Journal of Fluorine Chemistry 178 (2015) 142–153

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

An effective two-step synthesis, fluorescent properties, antioxidant activity and cytotoxicity evaluation of benzene-fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones

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ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form 2 July 2015
Accepted 8 July 2015
Available online 19 July 2015

Keywords:
Sonogashira coupling
PTSA-catalyzed cyclocondensation
Benzene-fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones
Photophysical properties
Cytotoxicity against cancer cells
Mutagenic and antioxidant properties

ABSTRACT

This study describes a simple and efficient procedure to synthesize a series of benzene-fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones by the PTSA-catalyzed cyclocondensation reaction of the corresponding *ortho*-alkynylanilines prepared by the Sonogashira reaction of fluoro-substituted 2-iodanilines with 2-methylbut-3-yn-2-ol. The photofluorescent properties (shape of bands, λ_{ex}, λ_{em}, Stokes shift) of the new compounds were investigated. It has been revealed that the 2,3-dihydroquinolinones with different number of fluoro-substituents have almost the same fluorescence properties. The cytotoxicity evaluation of the 2,3-dihydroquinolinones against human myeloma, human mammary adenocarcinoma, human hepatocellular carcinoma HepG2 epithelial tumor cells, normal mouse fibroblasts and Chinese hamster Ag 17 cells was performed. It has been found that the benzo-perfluorinated derivatives of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones show enhanced cytotoxic effect against the tumor RPMI (human myeloma) cells line compared with the normal cells. Mutagenic and antioxidant properties of the compounds using *Salmonella* tester strain were studied. It has been shown, that the compounds are well antioxidants.

1. Introduction

Quinolone-based compounds are considered as one of the highly privileged building blocks in medicinal chemistry, thus widely known fluorine-containing antibacterial drugs, including Ciprofloxacin [1]. On the contrary, the bioactivity properties of some quinolone derivatives, namely 2,3-dihydro-4(1*H*)-quinolinones, are insufficiently studied. Nevertheless, since products containing this scaffold have demonstrated interesting biological properties, this demanded developing new general methods of quinolinone synthesis [2]. In our synthetic planning to generate diverse quinolinone-based compounds, the objective was to synthesize fluorinated 2,3-dihydro-4(1*H*)-quinolinones **1**. From the one side, compounds of such structural backbone are known to possess a broad scope of pharmacological properties including anti-ulcer, antiinflammatory and anticancer activity (**A**) [3–5], high binding affinities for 5-HT_6 receptor with good selectivity over other serotonin and dopamine receptors (**B**) [6], and, as the last example, pain-blocking properties (**C**) [7].

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http://dx.doi.org/10.1016/j.jfluchem.2015.07.006
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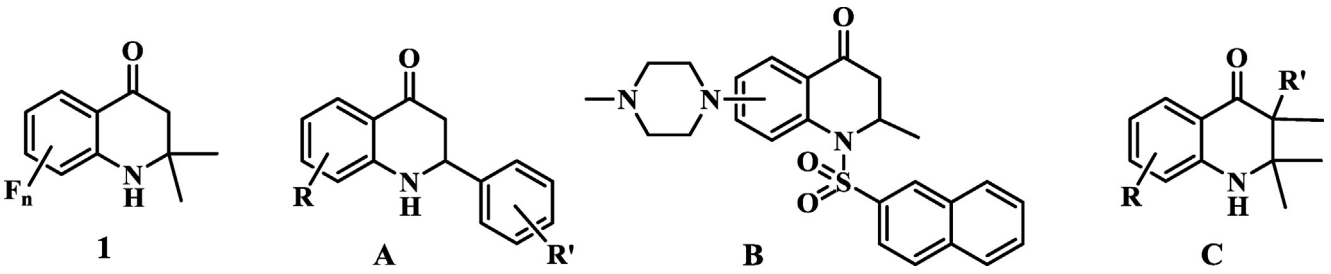


From the other side, aromatic fluorine is a unique modulator of the biological properties of organic compounds [8] that is directly associated with the specific electronic and structural features of a fluorine atom, such as a high electronegativity, relatively small size, a low polarizability of the C–F-bond and, in many cases, increased lipophilicity of fluorine containing organic molecules [9]. From synthetic point of view, the presence of several fluorine atoms in the benzene moiety of benzoheterocycles opens ample opportunities for a scaffold functionalization by the nucleophilic substitution of fluorine atoms, as was shown in [6] by the synthesis of piperazinyl derivatives of 1-(arylsulfonyl)-2,3-dihydro-1*H*-quinolin-4-ones (**B**).

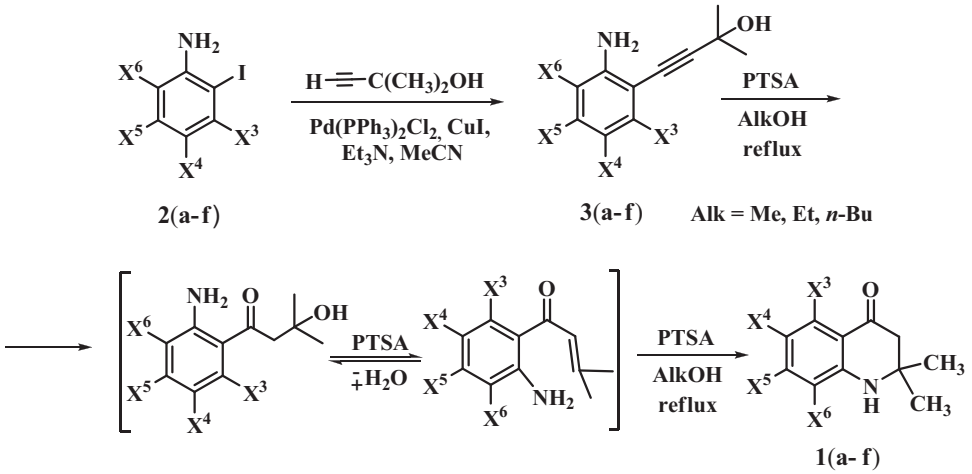
Herein, we wish to report a highly efficient synthesis of a broad range of fluorinated derivatives of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones, data about their cytotoxicity against cancer cells and fluorescent properties as well, depending on the number and the location of fluorine atoms in the benzene moiety.

