately 40–60% of maximum colchicine binding occurs.

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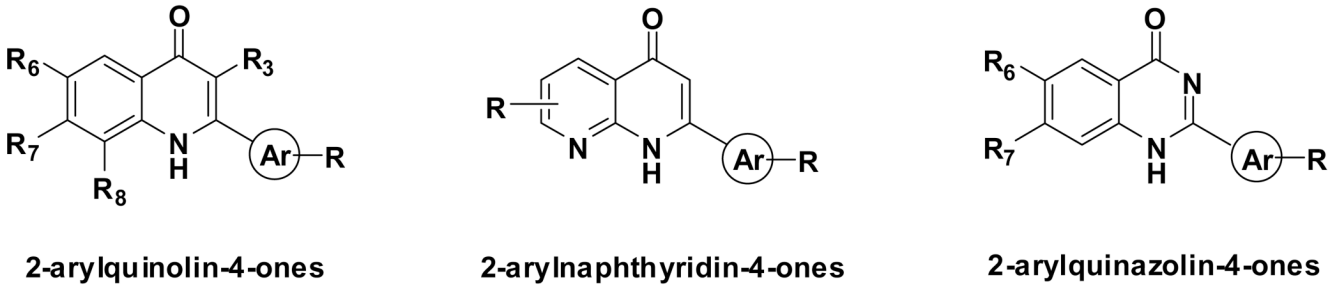


Figure 1.

Three series of structural skeletons of azo-flavonoids: 2-arylquinolone-4-ones, 2-arylnaphthyridin-4-ones, and 2-arylquinazolin-4-ones.

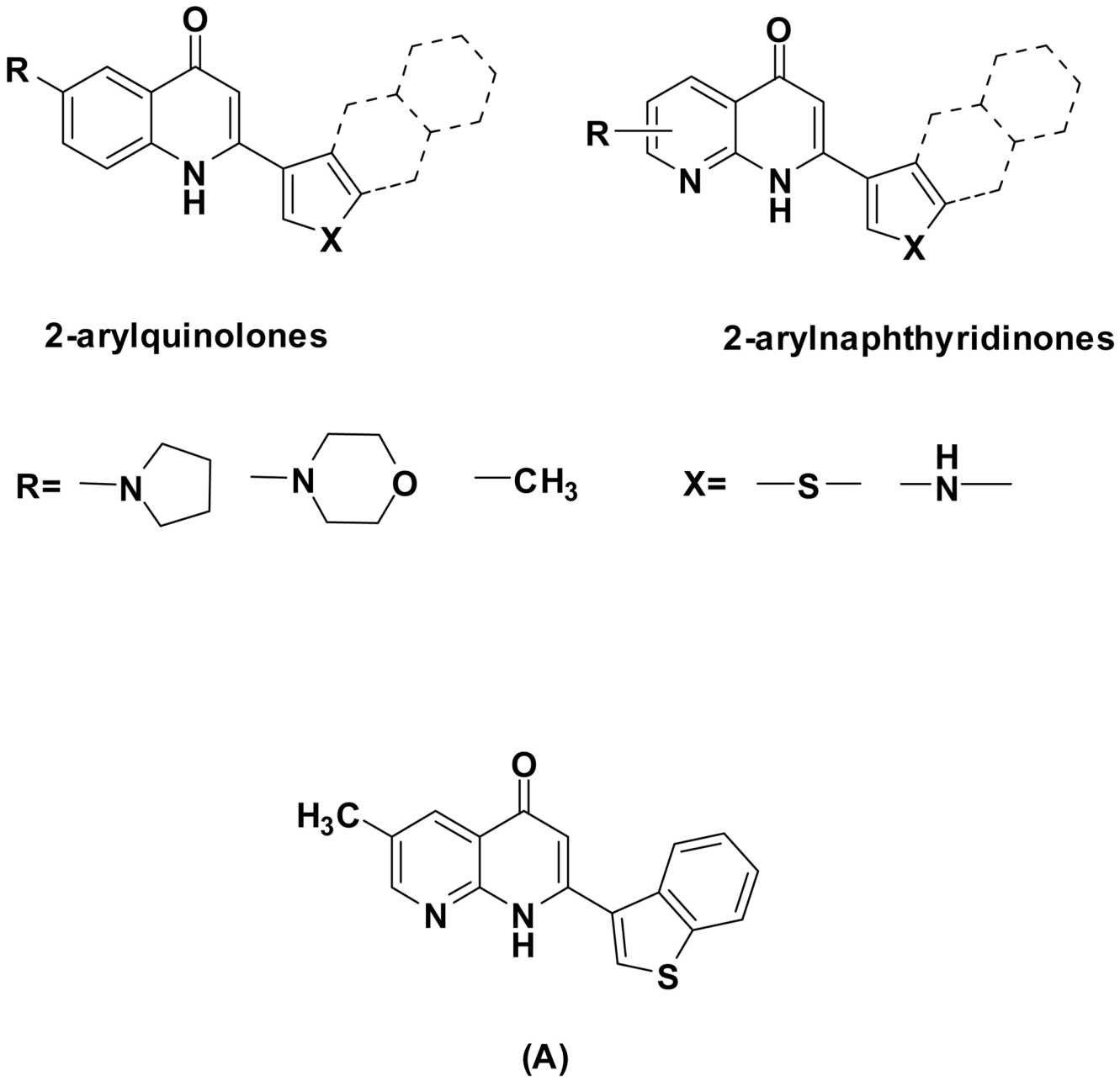
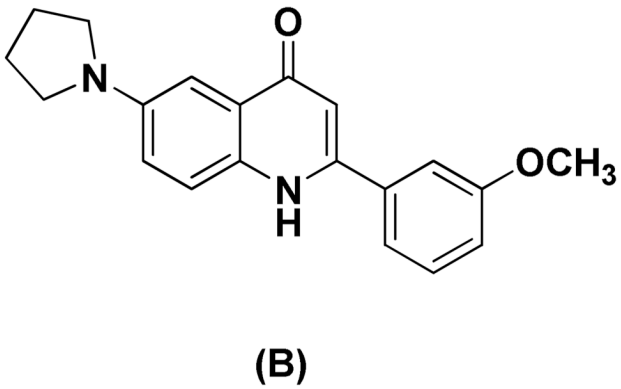
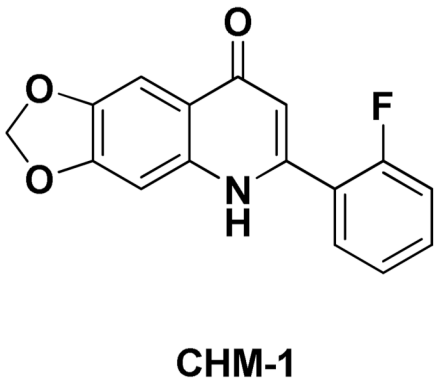


Figure 2.

Proposed models and newly designed compounds. Conventional Comparative Molecular Field Analysis (CoMFA) and g^2 GRS CoMFA are two computational programs performed to identify the essential structure requirements for increasing affinity at the colchicine site of tubulin. Based on the results of the QSAR study, we proposed two concept models. As the proof of concept, analog **A** was synthesized and tested against cancer cell lines and for binding to tubulin.



(B)



CHM-1

Figure 3.
Structures of compound **B** and **CHM-1**.

