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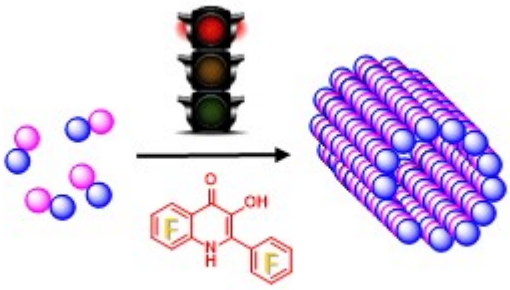


Fluorinated derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone as tubulin polymerization inhibitors

Jiří Řehulka[a#], Kristýna Vychodilová[b#], Petr Krejčí[b], Soňa Gurská[a], Pavel Hradil[b], Marián Hajdúch[a], Petr Džubák[a*], Jan Hlaváč[b*]

[a]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 5, 779 00, Olomouc, Czech Republic

[b]Department of Organic Chemistry, Faculty of Science, Palacký University, Tř. 17. listopadu 12, 771 46, Olomouc, Czech Republic

[#] both authors contributed equally

*Corresponding authors: Hněvotínská 5, 779 00, Olomouc, Czech Republic. Email address: petr.drzubak@upol.cz (P. Džubák)

Tř. 17. listopadu 12, 771 46, Olomouc, Czech Republic. Email address: jan.hlavac@upol.cz (J. Hlaváč)

Abstract

We have synthesized a series of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone derivatives substituted with one or more fluorine atoms on the quinolone backbone as well as on phenyl ring. The derivatives bearing more fluorine atoms were subjected to modification by nucleophilic substitutions by thiophenol, morpholine, and piperazine derivative. We have tested the prepared compounds in cytotoxic activity assay against cancer cell lines. Four derivatives exhibited micromolar values of IC_{50} against some of the cancer cell lines, and we have subjected them to cell cycle analysis on CCRF-CEM. Moreover, most active 7-fluoro-3-hydroxy-2-phenyl-6-(phenylthio)quinolin-4(1*H*)-one inhibits mitosis progression. Cell cycle analysis, in vitro tubulin polymerization assay, and tubulin imaging in cells indicated that the anticancer activity of thiophenol derivative is associated with its ability to inhibit microtubule formation.

Keywords: 2-phenyl-3-hydroxy-4(1*H*)-quinolinone, fluorine implementation, cytotoxic activity, tubulin

1. Introduction

The previous studies of the derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone (3-HQs) recognized these compounds to exhibit anticancer activity *in vitro* in the native state[1-7] or as mixed-ligand complexes with various metals[8-12]. Although these studies described the number of derivatives to have exciting anticancer activity, only two papers revealed a possible molecular target. In the first study, Sui et al.[13] reported the inhibition of topoisomerase as an expected molecular target. The authors found some derivatives with higher activity against mammalian topoisomerase than ellipticine. Recently published interaction of other 3-HQs derivatives with

elongation factor eEF1A1 inside the cancer cells revealed another possible mechanism of anticancer activity[14].

One of the frequently used strategies in the modification of experimental therapeutics to improve the biological activity and the pharmacological profile is an implementation of a fluorine atom to the structure[15]. The main reason for fluorine employment into a drug is increasing of the lipophilicity resulting in enhanced membrane penetration.. Although this phenomenon is well known, the literature does not introduce a broader study of the synthesis, transformation, and biological activity of fluorinated 3-HQs.

Till now, the biological activity was studied only for a few 3-HQs substituted by fluorine atoms. Although the derivatives **A-C** (**Figure 1**) bearing fluorine in the 2-phenyl ring inhibited topoisomerase II, and derivatives **D-H** (**Figure 1**) exhibited an activity in the cytotoxicity assays against cancer cell lines[13], the authors did not report an advanced evaluation of biological properties.

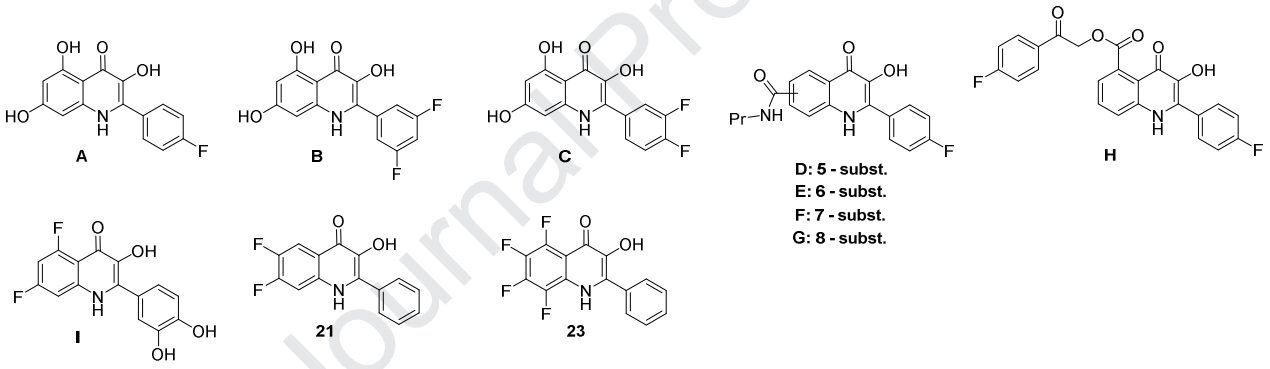


Figure 1. Biologically active derivatives of 3-HQs substituted by fluorine

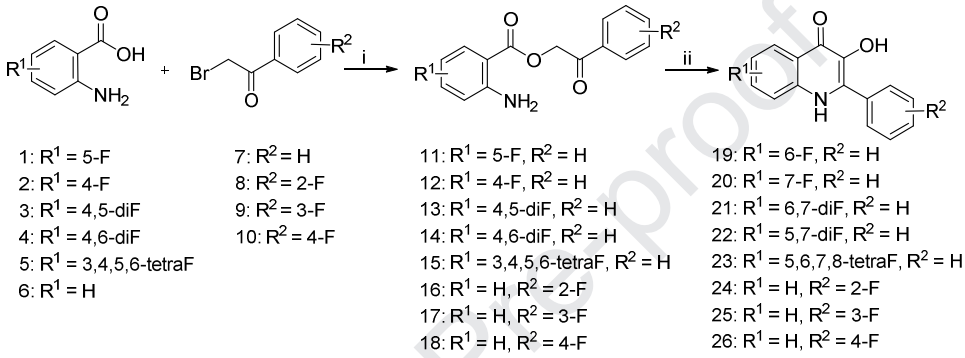
The biological activity of 3-HQ derivatives bearing fluorine atoms in the quinolinone benzene ring was studied only for three derivatives **I**, **21**, and **23**. The derivative **I** slightly inhibited Topoisomerase II[16], and even less effectively bacterial DNA gyrase. Recently, we described the biological effects of derivatives **21** and **23** on Na^+/K^+-ATPase[17]. However, the anticancer activity evaluation of fluorinated 3-HQs is still missing.

In this study, we describe synthetic modification and preparation of mono and polyfluoro analogs of 3-HQs bearing fluorine atoms in quinolone scaffold as well as in its 2-phenyl ring and subsequent study of cytotoxic activity against cancer cells followed by elucidation of the mode of action for the most active derivative.