2. Results and discussion

2.1. The synthesis of fluorinated 2,2-dimethyl-2,3-dihydro-1H-quinolin-4-ones

The synthesis is outlined in Scheme 1. On the first step, fluorinated *o*-iodoanilines **2**(**a**–**f**) were cross-coupled with 2-methyl-3-butyn-2-ol in MeCN in the presence of $Pd(PPh_3)_2Cl_2$ (4 mol %), CuI (9 mol%) and Et_3N. Then, the prepared acetylene derivatives **3**(**a**–**f**) were cyclized in boiling AlkOH (Alk = Me, Et, *n*-Bu) in the presence of PTSA as a catalyst to give 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**f**) with good and excellent yields calculated for the two-step procedure (Scheme 1, Table 1). The acid-mediated cyclization is generally accepted to proceed via regioselective hydrative–dehydrative rearrangement of the alkyne moiety through intermediate 1-(2-aminophenyl)-3-methylbut-2-en-1-ones [10,11].

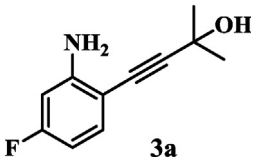
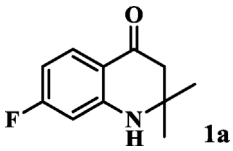
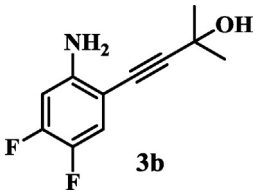
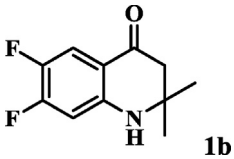
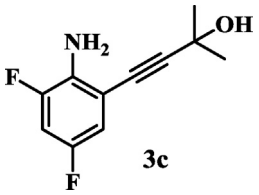
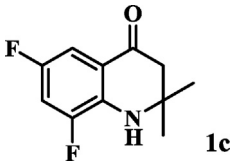
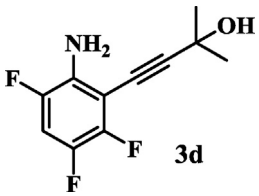
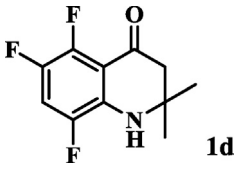
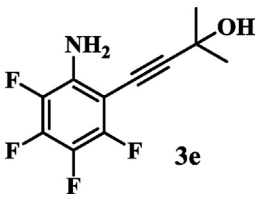
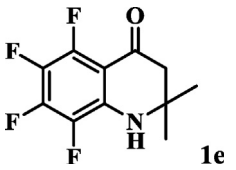
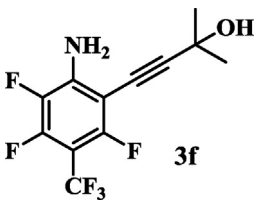
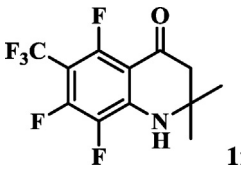
According to [13], aliphatic alcohols are preferable for using as solvents in the acid-catalyzed hydration of arylalkynes. In case of our fluoro-derivative to identify the optimal conditions, different alcohols were screened. We have revealed that boiling of ethanolic solution of **3c** in the presence of PTSA during 8 h resulted in the totally gumming of the reaction mixture, whereas using MeOH gave **1c** with acceptable yield (Table 1, entry 3). An attempt to prepare **1e** in boiling EtOH led mainly to earlier known [14] 2-substituted-4,5,6,7-tetrafluoroindole (~90% according to ^{19}F NMR and GC–MS analysis data) as result of acid-catalyzed intramolecular cyclization of the starting 2-alkynylaniline **3e** [15]. Using MeOH suppressed the backside process and gave **3e** with acceptable yields (Table 1, entry 5). As to **3f**, only using high-boiling *n*-BuOH allowed us to convert **3f** into the corresponding 2,3-dihydro-1*H*-quinolin-4-ones (Table 1, entry 6).



	X^3	X^4	X^5	X^6
a	H	H	F	H
b	H	F	F	H
c	H	F	H	F
d	F	F	H	F
e	F	F	F	F
f	F	CF_3	F	F

Scheme 1. Synthesis of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**f**).

Table 1
Synthesized 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**f**).

Entry	Substrate	PTSA (equiv)	Alk	Time, h	Product	Yield, %
1	 **3a**	1.5	Et	10	 **1a**	89
2	 **3b**	1	Et	8.5	 **1b**	70[a]
3	 **3c**	2	Me	15	 **1c**	38[a]
4	 **3d**	2	Et	17	 **1d**	43[a]
5	 **3e**	1	Me	40	 **1e**	57[a]
6	 **3f**	2	*n*-Bu	17.5	 **1f**	55[a]

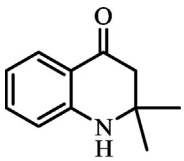
[a] Isolated yield of pure product based on **2** (Compounds **3**(**b**–**f**) were used without purification, their ^{1}H and ^{19}F NMR spectra correspond to those reported previously [12]).