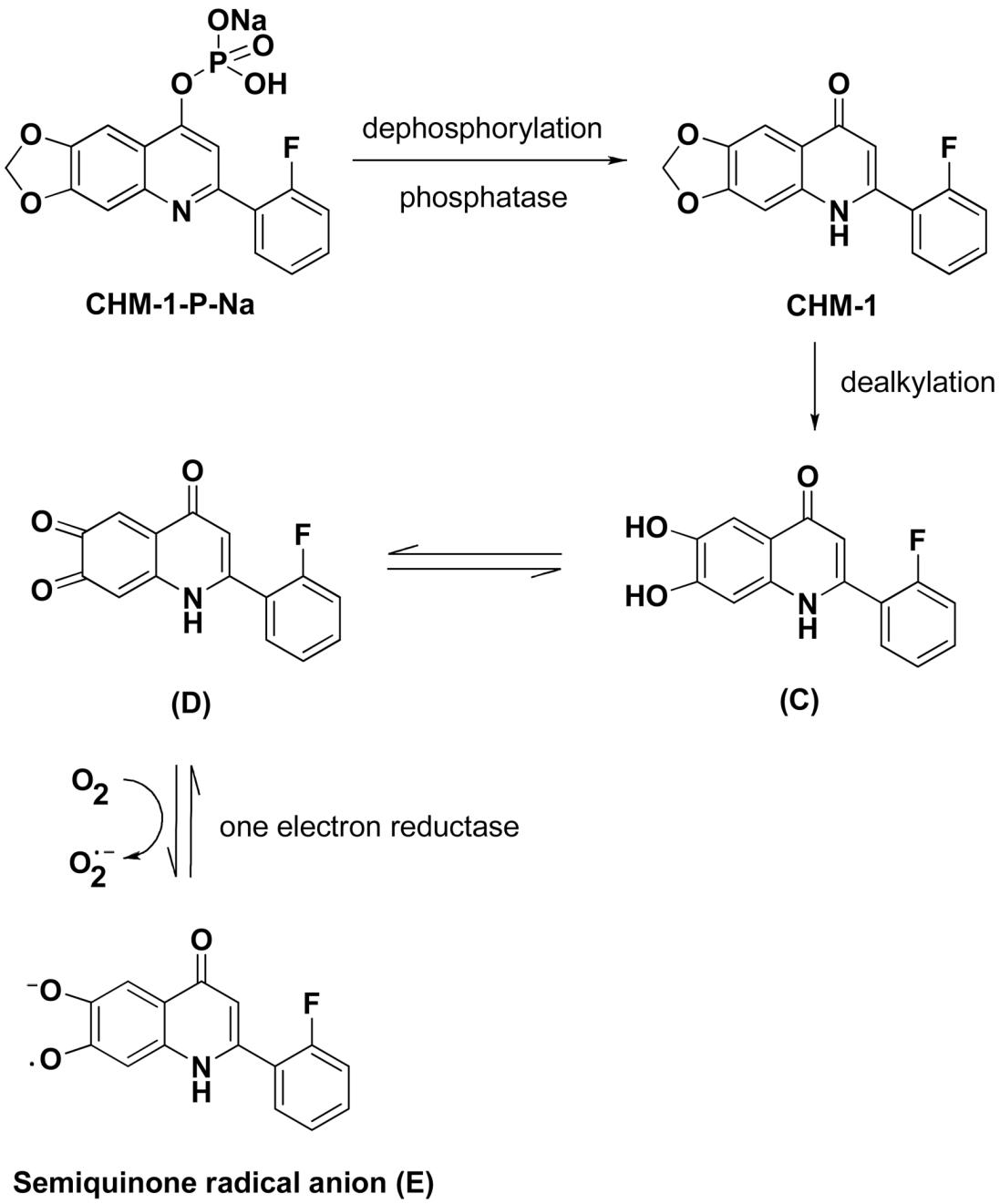


Figure 4.
Metabolic pathway of **CHM-1-P-Na**.

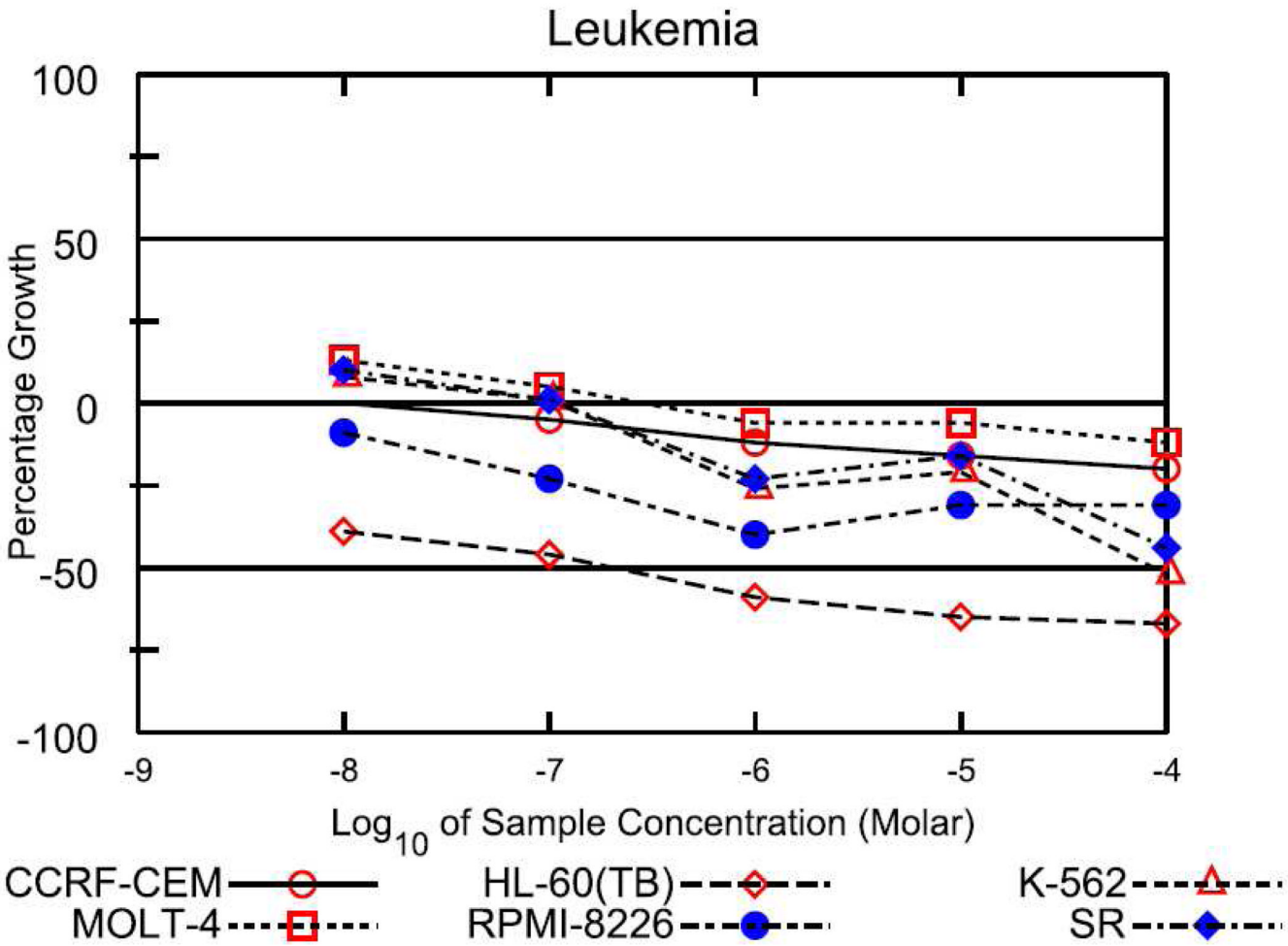
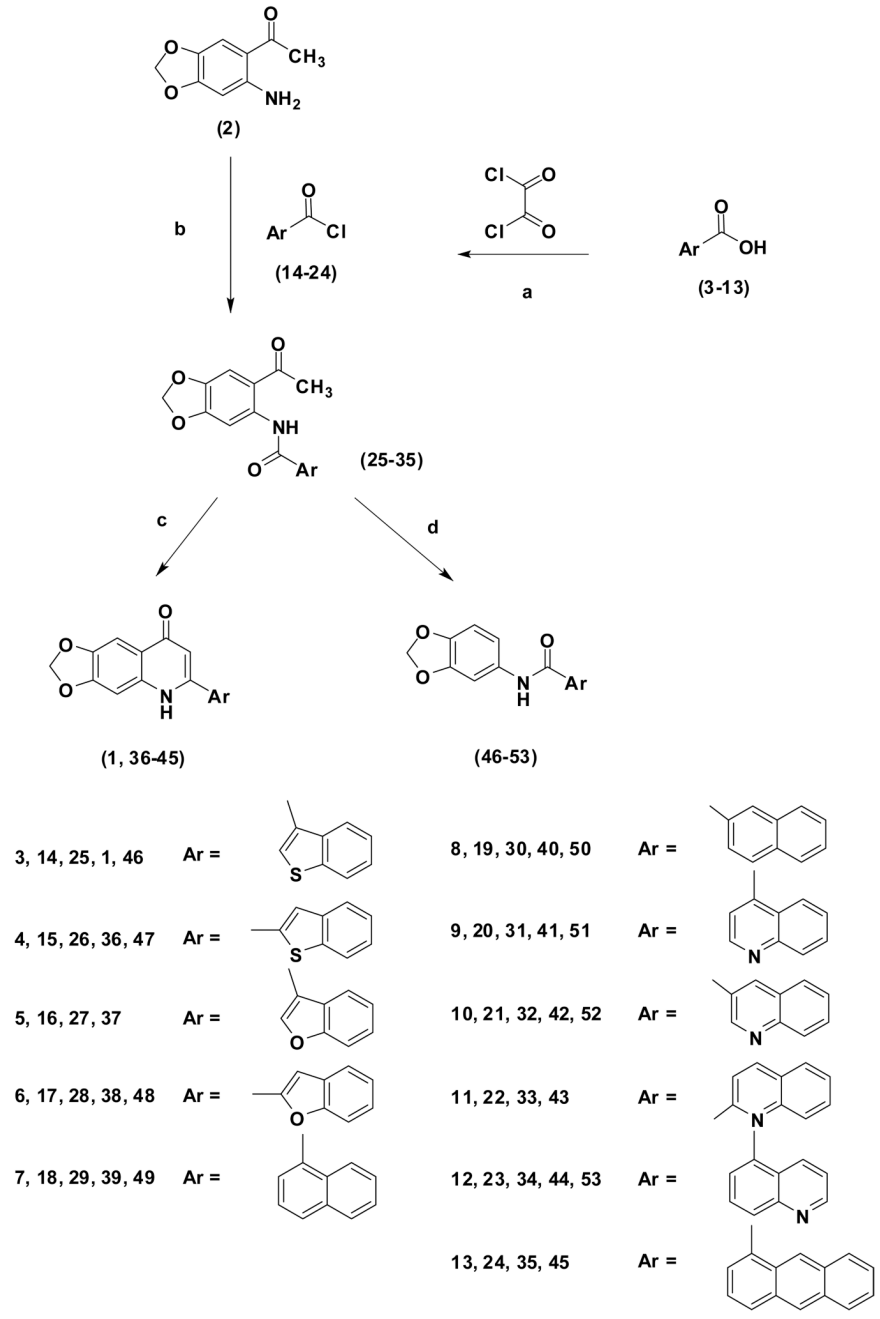
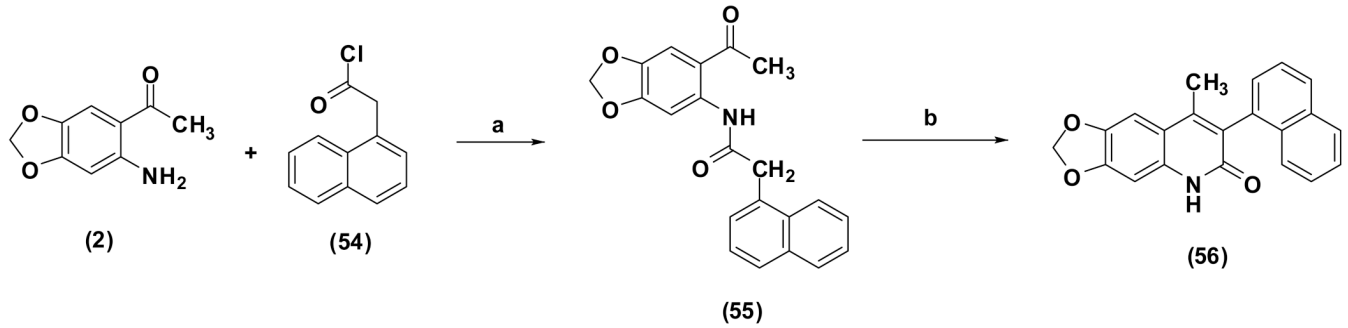