2. Results and discussion

2.1. Chemistry

The synthesis of 3-HQ derivatives follows the originally developed procedure[18] based on cyclization of phenacyl esters of anthranilic acids **11-18** (**Scheme 1**).



i) Na_2CO_3, DMF, 90min, 50-65 °C; ii) acetic acid, 2-16h, reflux

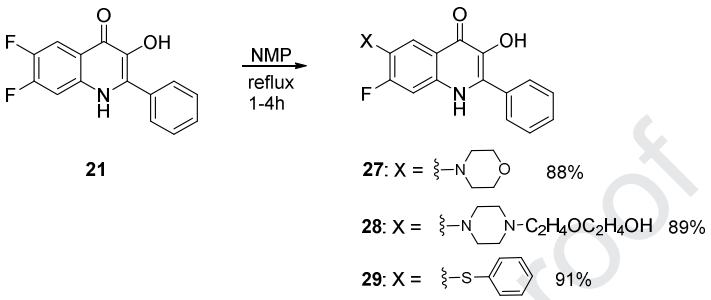
Scheme 1. The synthetic pathways for preparation of desired quinolinones **19-26**.

Final cyclization performed in glacial acetic acid under reflux afforded quinolinones **19-26** (for details see Experimental part).

The fluorine substitution comprises monosubstituted, disubstituted, and tetrasubstituted derivatives in the quinolinone scaffold regarding the commercial availability of fluorinated anthranilic acids and anilines. The fluorine substitution of the 2-phenyl ring was limited to monofluorinated derivatives due to the cost-efficient availability of appropriate acetophenones. As described previously, the unsubstituted derivative of 3-HQ had not exhibited any cytotoxic activity against cancer cells[19], so it was interesting to explore if the implementation of fluorine atoms can increase the anticancer activity.

The present fluorine atoms can allow further modification of the skeleton via nucleophilic substitution. We studied this possibility in the reaction of both monofluorinated derivatives **19**, **20** and **24-26**, 6,7-difluoro derivative **21**, and 5,6,7,8-tetrafluoro derivative **23** of 3-HQs with morpholine, 2-(2-(piperazine-1-yl)ethoxy)ethanol and thiophenol selected as model representatives of biologically relevant substituents[20, 21]. Monosubstituted fluoro derivatives **19**, **20** and **24-26** did not undergo any substitution with any nucleophile. When we used 6,7-difluoro derivative **21**

instead, the substitution was successful with all the nucleophiles proving the expected activation role of the second fluorine. Because the reaction proceeds only at a temperature higher than 90 °C, the substitution with amines proceeded at the boiling temperature of appropriate amine. In the case of thiophenol, we performed the reaction in *n*-butanol under reflux, but even after several hours, the reaction did not proceed. When we used refluxing in *N*-methyl pyrrolidone (NMP) instead, the reaction has finished within one hour. According to NMR, the substitution proceeded in position 6 (see **Scheme 2**).



Scheme 2. Nucleophilic substitution of 3-HQ **21** with various nucleophiles

In the case of tetrafluoro derivative **23** we obtained a complex mixture in reaction with the nucleophiles thwarting isolation of the final compounds.

2.2. Biological studies

2.2.1. Cytotoxicity assay

The cytotoxic activity of the prepared compounds against human cell lines was investigated using 72-hours MTS cytotoxicity assay. The cell lines panel consisted of CCRF-CEM (acute lymphoblastic leukemia), K562 (chronic myelogenous leukemia), A549 (lung adenocarcinoma), colorectal carcinoma cell lines HCT116 with functional p53 protein and p53-deficient cell line HCT116p53-/-. The panel also included chemoresistant subclones CCRF-CEM-DNR (resistant to daunorubicine) and K562-Tax (resistant to paclitaxel) overexpressing P-glycoprotein and possibly lung resistance-related protein (LRP). To evaluate the toxicity against non-tumor cells, we used human skin fibroblast cell line BJ. The following table (**Table 1**) summarizes all the results of the cytotoxicity assay.

Table 1. IC_{50} values of the studied compounds against a panel of human cancer cell lines and normal human fibroblasts. IC_{50} values (μmol/L) from the means of three independent experiments with standard deviation typically up to 20% of the average value.

Compound	CCRF-CEM	CCRF-CEM-DNR	K562	K562-Tax	HCT116	HCT116p53-/-	A549	BJ
19	17.8	15.3	18.8	20.3	>50	17.4	18.8	>50
20	17.8	17.5	21.7	21.4	>50	43.9	19.5	>50
21	12.6	12.8	18.9	17.4	15.1	22.6	18.2	>50

22	4.6	18	21.6	19.8	22.5	>50	19.6	>50
23	19.5	>50	>50	16.8	>50	>50	>50	>50
24	17.4	50	33.7	22.2	35.1	34.2	31.7	>50
25	7.3	50	12.9	7.5	27.1	50	13.1	>50
26	2.4	9.4	40.4	8.6	34.0	36.5	18.1	>50
27	>50	11.7	19.3	12.8	5.2	>50	13.6	>50
28	19.8	>50	47.7	>50	>50	29.4	>50	>50
29	3.3	3.7	1.3	2.9	1.3	4	3.6	>50

Substituted 3-HQs derivatives **19** and **20** with one fluorine atom in the quinolinone skeleton possess only moderate activity against some cancer cell lines. Disubstitution (derivatives **21** and **22)** increased the activity against CCRF-CEM leukemia cells only for the combination of fluorine atoms in positions 5 and 7 (derivative **22**). The 5,6,7,8-tetrafluoro derivative **23** exhibited low activity against selected cell lines. Derivatives **24-26** containing fluorine in 2-phenyl ring display moderate activity against cancer cell line panel except acute lymphoblastic leukemia CCRF-CEM cell line that was slightly more sensitive to 3-fluoro and 4-fluoro substituted compounds **25** and **26**. The substitution of the 6,7-difluoro derivative **21** with all three model nucleophiles in position 6 (morpholine, 2-(2-(piperazine-1-yl)ethoxy)ethanol and thiophenol) giving appropriate derivatives **27-29** caused a significant increase of the cytotoxic activity only for the last one. We observed marked sensitivity of all tested human cancer cell lines to compound **29** with the lowest IC_{50} value 1.3 µmol/L in HCT116 and K562 cell lines. In comparison with other derivatives, **29** displays a stable profile of cytotoxic activity against the cancer cell line panel. Normal human skin fibroblast cell line BJ was less sensitive to all tested derivatives with IC_{50} values above 50 µmol/L, which indicates an acceptable therapeutic index. The comparable sensitivity of resistant cell lines (CCRF-CEM-DNR, K562-Tax) and their parent ones (CCRF-CEM, K562) imply low involvement of the most common cellular mechanisms of drug resistance in response to the derivatives **19-21** and **29**. To predict the response of p53-null cancers, we tested the compounds against p53 deficient cell line HCT116p53-/- and HCT116 cell line with functional p53. Loss of p53 leads to dysregulation of spindle assembly checkpoint, tetraploidy and is frequently associated with a poor prognosis[22]. While the cell line HCT116p53-/- did not responded to derivatives **22** and **27**, p53-/- cells were comparably sensitive towards derivative **29**, indicating its promising activity in p53-null cancers as well. Also, **29** was the only derivative active in low micromolar concentration against A549. The overall results hint that the tested set of derivatives possesses low efficacy against cell lines derived from solid cancers except compound **29**.