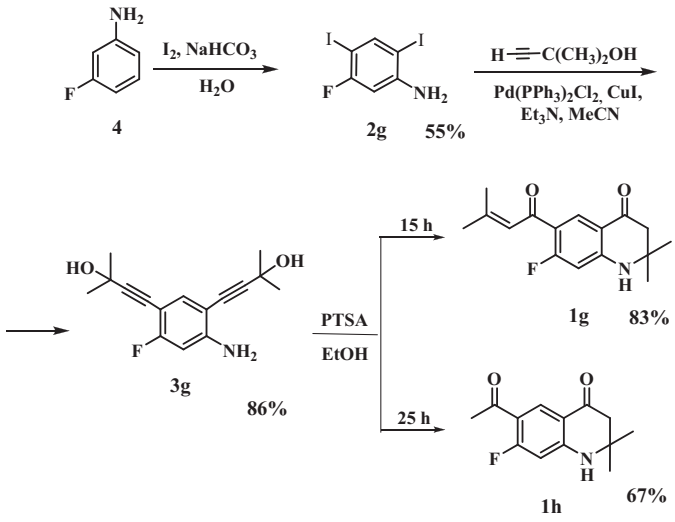
The special case was the hydration of **3g** having two acetylenic groups, which was prepared by iodination of 3-fluoroaniline **4** followed by the Sonogashira reaction of 5-fluoro-2,4-diiodoaniline **2g** with 2-methylbut-3-yn-2-ol (Scheme 2). The reaction of **3g** in the presence of 4 equivalents of PTSA in boiling EtOH for 15 h gave the substituted 2,3-dihydro-1*H*-quinolin-4-one **1g** in 83% yield. The increase of this reaction duration resulted in obtaining 6-acetyl-7-fluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1*H*)-one (**1h**) as a single product in 67% yield (Scheme 2).

The fact that the formation of 2,3-dihydroquinolinone nucleus in **1g** is accompanied by rearrangement of the tertiary acetylenic alcohol into the corresponding vinyl ketone proves the mechanism of heterocyclisation depicted on the Scheme 1. When the reaction time was prolonged, compound **3g** completely transformed into compound **1h**, probably due to the acid catalyzed retro-aldol cleavage (Scheme 3). The cleavage also proceeds on silica during TLC that leads to complete conversion of **1g** into compound **1h**.

*2.2. UV–vis, absorption and fluorescence spectra for 2,3-dihydroquinolinones **1**(**a**–**h**)*

In general 2,3-dihydro-1*H*-quinolin-4-one derivatives are of special interest in terms of their fluorescent properties [16]. The most of the fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**h**) show an intense green fluorescence in dichloromethane that is clearly visible by naked eye. The objective of this story was to determine whether the fluoro atoms introduced into the benzene ring can influence on photoluminescence of the 2,3-dihydroquinolinone. In this regard it was important to compare the photophysical properties of compounds **1**(**a**–**h**) with similar data for their non-fluorinated analog. For this purpose, we synthesized 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones (**5**) via the same procedure (Scheme 1) in 90% yield based on *o*-iodoaniline (see Section 4).


5



Scheme 2. Synthesis of 2,3-dihydroquinolinones **1**(**g**, **h**).

The UV–vis absorption spectra for the fluorinated derivative **1**(**a**–**h**) and **5** were measured in MeCN and EtOH. One can see that for the given compounds the line pattern and absorption maxima were almost similar in both solvents (Table 2, Figs. 1 and 2 and SI). All compounds are characterized by an intense band with a maximum at 220–236 nm and two less intense bands between 241 and 386 nm, whose positions varied only slightly and non-systematically. The UV–vis spectra of the 2,3-dihydroquinolinones **1g** and **1h** with the extended conjugated systems exhibit an additional intense absorption band with a maximum at 302–314 nm and an increased intensity of the second short-wavelength band as compared with the band for the compounds **1**(**a**–**g**) and **5**. The observed appearance of the additional intense absorption band is related with the light-induced intramolecular charge transfer from the amino-group to the benzene carbonyl substituent.

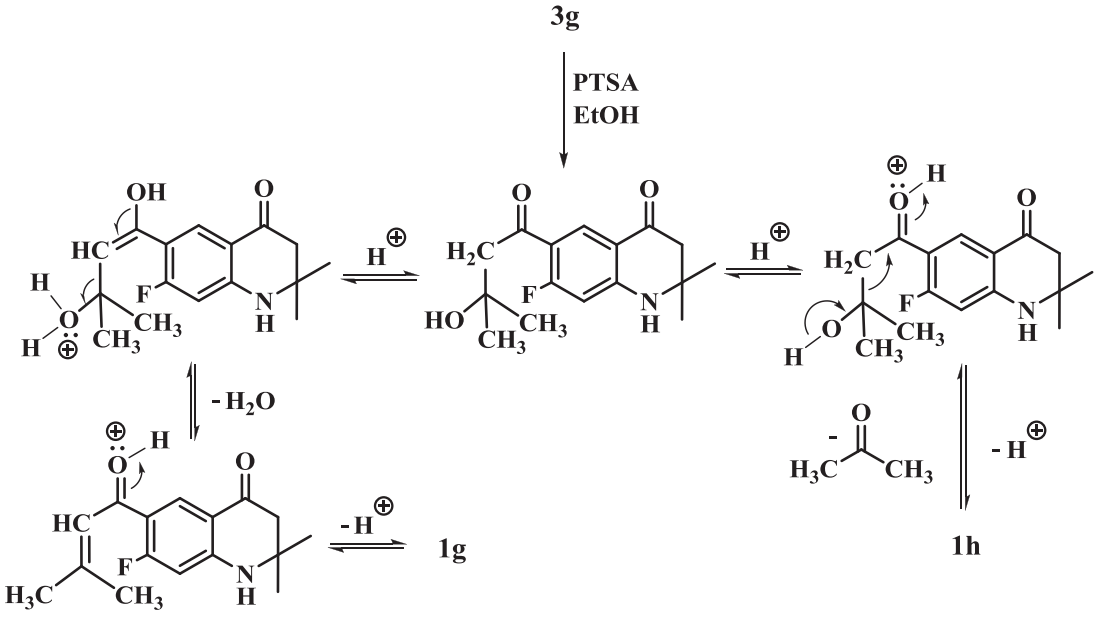
The fluorescence properties of **1**(**a**–**h**) and **5** were studied in MeCN and EtOH (Figs. 3 and 4 and SI). The absorption maxima, emission maxima and Stokes's shift of compounds **1**(**a**–**h**) and **5** are summarized in Table 3. It can be seen that, compared to acetonitrile solutions, alcohol solutions exhibit bathochromic shift (red shift) both in absorption and fluorescence maxima. Of particular notice is the Stokes shift in going from MeCN to EtOH, which is clearly indicative of higher stabilization of the photo-excited state of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**h**) compared to their ground electronic state.