Figure 5.
Dose response curves of **37** against different leukemia cell lines.



Scheme 1.

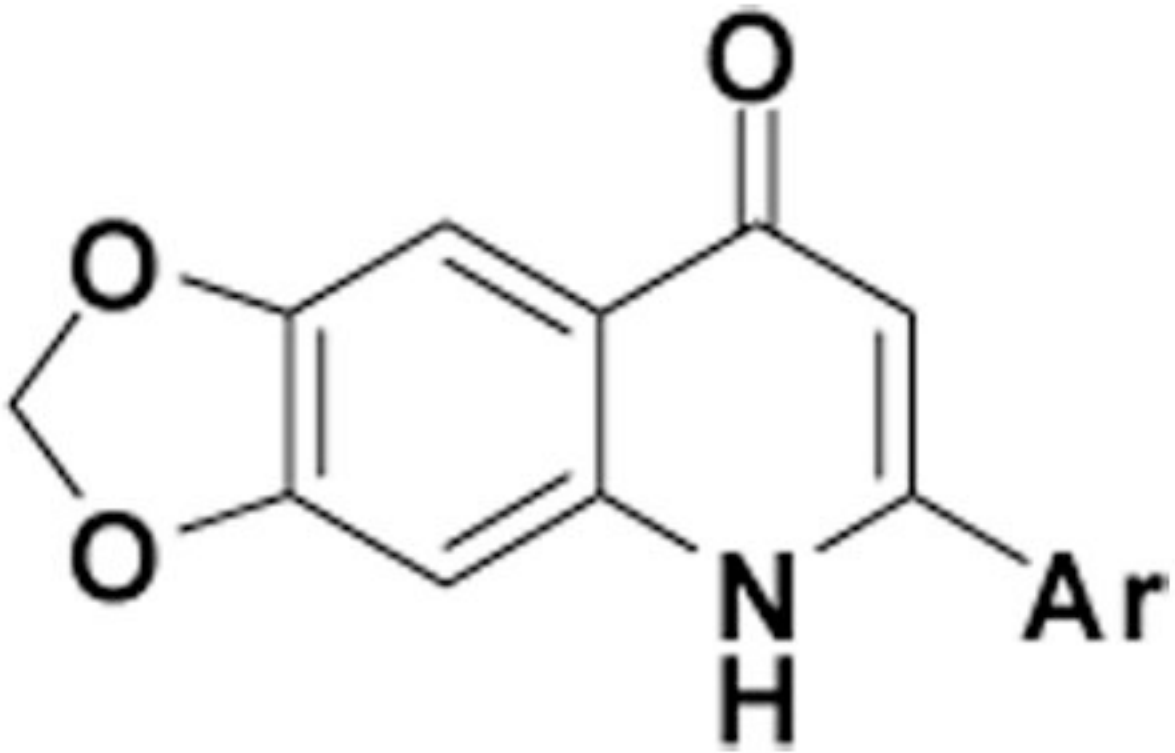
Reagents and conditions: (a) toluene/DMF, 22–25 °C; (b) toluene/triethylamine, 22–25 °C; (c) *t*-BuOK/ *t*-BuOH or NaOH/1,4-dioxane, reflux; (d) *t*-BuOK/toluene, 90 °C, 72 h.



Scheme 2.
Reagents and conditions: (a) toluene/triethylamine, 22–25 °C; (b) NaOH/1,4-dioxane, reflux.

Table 1

Values from *In Vitro* Cytotoxicity Testing of **1**, **36**-**45** and **56**.