2.2.2. Cell cycle analysis

For a more detailed description of the biological activity of studied 3-HQs, we performed cell cycle analysis of CCRF-CEM after 24-hours treatment with cytotoxic compounds **22**, **25**, **26** and **29**

(**Table 2**). Following incubation with 1xIC_{50} concentrations, CCRF-CEM cells were viable and the sub $G_{0/1}$ populations were comparable to controls, whereas 5xIC_{50} concentration treatment typically caused an increase in $G_{0/1}$ phase cells and higher DNA fragmentation except for compound **22**. Compound **22** induced an increase in the S-phase population, while treatment with derivatives **25**, **26** caused accumulation of cells in the G_2/M cell cycle phase. 1xIC_{50} concentration of **29** induced depletion of cells in G_1-phase, increased the G_2/M population and percentage of mitosis-specific phosphorylation of histone H3 at Ser10 ($pH3^{Ser10}$) indicating cell cycle arrest in mitosis.

Interestingly, we did not observe an increase of $pH3^{Ser10}$ in cells treated with **25** and **26** which indicates G_2 arrest. BrdU (5-bromo-2-deoxyuridine) is incorporated into newly synthesized DNA and BrdU pulse labeling is therefore commonly used as a proliferation marker. High BrdU incorporation in cells treated with **22** and 1xIC_{50} of **29** reflected S-phase accumulation, whereas the decrease of BrdU following the treatment by **25**, **26** and 5xIC_{50} of **29** is associated with lower proliferative potential. Percentage of BrU positive cells incorporating 5-bromouridine is proportional to the transcriptional activity of CCRF-CEM cells. These values significantly decreased upon treatment with 5xIC_{50} concentrations of **25**, **26** and **29**.

Table 2. Cell cycle analysis, DNA, and RNA synthesis of CCRF-CEM cell line following the treatment by 1×IC_{50}[a] and 5×IC_{50}[b] of the compounds **22**, **25**, **26** and **29** presented as a percentage of the total cellular population. $G_{0/1}$, S, G_2/M, $pH3^{Ser10}$, BrdU, and BrU are calculated as a percentage from the gated population of viable cells.

	Concentration (μM)	< $G_{0/1}$	$G_{0/1}$	S	G_2/M	$pH3^{Ser10}$	BrdU	BrU
control	0	2.0	35.2	42.2	22.6	2.0	60.0	52.2
22[a]	4.6	0.8	33.0	48.4	18.6	2.1	65.7	57.2
22[b]	23.0	1.4	26.6	51.6	21.8	0.9	79.0	53.7
25[a]	7.3	5.4	34.9	32.6	32.6	1.0	26.7	33.7
25[b]	36.5	15.9	52.6	19.9	27.5	0.2	22.4	3.8
26[a]	2.4	4.4	39.6	24.2	36.2	1.5	35.5	31.3
26[b]	12.0	8.4	45.9	13.5	40.7	1.6	25.2	32.1
29[a]	3.3	5.8	1.8	56.7	41.5	9.4	74.2	67.4
29[b]	16.5	29.7	58.2	21.6	20.2	4.9	18.7	11.4

In addition to leukemia cell line CCRF-CEM, we tested the most active derivative **29** against colorectal adenocarcinoma HCT116 cell line (**Table 3**) used as a model adherent cell line for the intended microscopic imaging. In agreement with CCRF-CEM data, there was substantial increase in the G_2/M phase and mitotic marker $pH3^{Ser10}$ positive cell population, which strongly suggests the antimitotic activity of **29**. In contrast to results obtained for CCRF-CEM, the percentage of S phase and BrdU positive HCT116 cell population remained comparable with untreated control.

Table 3. Cell cycle analysis, DNA, and RNA synthesis of HCT116 cell line following the treatment by $1\times IC_{50}$[a] and $5\times IC_{50}$[b] of compound **29** presented as a percentage of the total cellular population. $G_{0/1}$, S, G_2/M, $pH3^{Ser10}$, BrdU, and BrU are calculated as a percentage from the gated population of viable cells.

		Concentration (μM)	< $G_{0/1}$	$G_{0/1}$	S	G_2/M	$pH3^{Ser10}$	BrdU	BrU
control		0	1.6	35.3	37.7	27.0	3.4	48.6	54.0
LEM 389[a]	**29**	1.3	3.4	33.3	33.5	33.2	6.2	42.1	65.4
LEM 389[b]	**29**	6.5	8.2	2.7	33.2	64.1	15.5	41.6	63.9

2.2.3. Effect of **29** on spindle assembly checkpoint proteins

To gain further insight into the cell cycle arrest induced by **29**, we assessed the level of spindle assembly checkpoint (SAC) and apoptosis regulators such as cyclin B1, its partner kinase Cdc2, antiapoptotic and proapoptotic Bcl-2 family members Bcl-2 and Bax in kinetics over 36 hours. In parallel, we monitored the cell cycle profile with the use of the same concentration and incubation time (**Figure 2**). The $2xIC_{50}$ concentration allows the manifestation of the cell cycle events without causing severe cytotoxicity during the experiment. After 6 and 12 hours of treatment, there were elevated levels of mitosis marker $pH3^{Ser10}$, cyclin B1, and the apparent increase in the G_2/M phase population that indicates mitotic arrest and SAC activation (**Figure 2B**). After 24 hours, the protein levels consequently decreased, whereas the Cdc2 level remained stable throughout the incubation. High cyclin B1 level prevents premature exit from mitosis; however, after prolonged incubation, a small fraction of polyploid cells evolved (**Figure 2A**). Next, we assessed the level of pro-apoptotic and anti-apoptotic Bcl-2 family members represented by Bax and Bcl-2, respectively. We found a rapid induction of a pro-apoptotic member Bax, whereas Bcl-2 level increased later due to the arrest of the entire cell population in G_2/M.