Now it is reasonable to compare the fluorescence properties of fluorinated derivatives **1**(**a**–**h**) with that of non-fluorinated 2,2-dimethyl-2,3-dihydro-1H-quinolin-4-ones **5**. The replacement of the hydrogen atom in compounds **5** with a π-electron donor fluorine atom in position 7 (compounds **1a**) led to a blue shift of fluorescence spectra. At the same time, the introduction of two fluorine atoms at position 6 and 8 (compound **1c**) resulted in a red shift of the bands compared with that for the compound **5**. It is noteworthy that the fluorescence spectra of benzo-perfluorinated compound **1e** and unsubstituted 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **5** were similar. Therefore, the fluorination of the benzene ring in **5** has a relatively weak effect on the fluorescence properties that can be explained by un-co-operation of the substituents on the transfer of electron density from the nitrogen atom to the carbonyl group in the excited state of the molecule.

2.3. The cytotoxicity of 2,3-dihydroquinolinones *1*(*a*–*h*), *5*

The benzene-fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**h**) as well as non-fluorinated analog **5** were examined for their ability to inhibit the growth of three mammalian cell lines: tumor cell lines from human mammary adenocarcinoma (MCF-7), human hepatocellular carcinoma HepG2 epithelial tumor cells (HEP), human myeloma (RPMI 8226) as well as normal mouse fibroblasts (LMTK) and normal Chinese hamster Ag 17 cells (AG). Fig. 5 shows schematically the effect (relative activity, RA) of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**h**) and **5** at max concentration of 300 μM on the viability of all types of cells (%).

It can be seen, the non-fluorinated **5** did not suppress the growth of cells at all. Compounds **1**(**b**, **g**, **h**), containing substituents at the 6 and 7 positions of the aromatic ring and the hydrogen



Scheme 3. Proposed mechanism for the acid promoted formation of 2,3-dihydroquinolinones **1**(**g**, **h**).

Table 2
UV–vis absorption spectra for 1.0×10^{-4} mol/L solutions of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**h**) and **5** in MeCN and EtOH.[a]

Compd	$^1\lambda_{ex}$, nm (^{1}I, u.e.)		$^2\lambda_{ex}$, nm (^{2}I, u.e.)		$^3\lambda_{ex}$, nm (^{2}I, u.e.)		$^4\lambda_{ex}$, nm (^{3}I, u.e.)	
	MeCN	EtOH	MeCN	EtOH	MeCN	EtOH	MeCN	EtOH
5	233 (2.21)	236 (2.06)	258 (0.72)	260 (0.67)			372 (0.41)	383 (0.39)
1a	228 (2.19)	231 (2.01)	259 (0.75)	262 (0.75)			356 (0.41)	368 (0.42)
1b	229 (1.50)	233 (1.46)	258 (0.56)	260 (0.59)			369 (0.42)	381 (0.44)
1c	225 (2.02)	225 (2.16)	250 (0.54)	250 (0.56)			377 (0.39)	386 (0.41)
1d	222 (1.78)	220 (2.03)	251 (0.51)	254 (0.50)			374 (0.38)	384 (0.38)
1e	225 (2.44)	222 (3.19)	244 (0.59)	247 (0.94)			360 (0.38)	368 (0.61)
1f	230 (2.65)	233 (2.60)	241 (0.35)	263 (1.11)			341 (0.35)	347 (0.37)
1g	231 (0.97)	233 (1.30)	252 (2.21)	255 (1.66)	314 (1.18)	325 (2.02)	356 (0.38)	374 (0.60)
1h	~226 (0.63) (sh)	~230 (0.53) (sh)	244 (0.96)	245 (0.78)	302 (1.11)	308 (0.94)	352 (0.19)	355 (0.19)

[a] λ_{ex} – max excitation band wavelength, I–max absorption band intensity.

atoms at 5 and 8 positions, showed partially selective cytotoxic activity toward RPMI cells, which being more profound in the case of the compounds **1g**. Compound **1g** exhibited also the high cytotoxic activity toward HEP tumor cells. Benzene-perfluorinated derivatives of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1e** and **1f** demonstrated the best cytotoxicity in all tested cell lines. The results (IC_{50}) obtained for 2,3-dihydro-1*H*-quinolin-4-ones **1**(**e**, **f**) with all types of cells are summarized in Table 4. IC_{50} values for these compounds were comparable.

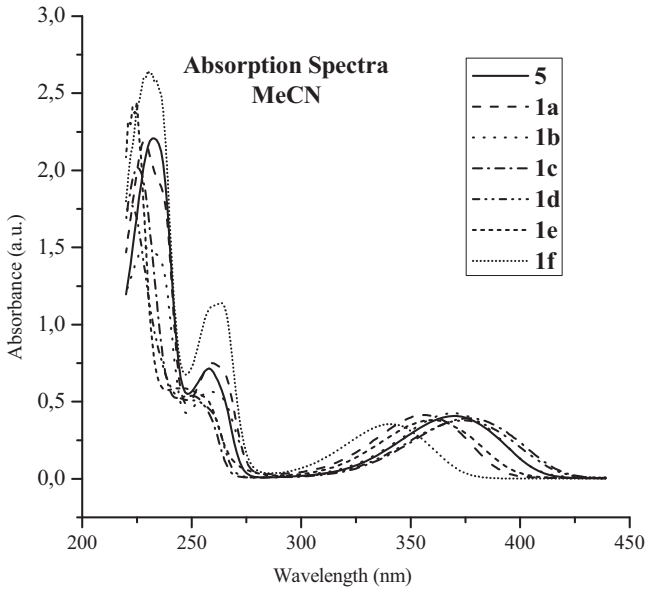


Fig. 1. UV–vis spectra for 1.0×10^{-4} mol/L solutions of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **5**, **1**(**a**–**f**) in MeCN.