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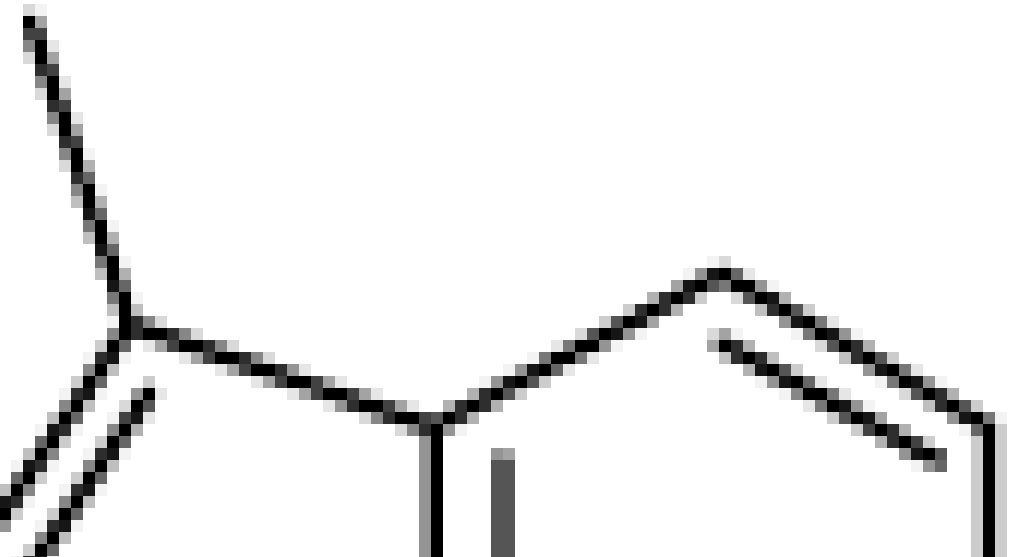
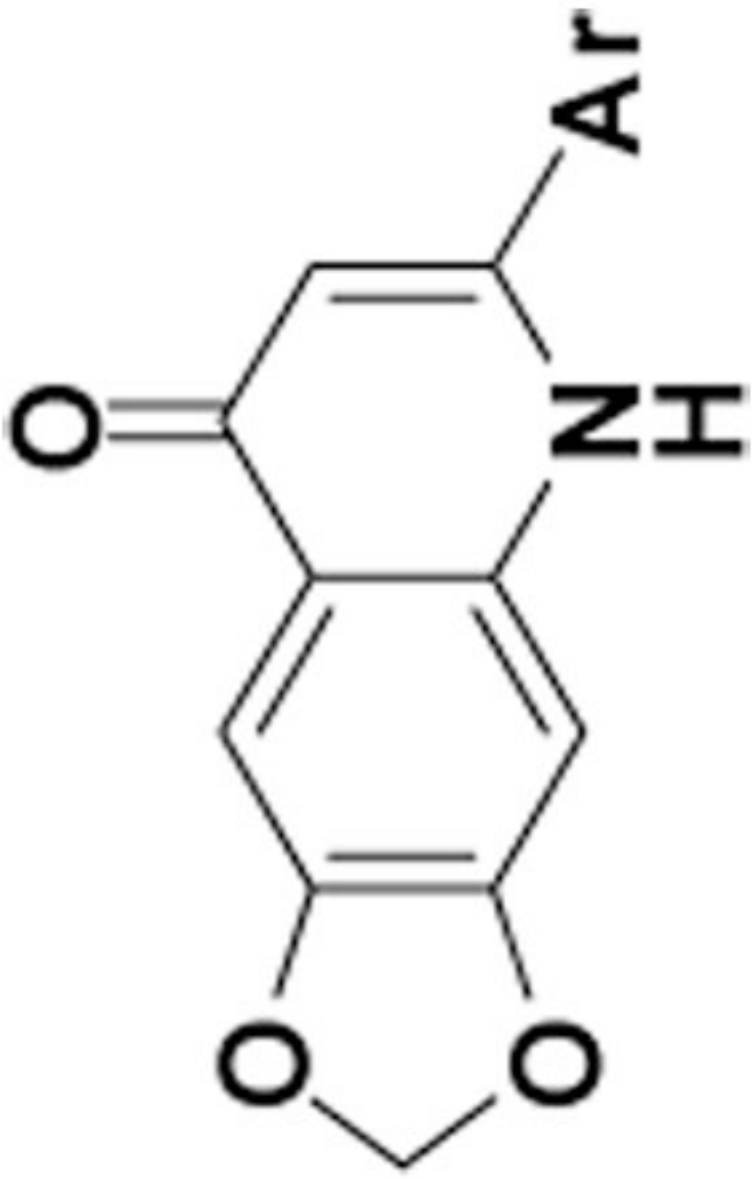
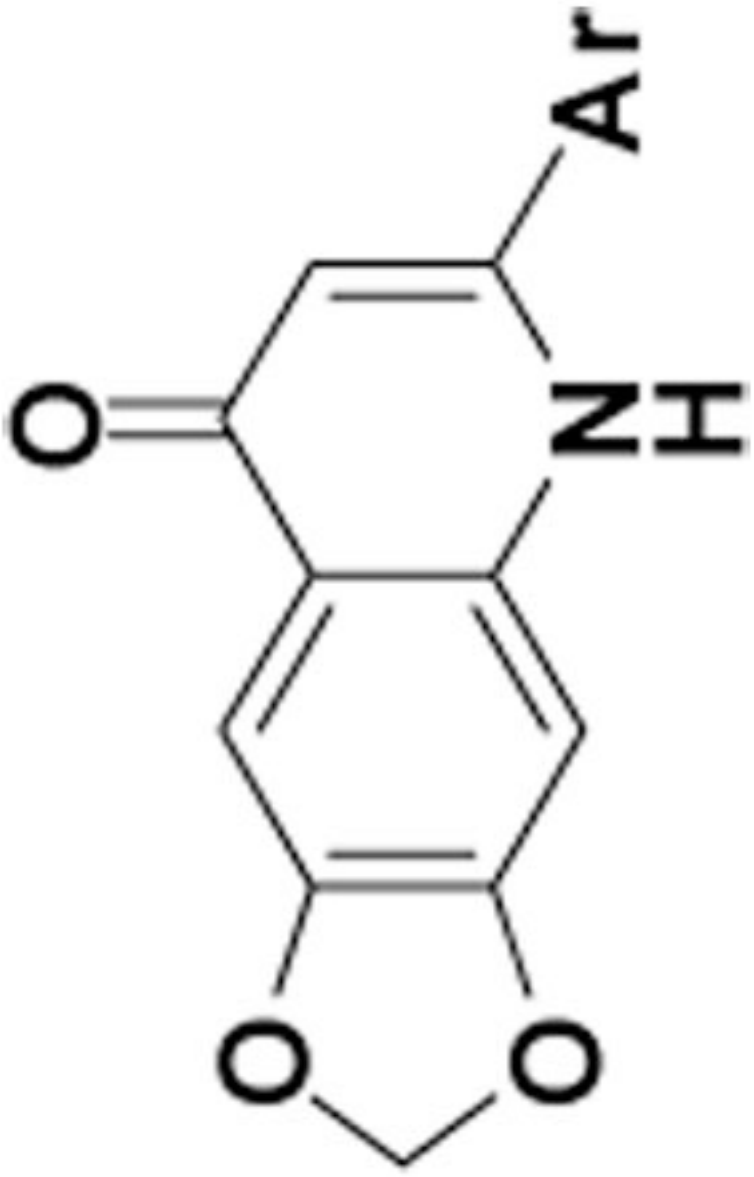
	0.17	0.14	>0.25	0.06	>0.25	>0.25	>0.25



Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145



Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
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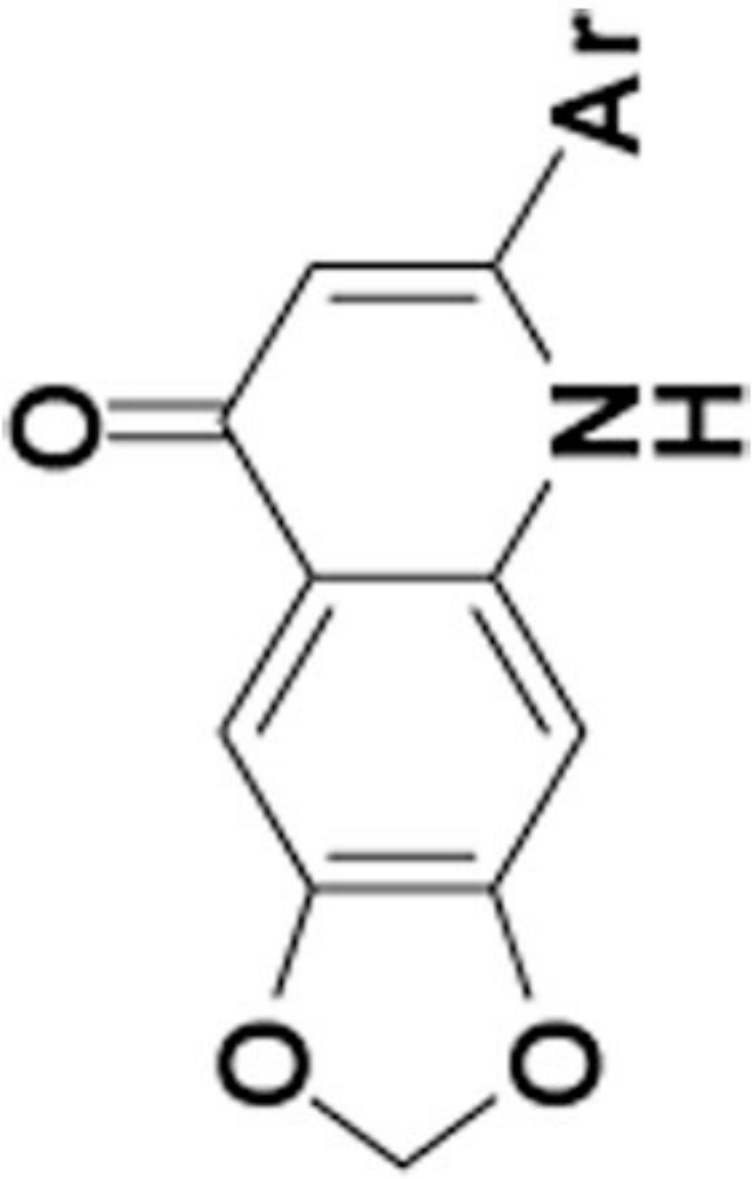
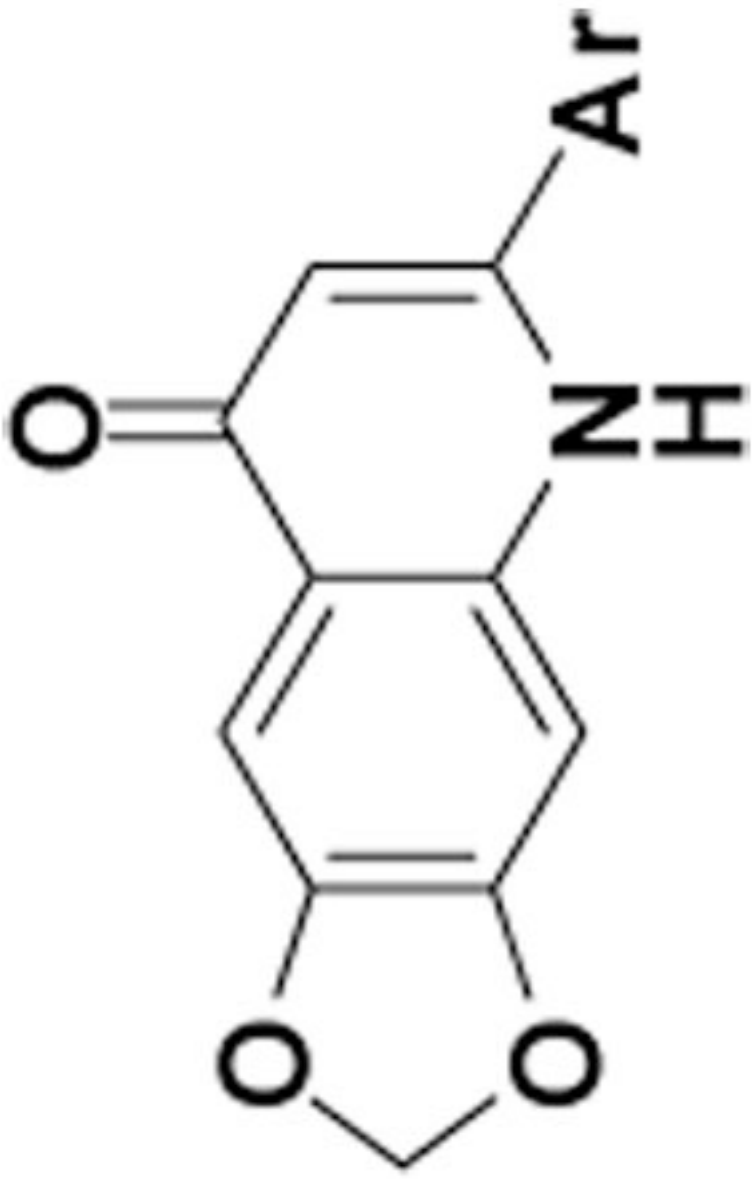
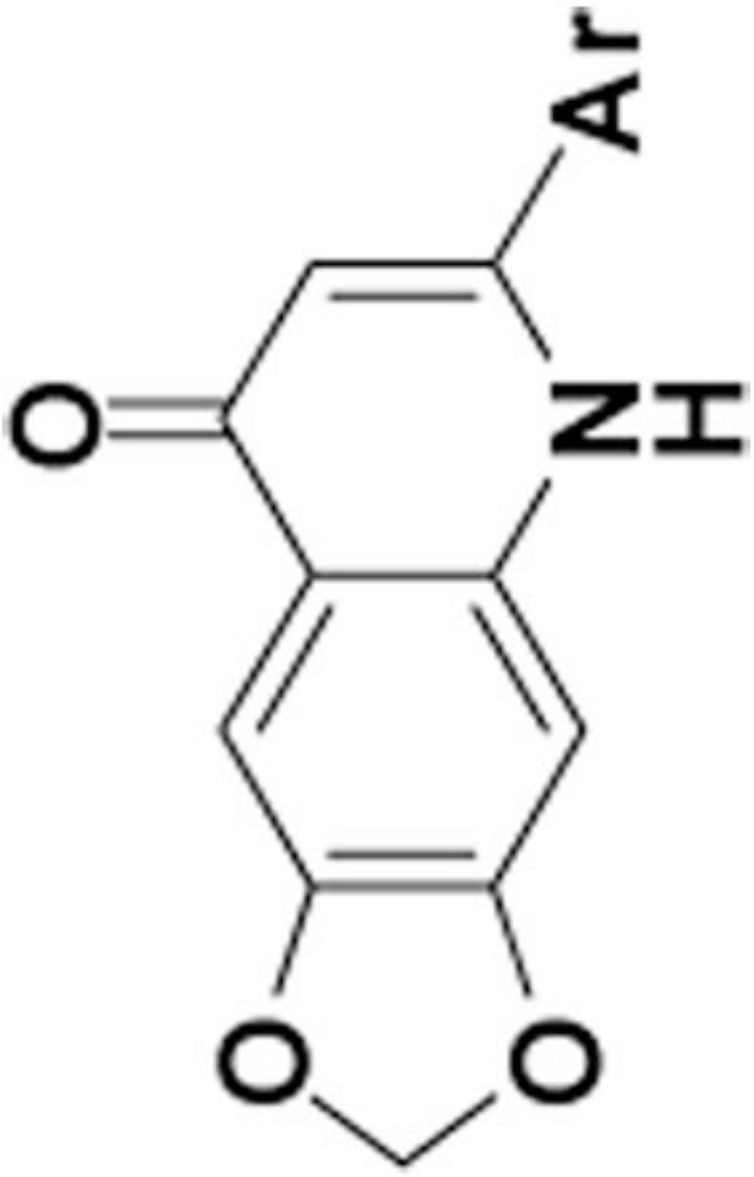


Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145

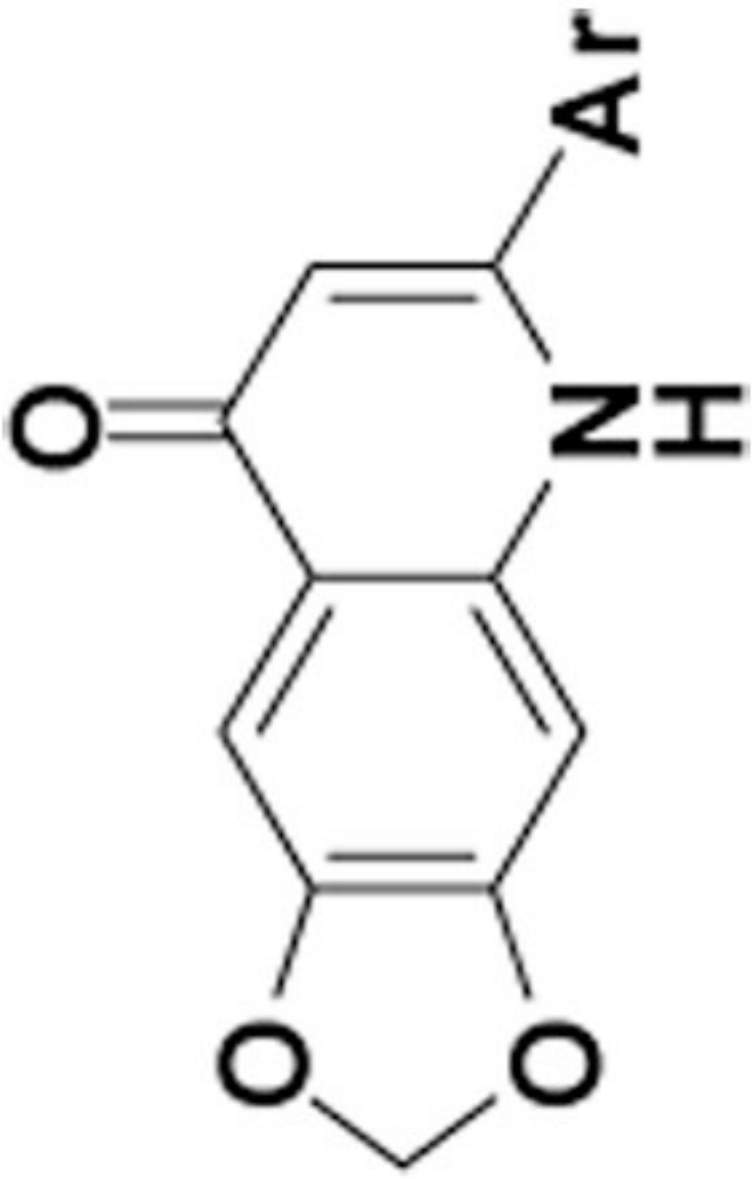
Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
	0.03	0.05	2.98	0.09	1.05	0.59	1.87



Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145

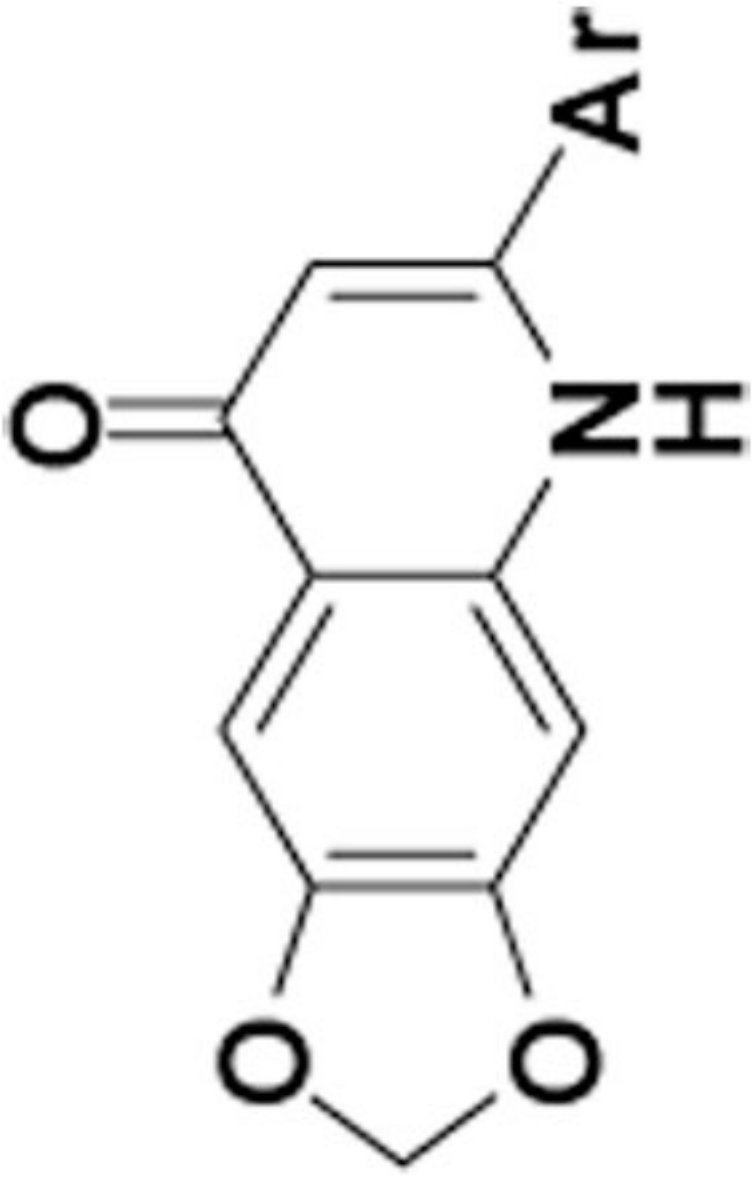


Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00

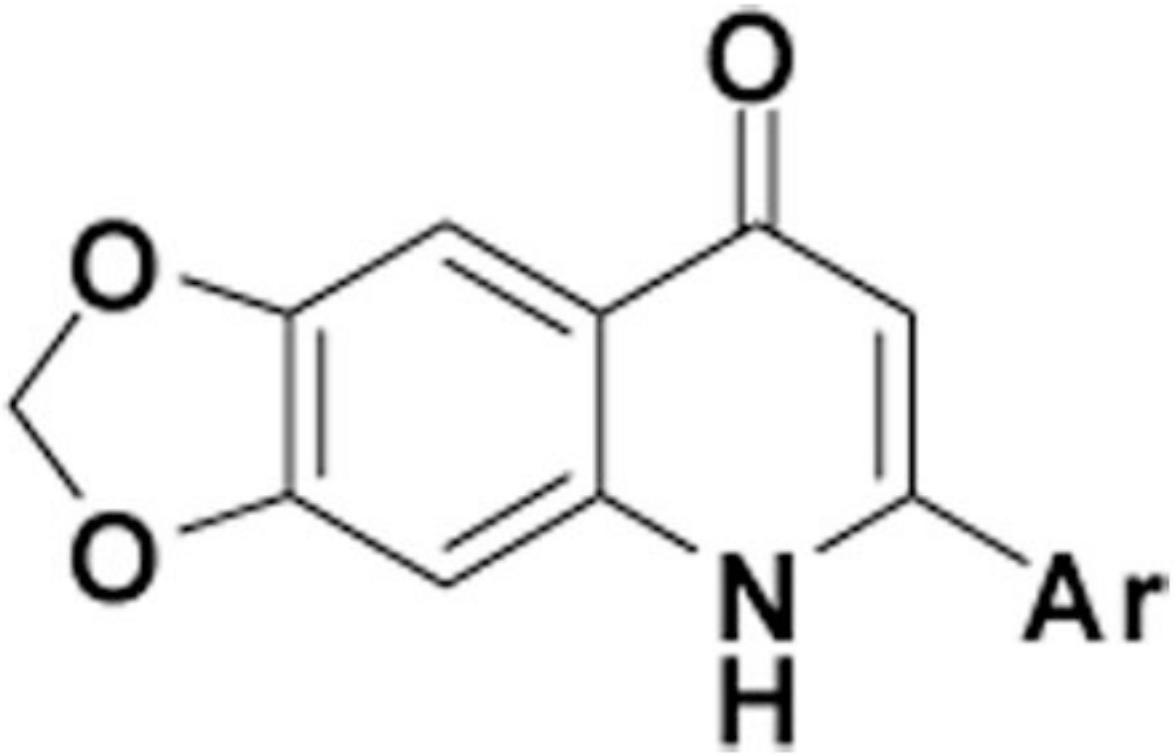


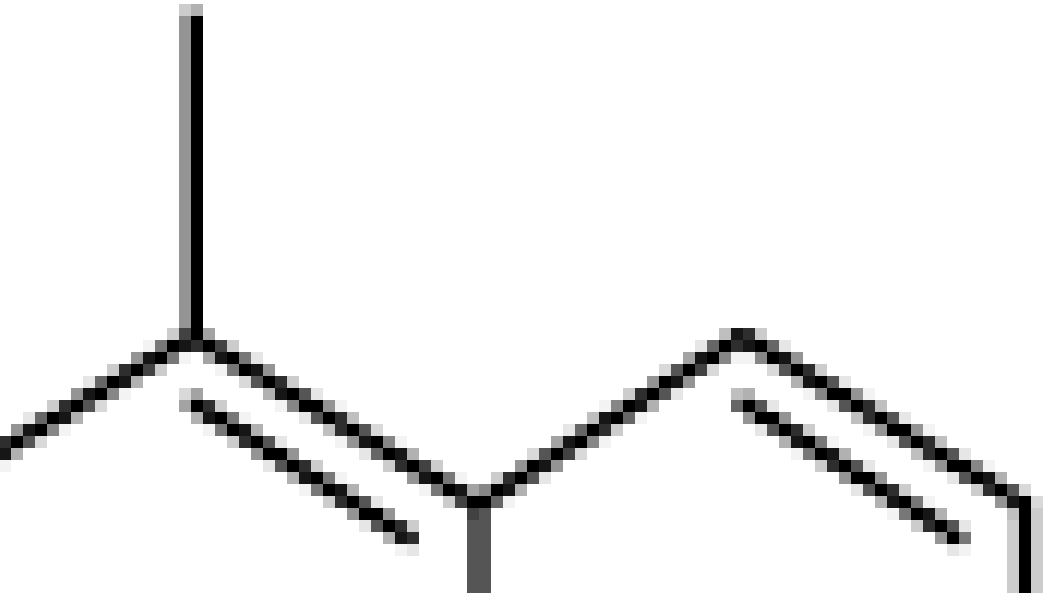
Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145

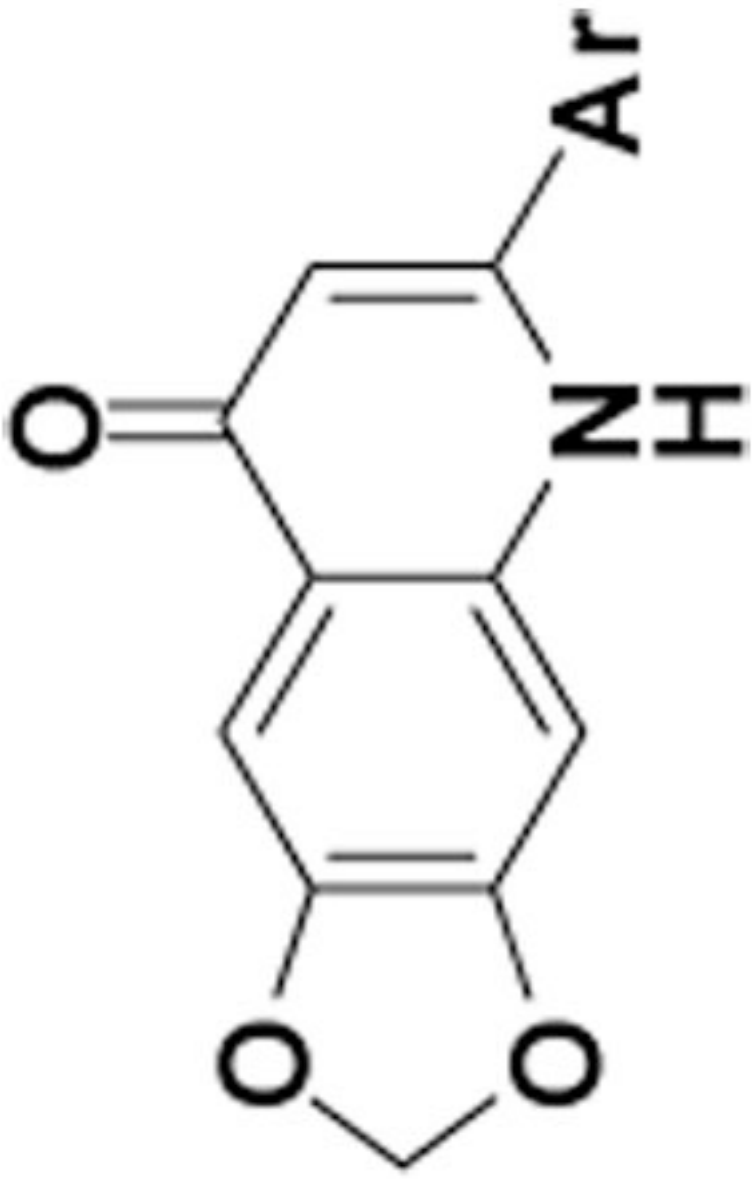
Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
	0.07	0.07	0.13	0.07	0.13	0.19	0.13



Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
	0.50	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00



Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00



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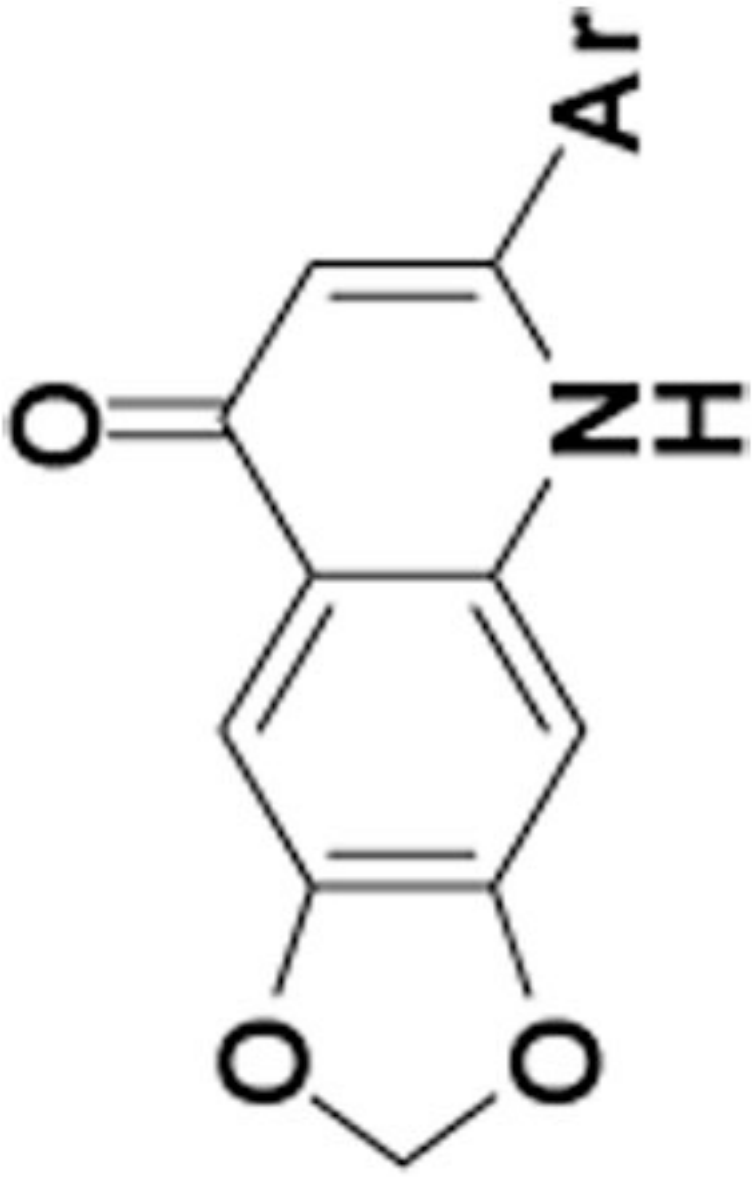
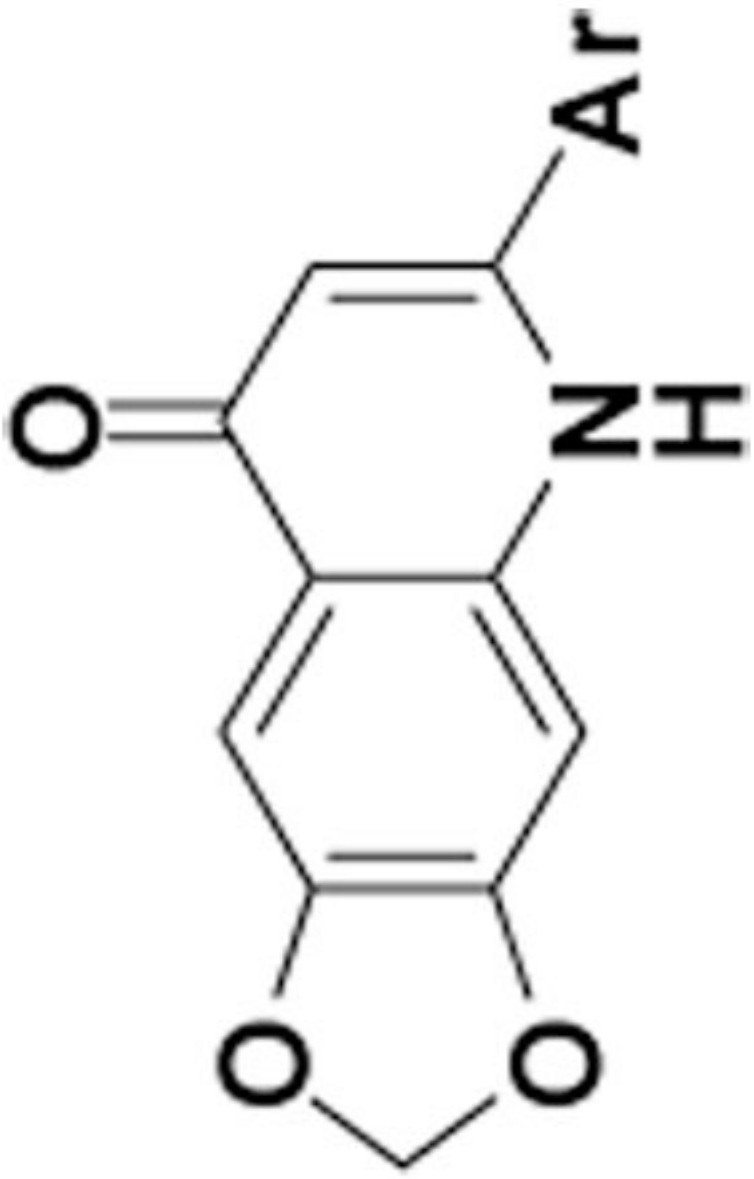


Ar	HL-60	HCT-116	A549	Hep 3B	KB	Kb-VIN	DU145
	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10



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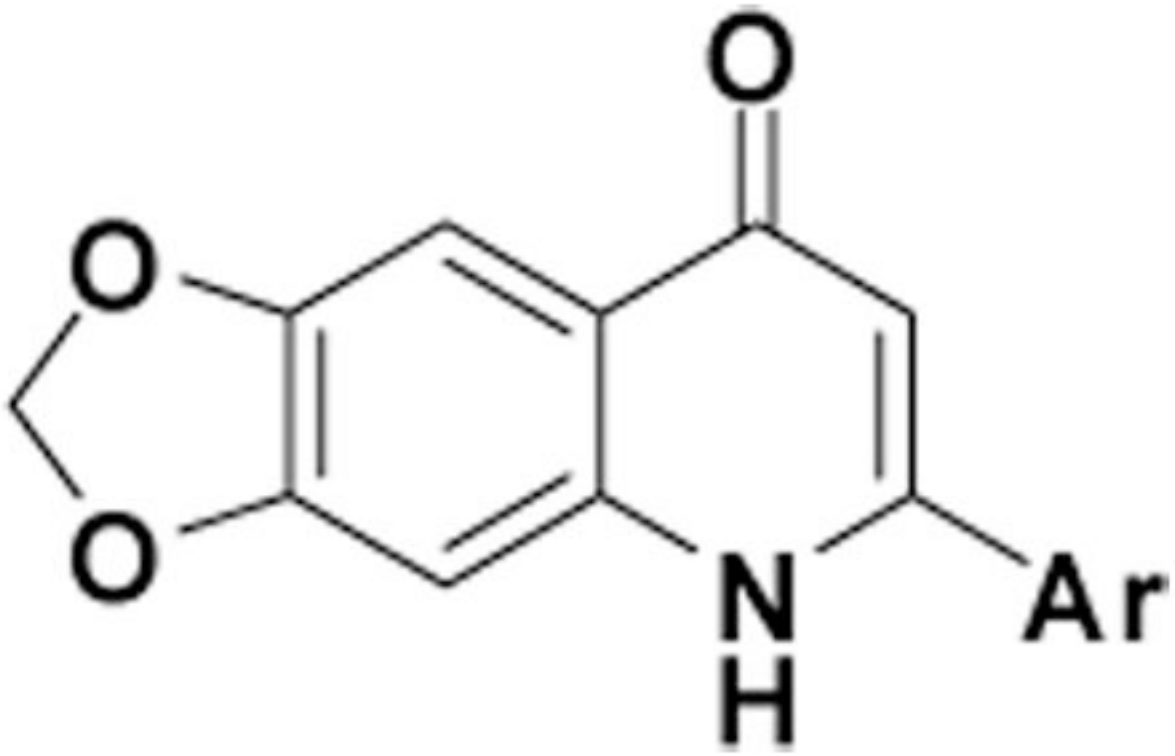