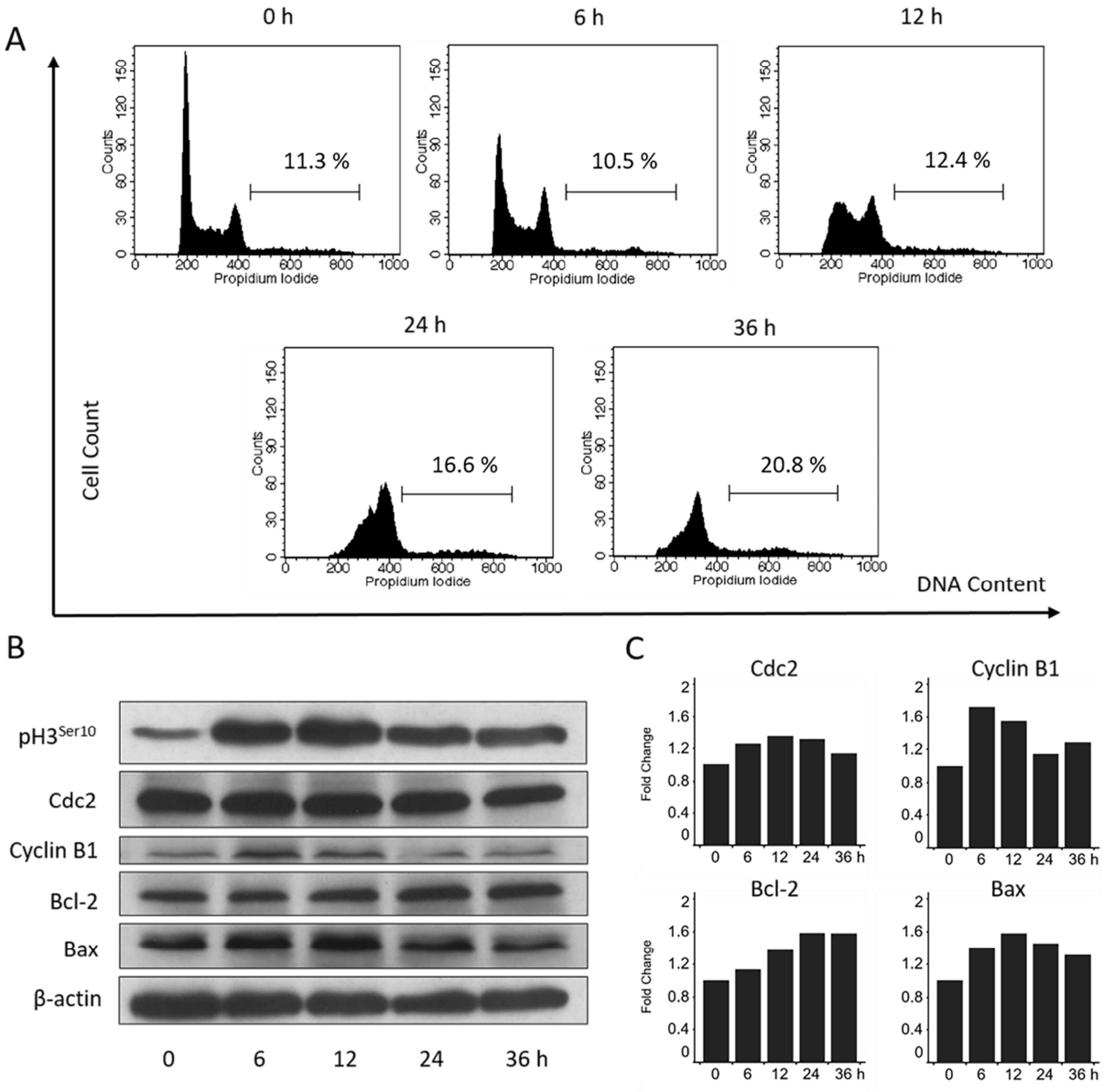


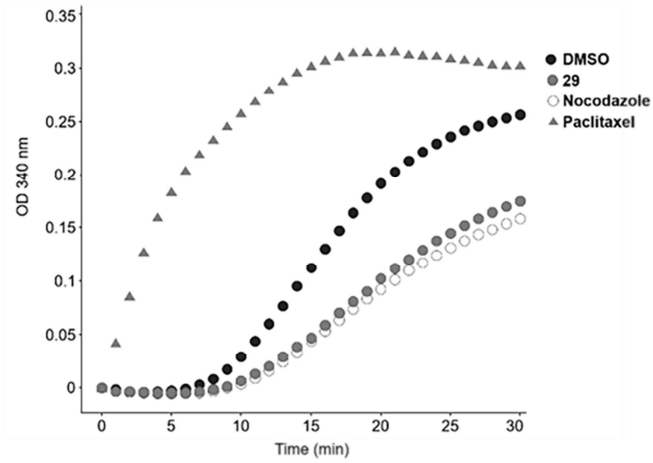
Figure 2. Derivative **29** induces mitotic arrest via spindle assembly checkpoint. (A) Cell cycle profile of CCRF-CEM cells in different incubation times after the treatment with **29**. The percentage of polyploid cells was calculated from the gated population of viable cells. (B) Immunoblots in the corresponding timepoints showing alterations in cell cycle-specific markers $pH3^{Ser10}$, Cdc2 and cyclin B1 and rapid induction of Bax. (C) Relative quantification of Cdc2, cyclin B1, Bcl-2, and Bax protein expression from the immunoblot.

2.2.4. Effect of **29** on tubulin assembly

To check whether **29** acts as a microtubule targeting agent (MTA), similarly to other known compounds inducing SAC, we subjected it to an *in vitro* tubulin polymerization assay. We performed the assay in parallel with the use of known MTAs that either promote microtubule assembly such as paclitaxel or inhibit the polymerization of tubulin subunits such as nocodazole. *In vitro,* the turbidometric assay revealed a strong inhibitory activity of **29**. The polymerization curves

and Vmax values were highly comparable to the activity of nocodazole. Next, we used immunofluorescence and confocal microscopy to visualize the effect of the tested compounds on the microtubules in HCT116 cells. Following 24 h incubation with **29**, we observed mitotic cells with aberrant mitotic spindle morphology. The shortened microtubules, similarly, as in nocodazole treated cells, disable proper alignment and lead to M-phase cell cycle arrest **(Figure 3)**.

A



B

Compound	Vmax (mOD/min)
DMSO	16.8 ± 0.2
29	8.3 ± 1.1
Nocodazole	7.6 ± 1.9
Paclitaxel	63.3 ± 29.3

C

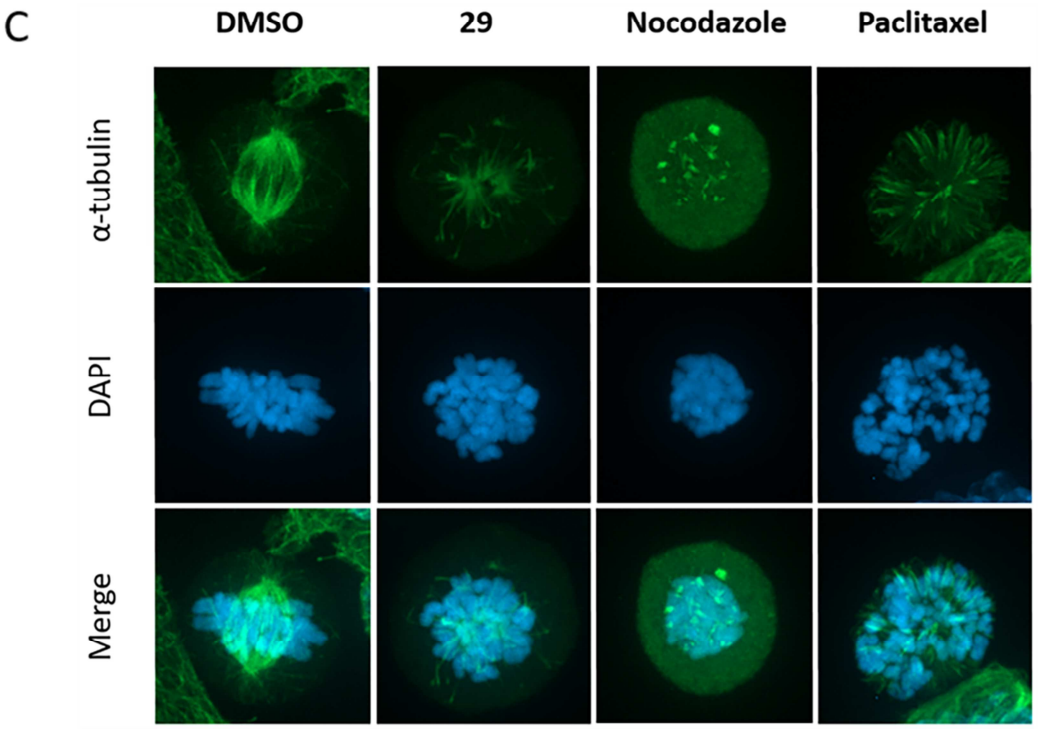


Figure 3. Derivative **29** targets mitotic spindle assembly. (A) *In vitro* porcine tubulin (99%) assembly in the presence of 10 µmol/L compounds, 10 µmol/L nocodazole, 10 µmol/L paclitaxel or corresponding volume of DMSO. (B) Maximal velocity of polymerization values (Vmax) calculated from appropriate tubulin polymerization curves. (C) Immunofluorescence images of

HCT116 colorectal cancer cells after 24 h treatment with 5 x IC_{50} of **29**, 1 µmol/L nocodazole, 1 µmol/L paclitaxel, and DMSO (control). α-tubulin was visualized by FITC and nuclear DNA stained with DAPI, 100 x objective after the fixation of the cells.