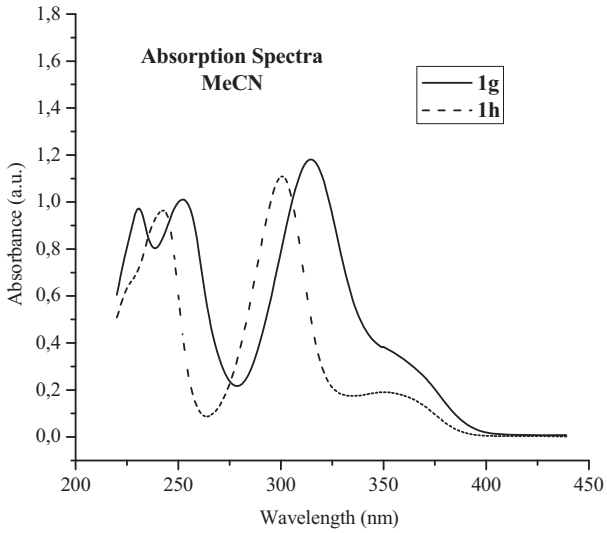


Fig. 2. UV–vis spectra for 1.0×10^{-4} mol/L solutions of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**g**, **h**) in MeCN.

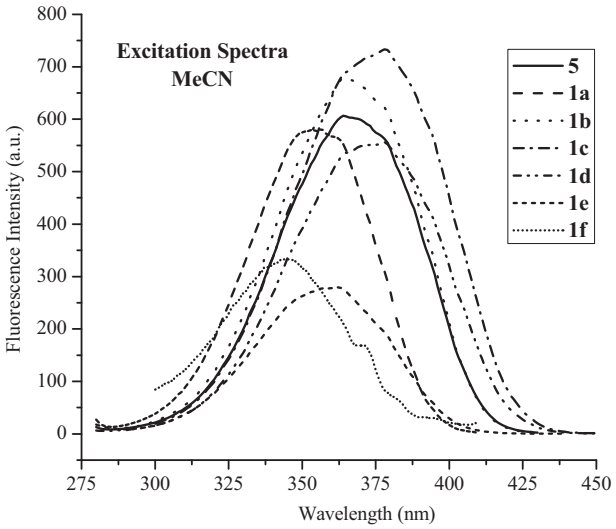


Fig. 3. Fluorescence excitation spectra of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **5** and **1**(**a**–**f**) in MeCN.

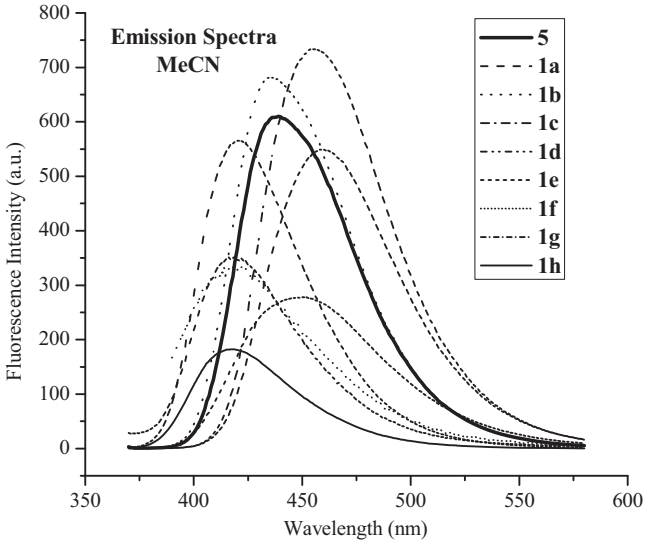


Fig. 4. Fluorescence emission spectra of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **5** and **1**(**a**–**h**) in MeCN.

Table 3
Solvent shift data for excitation and emission fluorescence spectra for 1.0×10^{-4} mol/L solutions of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **5** and **1**(**a**–**h**) in MeCN and EtOH.[a]

Compd	λ_{ex}, nm		λ_{em}, nm		Stokes shift, nm	
	MeCN	EtOH	MeCN	EtOH	MeCN	EtOH
5	364	379	439	483	75	104
1a	356	364	421	461	65	97
1b	364	379	435	472	71	93
1c	378	386	455	492	77	106
1d	378	380	459	499	81	119
1e	362	364	449	479	87	115
1f	345	347	419	453	74	106
1g	302, 352	311, 362 (sh)	422	459	70	97
1h	310, 350 (sh)	301, 348 (sh)	417	457	67	109

[a] λ_{ex} – position of the maximum of the long-wavelength excitation band, λ_{em} – position of the maxima of the emission band, Stokes shift ($\lambda_{em} - \lambda_{ex}$).

One can see that compounds **1e** and **1f** exhibited the highest cytotoxic activity toward human myeloma cell line, demonstrating the lowest IC_{50} values. Fig. 6 shows the change of the viability of RPMI cells (%) depending on the concentration (μM) of compounds **1e** and **1f**.

Antitumor drugs can be considered as useful when they are better suppressors of tumor than normal mammalian cells. Therefore, we further compared the effects of these compounds on three tumor cell lines and normal mouse fibroblasts LMTK and AG cells. Overall, the compounds **1e** and **1f** demonstrated various toxicity ratios in the suppression of tumor vs. normal cells. For the best inhibitor containing four fluorine atoms on the benzene fragment (5,6,7,8-tetrafluoro-2,2-dimethyl-2,3-dihydroquinolin-4-one **1e**), the average IC_{50} value toward tumor cell lines is approximately 2-fold lower than that toward normal cells. The best differences are observed in inhibition of human myeloma cell line (RPMI) and normal cells (LMTK, AG) (Fig. 7).