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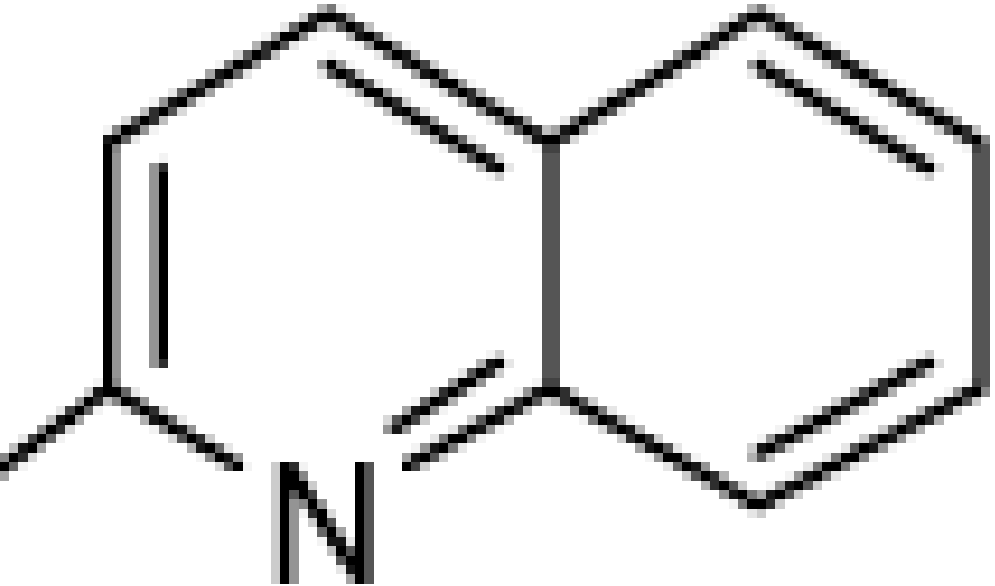
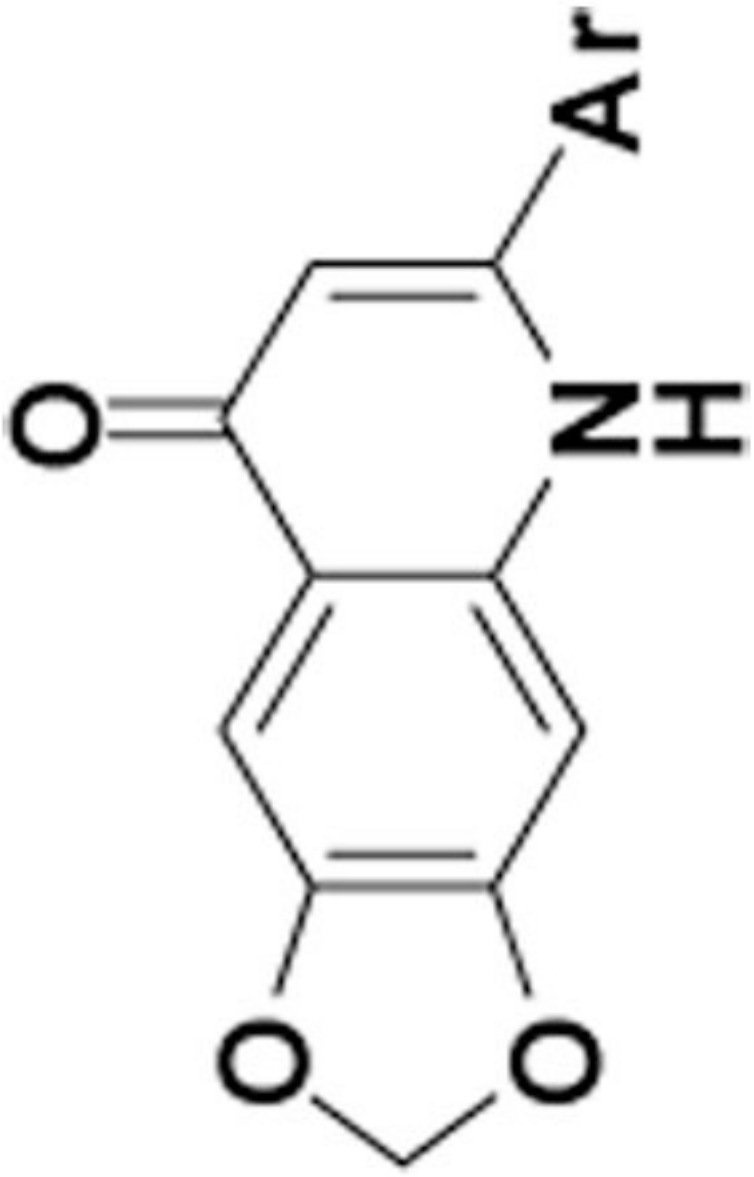
	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00



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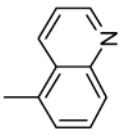	>10.00	>10.00	>10.00	>10.00	>10.00	>10.00	>10.00

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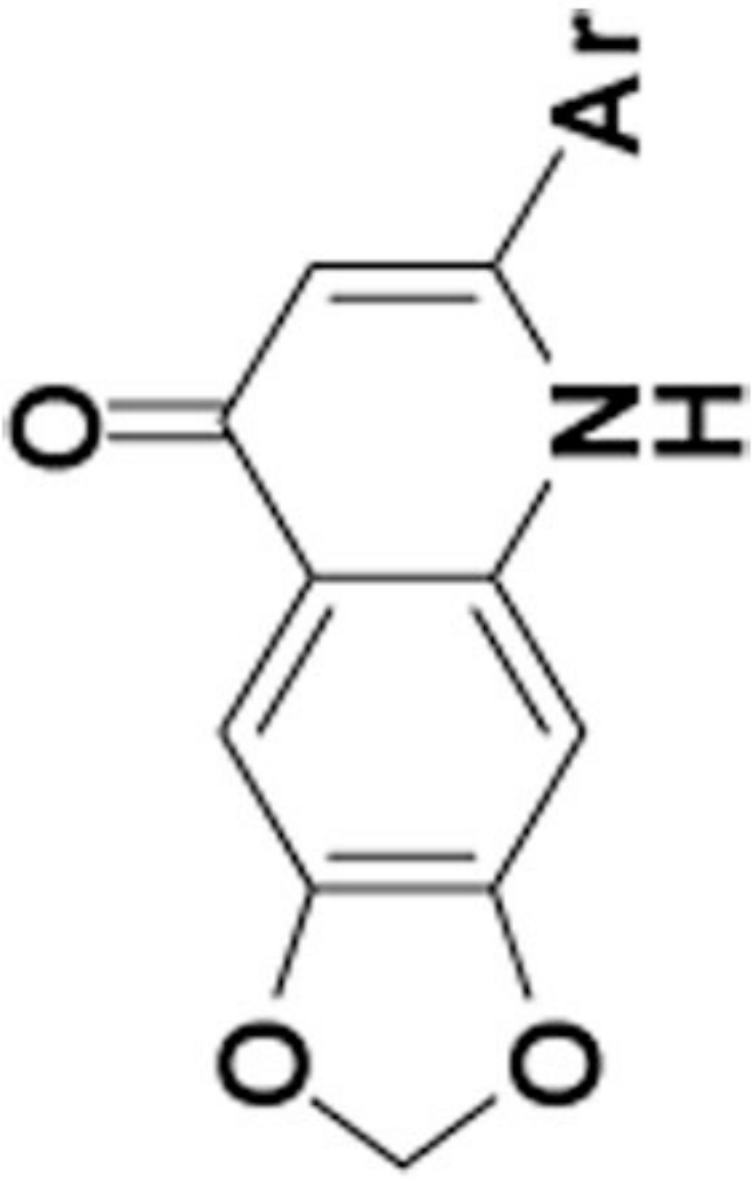
	0.53	1.60	NA*	2.20	NA	NA	NA



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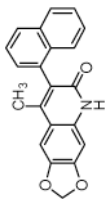	>10.00	>10.00	NA	4.30	NA	NA	NA

Table 2

Anti-tubulin Data for CA-4, **1** and **37-45**.

Compound	Inhibition of Tubulin Assembly[a] IC50 (μM) ± SD	Inhibition of Colchicine Binding[b] % inhibition at tested concentration	
		1 μM	5 μM
CA-4	1.2 ± 0.2	91	99
1	0.76 ± 0.06	25	61
37	0.58 ± 0.02	39	51
38	> 4	-	-
39	0.64 ± 0.07	33	67
40	> 40	-	-
41	> 40	-	-
42	> 4	-	-
43	> 40	-	-
44	27 ± 3	-	-
45	> 40	-	-

[a] Assembly assay contained 10 μM tubulin.

[b] Colchicine binding assay contained 1 μM tubulin and 5 μM [^{3}H]colchicine.