Presented microtubule interaction of **29** is in agreement with other quinolone derivatives found in the literature. 2-Phenyl-4-quinolone exhibited a comparable cytotoxic effect on cancer cell lines, including A549. Inhibition of tubulin assembly *in vitro* and *in situ* and induction of cyclin B1 expression are in concordance with the results of our study[23]. Other structurally related inhibitors of microtubule dynamics 2′-fluoro-6,7-methylenedioxy-2-phenyl-4-quinolone (CHM-1)[24] and 4-oxo-2-phenyl-1,4-dihydroquinoline-6-carboxylate (AS1712) enable to bind tubulin colchicine binding site with the induction of mitotic arrest and upregulation of cyclin B1 expression[25].

Since mitotic dysregulation is characteristic for 2-phenyl-4-quinolone[23], we can hypothesize that suitable substitution in the quinolone benzene ring together with the implementation of the hydroxy group to position 3 and replacing of hydrogen by fluorine atom in the 2-phenyl ring does not affect the interaction with mitotic assembly. Although we focused in this study primarily on the antimitotic activity, it brings up a rationale for further research. Compound **22** increased the percentage of cells in S-phase of the cell cycle and the percentage of cells incorporating BrdU into replicating DNA. It would be interesting to study the effect of this compound on DNA replication and topoisomerase activity. This evidence is supported by a high structural similarity of **22** to previously published topoisomerase inhibitor 2-phenyl-3-hydroxy-4-quinolone I[16]. Albeit there is a growing interest in research of Na^+/K^+-ATPase as a target for cancer treatment, the derivative **23** able to inhibit porcine kidney Na^+/K^+-ATPase[17] showed only weak inhibitory activity against the panel of cancer cell lines. Nevertheless, we cannot exclude stronger effects on other cell lines expressing different specific Na^+/K^+-ATPase isoenzyme[26, 27].

Conclusion

We have synthesized derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone bearing fluorine atoms in quinolinone benzene ring or phenyl in position 2. The 6,7-difluoro derivative treated with morpholine, substituted piperazine, or thiophenol as a nucleophile enabled substitution of the fluorine atom in position 6. The compounds **22**, **25**, **26** and **29** exhibited micromolar values of IC_{50} against selected cancer cell lines while stayed inactive against normal cells. The most active derivative **29** exhibited micromolar values of IC_{50} against all cancer cell lines regardless of histogenetic origin, p53 status, and chemoresistant phenotype. Our data demonstrate that **29** interfere with microtubules and mitotic spindle assembly and causes cell cycle arrest in mitosis. The results achieved in this study revealed a new molecular target for 3-HQs. Considering their

previously reported target proteins[13, 14, 17], we can confirm that the change of the 3-HQs substitution can cause the change of mode of action.

3. Experimental

3.1. General information

All chemicals and solvents for the synthesis were obtained from Sigma-Aldrich. Paclitaxel was purchased from Mylan Pharmaceuticals, nocodazole, 3-(4,5-dimethylthiazole-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), phenazine methosulfate (PMS), 5-bromo-2-deoxyuridine and 5-bromouridine were purchased from Sigma-Aldrich.

3.1.1. Structural analysis

NMR spectra were measured in DMSO-d_6 using a Jeol ECX-500 (500 MHz) spectrometer or a Bruker Avance (300 MHz) instrument. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*) are reported in Hertz (Hz). HRMS analysis was performed using an Orbitrap Elite high-resolution mass spectrometer (Thermo Fischer Scientific, MA, USA). The machine was operated at the positive full scan mode (120 000 FWMH) in the range of 200–900 m/z. The settings for electrospray ionization were as follows: oven temperature of 300 °C, sheath gas of 8 arb. units, and the source voltage of 1.5 kV. The acquired data were internally calibrated with diisooctyl phthalate as a contaminant in methanol (m/z 391.2843). Samples were diluted to a final concentration of 20 µmol/l with 0.1% formic acid in water and methanol (50:50, v/v). The samples were injected by direct infusion into the mass spectrometer.

3.1.2. Cell Lines

The cell lines were obtained from American Tissue Culture Collection (ATCC) if not indicated otherwise. HCT116 and HCT116p53-/- cell lines were purchased from Horizon Discovery. Resistant CEM-DNR bulk and K562-TAX sublines were selected by increasing doses of daunorubicin or paclitaxel, respectively. The CEM-DNR bulk cells have been shown to overexpress the MRP-1 and P-glycoprotein proteins, whereas K562-TAX cells overexpress only P-glycoprotein[28]. Cells were cultured in the cell culture medium according to ATCC or Horizon recommendations in the humidified incubator under the atmosphere of 95% air and 5% CO_2 at 37 °C. DMEM/RPMI 1640 (Sigma-Aldrich) was supplemented with 10% fetal calf serum (GBCO), 100 U/mL penicillin, and 100µg/mL streptomycin.

3.1.3. MTS Assay

Cells were seeded into 384-well microtiter plates in a volume of 30 μL. Next day the aliquots of compounds were transferred by Echo550 acoustic liquid handler (Labcyte) to obtain dose-response curves with dilution factor 4. The experiments were performed in technical duplicates and two or more biological replicates. After 72 h incubation in a humidified incubator, four μL of the MTS/PMS stock solution was pipetted into each well. Following an additional 1–4 h incubation absorbance at 490 nm was measured using EnVision Multilabel Plate Reader (PerkinElmer). The IC_{50} values were calculated from the appropriate dose-response curves in Dotmatics software.

3.1.4. Flow Cytometry

The cell cycle analysis, assessment of histone 3 phosphorylation and BrdU/BrU incorporation analyses were performed as described previously[29]. HCT116 and CCRF-CEM cells were seeded into cell culture dishes and after overnight incubation treated with 1 x and 5 x IC_{50} concentration of compounds for 24 hours. Cells were harvested, washed with cold PBS, and fixed. Following overnight fixation, cells were subjected to immunostaining for the desired marker, treated with RNase (0.5 mg/mL) and propidium iodide (0.1 mg/mL), and analyzed by FACSCalibur (Becton Dickinson) flow cytometer at 488 nm. For the BrdU/BrU incorporation methods, the cells were pulse-labeled for 30 min with 10 μM 5-bromo-2-deoxyuridine (BrdU) or 1 mM 5-bromouridine (BrU) before the harvesting. Anti-BrdU antibody was obtained from Exbio, anti-phospho-histone H3 (Ser10) antibody, and secondary anti-mouse-FITC antibody was obtained from Sigma-Aldrich. The cell cycle was analyzed in the program ModFitLT (Verity). The DNA fragmentation was assessed using the logarithmic model expressing the percentage of the particles with the propidium iodide content lower than cells in the G_0/G_1 phase ($<G_1$) of the cell cycle.

3.1.5. Western Blot

Anti-cyclin B1 and Cdc2 antibodies were obtained from Santa Cruz, anti-Bcl-2, and Bax were purchased from Cell Signalling and β-actin from Sigma-Aldrich. The densities of the bands were assessed using ImageJ and normalized to β-actin expression.