Table 4
Cytotoxicity (IC_{50}) of the benzo-perfluorinated derivatives of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**e**, **f**).

Compd	IC_{50} (μM) for different cell lines[a]				
	Tumor cells[b]			Control cells	
	MCF-7	HEP	RPMI	LMTK	AG
1e	59.5 ± 2.8	41.9 ± 8.1	17.5 ± 7.6	51.0 ± 8.2	95.0 ± 2.9
1f	60.9 ± 2.1	18.7 ± 9.4	13.0 ± 6.7	53.0 ± 3.9	73.0 ± 2.3

[a] Mean ± standard deviation from three independent experiments.
[b] MCF-7, human mammary adenocarcinoma; HEP, human hepatocellular carcinoma HepG2 epithelial tumor cells; RPMI, human myeloma; LMTK, normal mouse fibroblasts; AG, Chinese hamster Ag 17 cells.

2.4. Antioxidant properties of 2,3-dihydroquinolinones

It is known that some compounds interacting with many cell targets at the same time may be polyfunctional and possess cytoprotective properties, or, on the contrary, may be mutagenic or carcinogenic. Obviously, drugs are more successful when they are not mutagenic at least at the therapeutic concentrations. The *Salmonella typhimurium* TA102 strain is often used both for evaluation of mutagenicity of different compounds and for detection of antioxidant properties, as judged from suppression of spontaneous mutagenesis in this strain and from a decrease in mutagenicity of oxidants, usually H_2O_2 [17]. The mutagenic activity of several compounds was estimated in the Ames test [17] using *S. typhimurium* TA102 as reported by Kemeleva et al. [18]. The mutation induction in the Ames assay is estimated by calculating the frequency of reversion from histidine auxotrophy to prototrophy in response to the substance under testing [17,18].

Some antioxidant compounds are known to efficiently decrease the mutagenic effect of H_2O_2 [17,18]. In the Ames test, H_2O_2 was added to TA102 cells at the optimal concentration, 3 μM [17]. Fig. 8 shows the representative data for compounds **5** and **1e** in the suppression of spontaneous and the H_2O_2-dependent formation of mutants. At low concentrations compounds **5** and **1e** efficiently

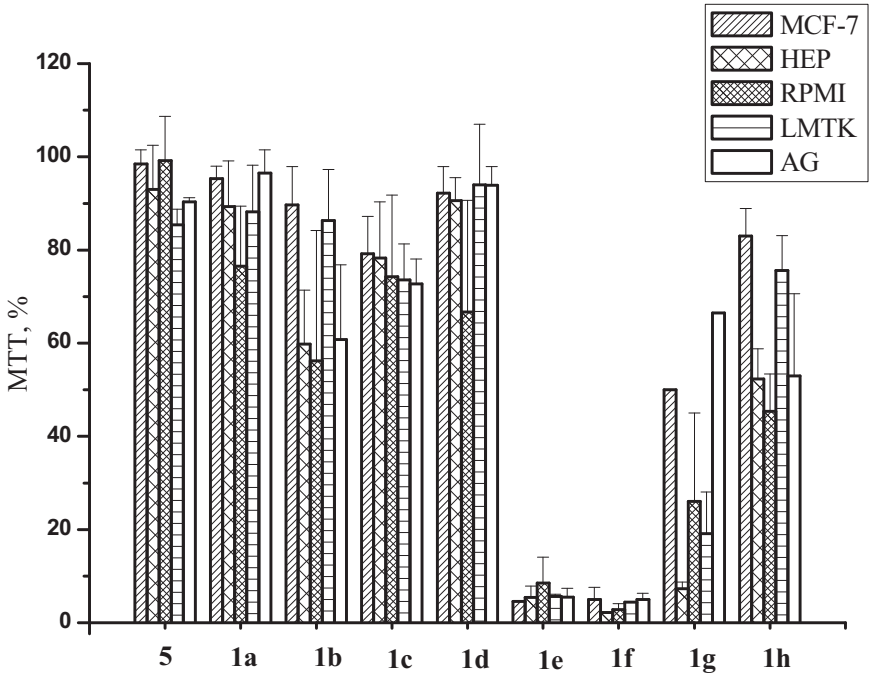


Fig. 5. Living cell count (%) by action of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **5**, **1**(**a**–**h**) in the concentration of 300 μM.

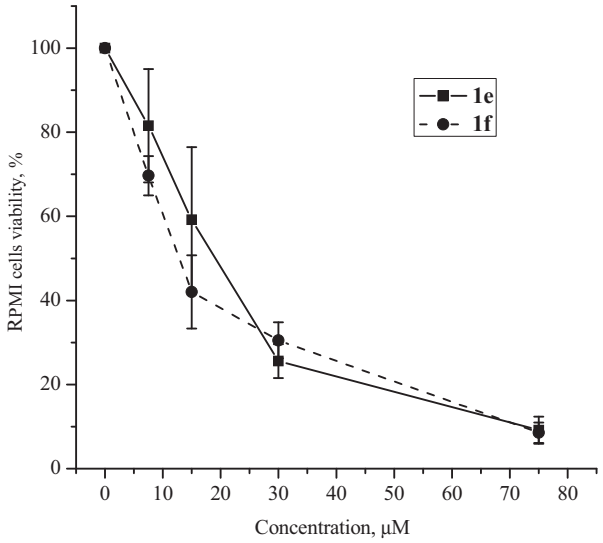


Fig. 6. The effect of the benzo-perfluorinated derivatives of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**e**,**f**) on the growth of RPMI cells (%).

suppressed the H_2O_2-dependent formation of mutants from 130 to 100% and at higher concentration from 100 to 50% of revertants (the number of revertants observed in controls without H_2O_2 was taken for 100%). The data for six analyzed compounds are summarized in Table 5.