3.1.6. Tubulin Polymerization Assay

In vitro microtubule assembly curves were obtained using Tubulin Polymerization Assay Kit (Cytoskeleton) as described previously[30]. Porcine brain tubulin (> 99% pure) was dissolved to a final concentration 3 mg/mL in Tubulin Polymerization Buffer containing 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP, 10.2% glycerol. The absorbance was measured at 340 nm using EnVision Multilabel Plate Reader (PerkinElmer) at 37 °C and recorded every 60 s for 30 min.

3.1.7. Immunofluorescence

The visualization of microtubules was previously described[30]. Briefly, the cells grown on coverslips were incubated for 24 hours with compounds, washed in buffer containing 10 mM MES, 150 mM NaCl, 5 mM EGTA, 5 mM $MgCl_2$, 5 mM glucose, pH 6.1 and fixed by 3% solution of paraformaldehyde with 0.3% Triton X-100 in the same buffer. Fixed cells were incubated with α-tubulin (DM1A) mouse monoclonal antibody (Cell Signaling) and Alexa Fluor-488-conjugated anti-mouse antibody (Sigma-Aldrich). Vectashield Mounting Medium with DAPI (Vector Laboratories) was used for DNA staining and sample preparation. Images were acquired using a spinning disk confocal microscope (Zeiss).

3.1.8. Synthesis of the compounds

General methods for the preparation of phenacyl esters

*Method A***:** TEA (3.2 mmol) was added to a solution of substituted anthranilic acid (3.0 mmol) in DMF (5 mL) at room temperature. After 3 min, appropriate bromo acetophenone (3.0 mmol) was added into the reaction mixture. The reaction mixture was stirred at 70 °C. After 40 min, the reaction mixture was poured to ice-cold water (100 mL). The precipitated product was filtered off and washed thoroughly with water, dried, and recrystallized from EtOAc/EtOH.

Method B: Na_2CO_3 (1.7 mmol) was added to a solution of substituted anthranilic acid (3.0 mmol) in DMF (5 mL) and the reaction mixture was stirred 30 min at 50 °C. Then it was cooled to room temperature, and appropriate bromo acetophenone (3.0 mmol) was added into the reaction mixture. The reaction mixture was stirred at 65-70 °C. After one hour, the reaction mixture was poured to ice-cold water (100 mL) and extracted with EtOAc. The organic phase was collected, washed with water, dried over Na_2SO_4, and evaporated to dryness. The obtained product was crystallized from EtOAc/EtOH.

Method C: Potassium carbonate (0.14 g, 1 mmol) was added to the solution of anthranilic acid (0.14 g, 1 mmol) was dissolved in DMF (5 ml). The mixture was stirred at 90 °C for 1h. Then it was cooled to RT, and fluorinated acetophenone (0.22 g, 1 mmol) was added. The mixture was stirred 1h at RT, and then it was poured to ice-cold water (10 mL). The precipitated product was filtered off, washed with water and dried.

2-oxo-2-phenylethyl 2-amino-5-fluorobenzoate **11:** TEA (3.2 mmol) was added to a solution of 5-fluoroanthranilic acid (3.0 mmol) in dimethylformamide (5 mL) at room temperature. After 3 min, 2-bromacetophenone (3.0 mmol) was added into the reaction mixture. The reaction mixture was stirred at 70 °C. After 40 min, the reaction mixture was poured to ice cold water (100 mL) and extracted with EtOAc. The organic phase was collected, washed with water, dried over Na_2SO_4 and

evaporated to dryness. Obtained product was crystallized from EtOAc/ethanol. Yield 770 mg (94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 – 8.00 (m, 2H), 7.74 – 7.69 (m, 1H), 7.61 – 7.56 (m, 2H), 7.52 – 7.48 (m, 1H), 7.27 – 7.21 (m, 1H), 6.84 (dd, J = 9.5, 4.9 Hz, 1H), 6.57 (bs, 2H), 5.69 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.52, 166.38, 154.0, 151.71, 149.06, 134.51 (d, J_{C-F} = 6 Hz), 129.50, 128.35, 123.23 (d, J_{C-F} = 23 Hz), 118.84 (d, J_{C-F} = 8 Hz), 115.42 (d, J_{C-F} = 23 Hz), 108.14 (d, J_{C-F} = 7 Hz), 67.25. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{13}FNO_3$ 274.0874; found: 274.0875.

2-oxo-2-phenylethyl 2-amino-4-fluorobenzoate **12:** Prepared from 4-fluoroanthranilic acid (3.0 mmol) and 2-bromacetophenone (3.0 mmol) according to the general method A. Yield 737 mg (90 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 – 7.98 (m, 2H), 7.89 (dd, J = 9.0, 6.9 Hz, 1H), 7.74 – 7.68 (m, 1H), 7.61 – 7.55 (m, 2H), 6.90 (bs, 2H), 6.57 (dd, J = 11.9, 2.6 Hz, 1H), 6.43 – 6.38 (m, 1H), 5.67 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.10, 167.25, 165.90, 164.78, 153.73 (d, J_{C-F} = 13 Hz), 133.93 (m), 128.91, 127.76, 105.41 (d, J_{C-F} = 1 Hz), 102.86 (d, J_{C-F} = 23 Hz) 101.38 (d, J_{C-F} = 24 Hz), 66.46. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{12}FNO_3$ 274.0874; found: 274.0874.

2-oxo-2-phenylethyl 2-amino-4,5-difluorobenzoate **13:** Prepared from 4,5-difluoroanthranilic acid (3.0 mmol) and 2-bromacetophenone (3.0 mmol) according to the general method A. Yield 847 mg (97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 – 7.99 (m, 2H), 7.75 – 7.68 (m, 2H), 7.61 – 7.55 (m, 2H), 6.83 – 6.75 (m, 3H), 5.68 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.88, 165.24 (d, J_{C-F} = 2 Hz), 149.76 (d, J_{C-F} = 12 Hz), 141.21 (d, J_{C-F} = 14 Hz), 138.88 (d, J_{C-F} = 14 Hz), 133.93 (d, J_{C-F} = 11 Hz), 128.93, 127.78, 118.25 (d, J_{C-F} = 3 Hz), 118.08 (d, J_{C-F} = 3 Hz), 103.81, 103.61, 66.75. HRMS (ESI) calcd for $[M-H]^-$ $C_{15}H_{10}F_2NO_3$ 290.0623; found: 290.0615.

2-oxo-2-phenylethyl 2-amino-4,6-difluorobenzoate **14:** Prepared from 4,6-difluoroanthranilic acid (3.0 mmol) and 2-bromoacetophenone (3.0 mmol) according to the general method B. Yield 777 mg (89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 – 7.99 (m, 2H), 7.74 – 7.67 (m, 1H), 7.58 (t, J = 7.7 Hz, 2H), 7.00 (bs, 2H), 6.46 – 6.32 (m, 2H), 5.72 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.95, 166.17 (d, J_{C-F} = 17 Hz), 164.27 (d, J_{C-F} = 3 Hz), 163.71 (d, J_{C-F} = 18 Hz), 162.58 (d, J_{C-F} = 17 Hz), 153.62 (m), 133.93 (d, J_{C-F} = 22 Hz), 128.93, 127.83, 97.42 (dd, J_{C-F} = 4, 25 Hz), 96.72 (dd, J_{C-F} = 3, 14 Hz), 91.45 (m), 66.84. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{12}F_2NO_3$ 292.0780; found: 292.0779.