Minimal effect of spontaneous mutagenesis suppression was observed for non-fluorinated compound **5** (IC_{50} = 1.40 μM) and this effect was increased for fluorinated ones in the following order: **1d** (1.10 μM) ≤ **1b** (1.00 μM) ≈ **1c** (1.00 μM) < **1 h** (0.60 μM) < **1e** (0.23 μM) (Table 5). The Compound **5** (IC_{50} = 1.40 μM) was also the worst suppressor of H_2O_2-induced mutagenesis from 130 to 100%, while other compound inhibited effect of H_2O_2 in an order: **1c** (0.55 μM) < **1b** (0.37 μM) < **1d** (0.21 μM) < **1e** (0.16 μM) < **1 h** (0.06 μM). The suppression of H_2O_2-induced mutagenesis from 100 to 50% was observed at higher concentrations and the effect was increased in the following order: **5** (1.80 μM) < **1c** (1.40 μM) ≤ **1b** (1.30 μM) ≈ **1d** (1.10 μM) < **1e** (0.97 μM) < **1 h** (0.81 μM) (Table 5). These data indicate that all analyzed compounds are not mutagenic themselves and efficiently decrease the level of spontaneous mutagenesis as well as the mutagenic effect of H_2O_2.

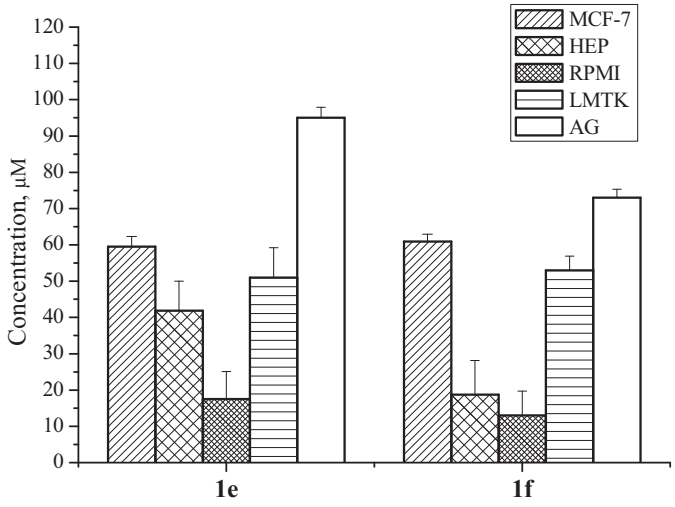


Fig. 7. IC_{50} values (μM) of the benzo-perfluorinated derivatives of 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**e**, **f**).

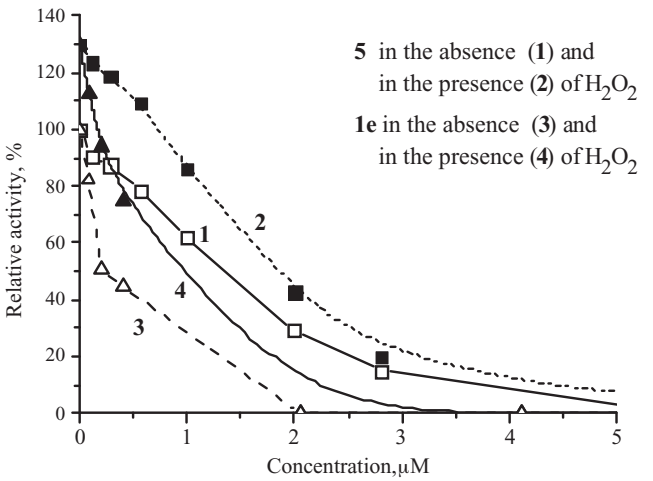


Fig. 8. Analysis of the mutagenic and antioxidant activity of compounds **5** (curves 1 and 2) and **1e** (curves 3 and 4) by a standard Ames test using the *S. typhimurium* strain TA102 in the absence (curves 1 and 3) and in the presence (curves 2 and 4) of 3 μM H_2O_2. The number of revertants in the absence of H_2O_2 was taken for 100%. The average error in three experiments for any compound concentration did not exceed 7–12%.

Table 5
IC_{50} values characterizing suppression of spontaneous and H_2O_2-induced mutagenesis by derivatives of benzene-fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones.

Compd	IC_{50}, μM[a]		
	Suppression of spontaneous mutagenesis (from 100 to 50%)	Suppression of H_2O_2-induced and spontaneous mutagenesis	
		From 130 to 100%	From 100 to 50%
Control 5	1.40 ± 0.07[a]	0.73 ± 0.05	1.80 ± 0.08
1b	1.00 ± 0.08	0.37 ± 0.03	1.30 ± 0.08
1c	1.00 ± 0.07	0.55 ± 0.04	1.40 ± 0.07
1d	1.10 ± 0.07	0.21 ± 0.02	1.10 ± 0.07
1e	0.23 ± 0.02	0.16 ± 0.01	0.97 ± 0.07
1 h	0.60 ± 0.04	0.06 ± 0.0004	0.81 ± 0.06

[a] Mean ± standard deviation from three independent experiments.

Interestingly, when non-fluorinated **5** is a relatively effective antioxidant it is not suppressing the growth of any tumor cells at all (Fig. 5). Compound **1e**, which is one of the best suppressors of all tumor cells, efficiently inhibits spontaneous and H_2O_2-induced mutagenesis. At the same time, **1h** also demonstrating high activity in the suppression spontaneous and H_2O_2-induced mutagenesis, but it possesses relatively low and selective effect on different tumor cells (Fig. 5). It is possible that all new compounds can play a double role, acting both as inhibitors of cell growth and as antioxidants and these functions probably only partially overlap.