2-oxo-2-phenylethyl 2-amino-3,4,5,6-tetrafluorobenzoate **15:** Prepared from 3,4,5,6-tetrafluoroanthranilic acid (3.0 mmol) and 2-bromacetophenone (3.0 mmol) according to the

general method B. Yield 873 mg (89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 – 8.00 (m, 2H), 7.75 – 7.69 (m, 1H), 7.59 (t, J = 7.7 Hz, 2H), 6.78 (bs, 2H), 5.81 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 192.58, 163.02 m, 136.83, 134.17, 133.63, 128.97, 127.88, 100.78, 99.45, 97.15, 67.55. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{10}F_4NO_3$ 328.0591; found: 328.0591.

2-(2-fluorophenyl)-2-oxoethyl 2-aminobenzoate **16:** The compound was prepared according to the general method C. Yield 221 mg (81%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92 (td, J = 7.5, 1.7 Hz, 1H), 7.80 (dd, J = 8.1, 1.55 Hz, 1H), 7.77 – 7.72 (m, 1H), 7.46 – 7.38 (m, 2H), 7.31 - 7.27 (m, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.65 (bs, 2H), 6.57 (t, J = 7.5 Hz, 1H), 5.48 (d, J = 2.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 191.18 (d, J_{C-F} = 4 Hz), 166.58, 162.39, 160.37, 151.56, 135.99 (d, J_{C-F} = 9 Hz), 134.36, 130.75, 130.12 (d, J_{C-F} = 3 Hz), 125.11 (d, J_{C-F} = 3 Hz), 122.35 (d, J_{C-F} = 14 Hz), 116.78 (m), 114.76, 108.08, 68.48. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{13}FNO_3$ 274.0874; found: 274.0873.

2-(3-fluorophenyl)-2-oxoethyl 2-aminobenzoate **17:** The compound was prepared according to the general method C. Yield 213 mg (78%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (dt, J = 7.7, 1.2 Hz, 1H), 7.83 – 7.79 (m, 2H), 7.67 – 7.62 (m, 1H), 7.60 – 7.54 (m, 1H), 7.32 – 7.27 (m, 1H), 6.80 (dd, J = 8.4, 0.8 Hz, 1H), 6.63 (bs, 2H), 6.59 – 6.55 (m, 1H), 5.67 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 192.39, 166.59, 163.15, 151.55, 136.07 (d, J_{C-F} = 6 Hz), 134.38, 131.20 (d, J_{C-F} = 8 Hz), 130.75, 124.03 (d, J_{C-F} = 3 Hz), 120.83 (d, J_{C-F} = 21 Hz), 116.59, 114.80, 114.37 (d, J_{C-F} = 22 Hz), 108.07, 66.47. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{13}FNO_3$ 274.0874; found: 274.0872.

2-(4-fluorophenyl)-2-oxoethyl 2-aminobenzoate **18:** The compound was prepared according to the general method C. Yield 186 mg (68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 – 8.08 (m, 2H), 7.81 (dd, J = 8.1, 1.5 Hz, 1H), 7.44 – 7.39 (m, 2H), 7.31 – 7.27 (m, 1H), 6.80 (d, J = 7.9 Hz, 1H), 6.63 (bs, 2H), 6.59 – 6.55 (m, 1H), 5.66 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 191.88, 166.63, 166.36, 164.35, 151.53, 134.35, 130.83 (m), 116.59, 116.09, 115.92, 114.79, 108.16, 66.26. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{13}FNO_3$ 274.0874; found: 274.0873.

The general method for the preparation of 3-HQs by cyclization reaction

The solution of phenacyl ester in glacial acetic acid (approx. 1 mmol of ester/ 4 ml acid) was stirred under reflux 3-16 h; the reaction was monitored by LC/MS. After the ester consumption, the reaction mixture was poured onto crushed ice, and the precipitated product was filtered off, washed thoroughly with water and cold acetone, followed by drying under vacuum. In case the precipitation

was not formed, the product was extracted to EtOAc, the organic phase was dried over the Na_2SO_4 and evaporated to dryness, followed by washing the powder by cold acetone.

6-fluoro-3-hydroxy-2-phenylquinolin-4(1H)-one **19:** Yield 568 mg (89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H), 7.93 (bs, 1H), 7.86 – 7.74 (m, 3H), 7.85 – 7.75 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.97, 169.17, 158.75, 156.35, 137.55, 134.89, 132.11 (d, J_{C-F} = 7 Hz), 129.33, 128.25, 122.56 (d, J_{C-F} = 7 Hz), 121.47 (d, J_{C-F} = 8 Hz), 119.78 (d, J_{C-F} = 26 Hz),107.71 (d, J_{C-F} = 22 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{11}FNO_2$ 256.0768; found: 256.0769.

7-fluoro-3-hydroxy-2-phenylquinolin-4(1H)-one **20:** Yield 478 mg (75%).^{1}H NMR (400 MHz, DMSO-d_6) δ 11.57 (s, 1H), 8.43 (bs, 1H), 8.21 (dd, J = 9.1, 6.4 Hz, 1H), 7.82 – 7.77 (m, 2H), 7.61 – 7.48 (m, 3H), 7.42 (dd, J = 10.6, 2.4 Hz, 1H), 7.14 (td, J = 8.8, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.83, 164.45, 161.99, 139.40 (d, J_{C-F} = 13 Hz) , 137.85, 132.17, 131.66, 129.20 (d, J_{C-F} = 19 Hz), 128.32, 127.82 (d, J_{C-F} = 11 Hz), 119.01, 111.25 (d, J_{C-F} = 24 Hz), 102.90 (d, J_{C-F} = 25 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{11}FNO_2$ 256.0768; found: 256.0768.

6,7-difluoro-3-hydroxy-2-phenylquinolin-4(1H)-one **21:** Yield 656 mg (96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.55 (s, 1H), 7.97 (dd, J = 11.0, 8.8 Hz, 1H), 7.77 (s, 2H), 7.66 – 7.48 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.05, 152.83 (d, J_{C-F} = 16 Hz), 150.34 (d, J_{C-F} = 16 Hz), 147.42 (d, J_{C-F} = 15 Hz), 144.99 (d, J_{C-F} = 15 Hz), 137.69, 134.81 (d, J_{C-F} = 11 Hz), 132.14 (d, J_{C-F} = 23 Hz), 129.26 (d, J_{C-F} = 28 Hz), 128.34, 118.71 (d, J_{C-F} = 5 Hz), 111.11 (d, J_{C-F} = 18 Hz), 106.01 (d, J_{C-F} = 21 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{10}F_2NO_2$ 274.0674; found: 274.0677.

5,7-difluoro-3-hydroxy-2-phenylquinolin-4(1H)-one **22:** Yield 649 mg (95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.46 (bs, 1H), 7.82 – 7.74 (m, 2H), 7.61 – 7.49 (m, 3H), 7.25 (d, J = 10.3 Hz, 1H), 7.06 – 6.96 (m, 1H).^{13}C NMR (101 MHz, DMSO-d_6) δ 168.59, 163.05 (dd, J_{C-F} = 15, 63 Hz), 160.52 (dd, J_{C-F} = 15, 77 Hz), 140.31 (m), 138.75, 131.68, 130.26, 129.41, 129.06, 128.34, 109.25 (d, J_{C-F} = 2 Hz), 99.17 (dd, J_{C-F} = 5, 25 Hz), 97.94 (m). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{10}F_2NO_2$ 274.0674; found: 274.0675.

5,6,7,8-tetrafluoro-3-hydroxy-2-phenylquinolin-4(1H)-one **23:** Yield: 726 mg (94%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.49-7.58 (m, 3H); 7.70-7.77 (m, 2H). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_7F_4NO_2$ 310.0484; found: 310.0486. ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.97, 138.84 m, 129.39 m, 128.06, 108.85 m.

3-hydroxy-2-(2´-fluoro)phenylquinolin-4(1H)-one **24:** Yield 130 mg (79%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.79 (s, 1H), 8.41 (bs, 1H), 8.18 (d, J = 8.0 Hz, 1H), 7.69 – 7.56 (m, 4H), 7.45 – 7.36 (m, 2H), 7.32 – 7.23 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.87, 160.31, 158.33, 138.68,

137.90, 131.87, 131.66 (d, J_{C-F} = 8 Hz), 130.63, 126.88, 124.45 (d, J_{C-F} = 28 Hz), 122.18, 121.88, 120.21 (d, J_{C-F} = 15 Hz), 118.18, 115.85 (d, J_{C-F} = 21 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{11}FNO_2$ 256.0768; found: 274.0768.

3-hydroxy-2-(3´-fluoro)phenylquinolin-4(1H)-one **25:** Yield 145 mg (83%).^{1}H NMR (500 MHz, DMSO-d_6) δ 11.66 (s, 1H), 8.18 – 8.15 (m, 1H), 7.75 – 7.72 (m, 1H), 7.69 – 7.59 (m, 4H), 7.40 – 7.34 (m, 1H), 7.31 – 7.27 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.77, 162.72, 160.78, 137.95 (d, J_{C-F} = 13 Hz), 134.34 (d, J_{C-F} = 9 Hz), 130.75, 130.33 (d, J_{C-F} = 8 Hz), 125.46 (d, J_{C-F} = 3 Hz), 124.37, 122.12, 121.71, 118.51, 116.17 (d, J_{C-F} = 7 Hz), 116.00 (d, J_{C-F} = 5 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{11}FNO_2$ 256.0768; found: 274.0768.

3-hydroxy-2-(4´-fluoro)phenylquinolin-4(1H)-one **26:** Yield 150 mg (84%).^{1}H NMR (500 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.16 (dd, J = 8.2, 1.3 Hz, 1H), 7.90 - 7.84 (m, 2H), 7.72 (d, J = 8.4 Hz, 1H), 7.64 – 7.58 (m, 1H), 7.45 – 7.39 (m, 2H), 7.32 – 7.27 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.43, 163.44, 161.48, 137.79 (d, J_{C-F} = 34 Hz), 131.63 (d, J_{C-F} = 7 Hz), 131.22, 130.64, 128.57 (d, J_{C-F} = 3 Hz), 124.33, 122.13, 121.69, 118.47, 115.26 (d, J_{C-F} = 21 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{11}FNO_2$ 256.0768; found: 274.0767.

7-fluoro-3-hydroxy-6-morfolino-2-phenylquinolin-4(1H)-one **27:** Quinolone **21** (150 mg, 0.55 mmol) was added into the solution of morpholine (4.0 mL, 45.9 mmol) in *N*-methyl-2-pyrrolidone (NMP, 1.0 mL). The reaction mixture was refluxed for 4 h. After that it was poured onto crushed ice (100 g) and pH was adjusted with diluted HCl to pH 7. Precipitated product was filtered off, washed thoroughly with water and recrystalized from 2-methoxyethanol after drying. Yield 164 mg (88%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 7.82 – 7.76 (m, 2H), 7.69 (d, J = 13.6 Hz, 1H), 7.59 – 7.47 (m, 3H), 7.23 (d, J = 7.6 Hz, 1H), 3.82 – 3.76 (m, 4H), 3.15 – 3.08 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.97, 152.93, 150.50, 143.26 (d, J_{C-F} = 11 Hz), 137.07, 135.88, 132.33, 130.81, 129.08 (d, J_{C-F} = 12 Hz), 128.26, 116.43 (d, J_{C-F} = 7 Hz), 108.98 (d, J_{C-F} = 22 Hz), 106.30 (d, J_{C-F} = 2 Hz), 65.95, 50.22. HRMS (ESI) calcd for $[M-H]^-$ $C_{19}H_{16}FN_2O_3$ 339.1139; found: 339.1127.

7-fluoro-3-hydroxy-6-(4-(2-(2-hydroxyethoxy)ethyl)piperazin-1-yl)2-phenylquinolin-4(1H)-one **28:** Quinolone **21** (250 g, 0.91 mmol) was added into the solution of 1-(2-(hydroxyethoxy)-ethyl)piperazine (0.78 mL, 4.5 mmol) in NMP (1.0 mL). The reaction mixture was refluxed for 3 h. After that it was poured onto crushed ice (100 g) and pH was adjusted with diluted HCl to pH 7. Precipitated product was filtred off, washed thoroughly with water and recrystalized from 2-

methoxyethanol after drying. Yield 350 mg (89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 7.79 (d, J = 7.1 Hz, 2H), 7.67 (d, J = 13.6 Hz, 1H), 7.58 – 7.47 (m, 3H), 7.22 (d, J = 7.6 Hz, 1H), 3.57 (t, J = 5.8 Hz, 2H), 3.53 – 3.48 (m, 2H), 3.45 – 3.41 (m, 2H), 3.13 (bs, 4H), 2.66 (bs, 4H), 2.58 (t, J = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.96, 152.97, 150.54, 143.32 (d, J_{C-F} = 12 Hz), 137.02, 135.91, 132.35, 130.69, 129.11, 129.02, 128.25, 116.26 (d, J_{C-F} = 7 Hz), 108.97, 108.75, 106.35, 72.22, 68.07, 60.23, 57.14. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{27}FN_3O_3$ 428.1980; found: 428.1981.

7-fluoro-3-hydroxy-2-phenyl-6-(phenylthio)quinolin-4(1H)-one **29:** NaH 80% (90.0 mg, 3.0 mmol) was added into the solution of thiophenol (308 μL, 3.0 mmol) in NMP (5.0 mL). After 5 min of stirring quinolone **21** (400 mg, 1.46 mmol) was added and the reaction mixture was refluxed for 1 h. Cooled reaction mixture was poured into cold water (100 mL) and pH was adjusted with diluted HCl to pH 7. Precipitated product was filtered off, washed thoroughly with water and recrystallized from acetone after drying. Yield 483 mg (91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 7.82 (d, J = 10.4 Hz, 1H), 7.71 (dd, J = 7.9, 1.3 Hz, 2H), 7.59 – 7.45 (m, 8H), 7.34 (d, J = 6.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.94, 155.48, 153.09, 137.64, 135.05, 133.60, 132.07, 131.95, 130.26, 130.03, 129.74 (d, J_{C-F} = 16 Hz), 129.36 (d, J_{C-F} = 7 Hz), 129.25, 128.27, 120.96 (d, J_{C-F} = 7 Hz), 118.93, 108.37 (d, J_{C-F} = 22 Hz). HRMS (ESI) calcd for $[M+H]^+$ $C_{21}H_{15}FNSO_2$ 364.0802; found: 364.0801.

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- Fluorine in 2-phenyl-3-hydroxy-4(1*H*)-quinolinone improves biological activity
- Difluoroderivatives of 3-hydroxy-4(1*H*)-quinolinone enable nucleophilic substitution
- 2-phenyl-3-hydroxy-4(1*H*)-quinolinones can inhibit tubulin polymerization

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Declarations of interest: none'