3. Conclusion

In summary, we have developed the two-step procedure for preparation of fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones including the Sonogashira cross-coupling of fluorinated *ortho*-iodanilines with 2-methylbut-3-yn-2-ol and the following PTSA-catalyzed hydration-cyclization of prepared *ortho*-alkynylanilines. A wide range of the fluoro-substituted *ortho*-iodanilines effectively participated in the reactions to give access to a variety of unknown fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones. The photophysical data indicated that fluorinated 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones are the intramolecular charge transfer fluorescent compounds and can have potential

application as novel electroluminescent materials. It has been revealed that the polyfluorinated compounds **1**(**e**, **f**) inhibit the growth of three tumor mammalian cell lines, the average IC_{50} value toward tumor cell lines being approximately 2-fold lower than that toward normal cells. In addition all fluorinated compounds efficiently suppress spontaneous and H_2O_2-induced mutagenesis of bacterial cells.

4. Experimental

4.1. General methods

All the cross-coupling reactions were carried out in oven-dried glassware under an argon atmosphere. All solvents were purified using the standard procedures and dried before use. Et_3N, MeCN were distilled and kept over CaH_2 before to use. 2-Iodo-4,5-difluoroaniline (**2b**) [19], 2-iodo-4,6-difluoroaniline (**2c**) [12], 2-iodo-3,4,6-trifluoroaniline (**2d**) [12], 2-iodo-3,4,5,6-tetrafluoroaniline (**2e**) [12], 2-iodo-3,5,6-trifluoro-4-(trifluoromethyl)aniline (**2f**) [12], $Pd(PPh_3)_2Cl_2$ [20] were prepared according to known procedures. Other starting materials were obtained from commercial supplies and used without purification.

Silica gel (100–300 mesh) was used for column chromatography and analytical thin-layer chromatography was performed on Merck precoated silica gel 60 PF_{254} containing gypsum or on Sorbfil plates (UV 254). The visualization of the developed chromatograms was performed by UV light. To obtain analytically pure samples, the synthesized compounds were crystallized from hexane or sublimed at 100–150 °C under vacuum (~15 Torr).

The NMR spectra were recorded on a Bruker Avance-300 (300.13 MHz for ^1H and 282.37 MHz for ^{19}F), Avance-400 (400.13 MHz for ^1H and 100.62 MHz for ^{13}C) and DRX-500 (125.76 MHz for ^{13}C) spectrometers. Deuterochloroform ($CDCl_3$) was used as the solvent, with residual $CHCl_3$ (δ_H = 7.26 ppm) or $CDCl_3$ (δ_C = 77.0 ppm) being employed as an internal standard. The ^{13}C NMR spectra were registered with C–H spin decoupling. The masses of molecular ions were determined by HRMS on a DFS Thermo scientific instrument (EI, 70 eV). The melting points were registered on a Mettler-Toledo FP81 Thermosystem apparatus. The IR spectra were recorded on a Bruker Vector 22 spectrometer (KBr). The elemental analyses were performed on a Euro EA-3000 CHNS analyzer, or on Carlo Erba 1106 CHN elemental analyzer. The UV–vis absorption spectra were taken on a spectrophotometer Hewlett Packard 4853. The fluorescence spectra were recorded on a Cary Eclipse Varian spectrofluorimeter equipped with a pulse xenon lamp and a scheme of the luminescence registration at an angle of 90° with the excitation and emission slits of 5 nm, quartz cells 10 mm thick, at room temperature.

4.1.1. 5-Ffluoro-2-iodoaniline (2a), 5-fluoro-2,4-diiodoaniline (2g) and 3-fluoro-4-iodoaniline(6)

$NaHCO_3$ (1.4 g, 13 mmol) and fine-ground I_2 (2.0 g, 8 mmol) were added to a stirred emulsion of 3-fluoroaniline (**4**) (1.0 g, 9 mmol) in H_2O (50 mL) at room temperature. The reaction mixture was maintained for 0.5 h with stirring. After the mixture was extracted with CH_2Cl_2 (3× 50 mL), the combined organic layers were washed with sat. aq. solutions of $Na_2S_2O_3$ (2× 30 mL), H_2O (50 mL) and dried ($MgSO_4$). After the evaporation of the solvent in vacuo, the crude products were separated by column chromatography (hexane/ethyl acetate, 10:1). The first fraction was evaporated to give 0.085 g (4%) of **2a** (R_f 0.71, hexane/ethyl acetate, 5:1) as an oil material. IR (liquid film): ν 3470, 3375, 3204, 3078, 2926, 1614, 1574, 1481, 1429, 1283, 1253, 1173, 1119, 1013, 970, 839, 783, 573; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.52 (dd, 1H, $J_H3,_H4$ = 8.6; $J_H3,_F$ = 6.2 Hz, H^3), 6.45 (dd, 1H, $J_H6,_F$ = 10.5; $J_H6,_H4$ = 2.8 Hz, H^6), 6.24 (ddd, 1H, $J_H4,_H3$ = 8.6; $J_H4,_F$ = 8.2; $J_H4,_H6$ = 2.8 Hz, H^4), 4.17 (br s, 2H, NH_2); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 164.0 (d, $J_C5,_F$ = 244.7 Hz, C^5), 148.1 (d, $J_C1,_F$ = 11.0 Hz, C^1), 139.7 (d, $J_C3,_F$ = 9.5 Hz, C^3), 107.2 (d, $J_C4,_F$ = 22.6 Hz, C^4), 101.5 (d, $J_C6,_F$ = 25.6 Hz, C^6), 77.0 (d, $J_C2,_F$ = 2.5 Hz, C^2); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −114.7 (ddd, 1F, $J_F,_H6$ = 10.5; $J_F,_H4$ = 8.2; $J_F,_H3$ = 6.2 Hz, F^5); HRMS (EI) calcd. for C_6H_5IFN (M^+) 236.9445, found 236.9452.

From the second fraction 0.20 g (6%) of **2g** (R_f 0.55, hexane/ethyl acetate, 5:1) was isolated, colorless crystals, m.p. 94.6 °C with decomposition from hexane. IR (KBr): ν 3445, 3354, 2926, 2853, 1607, 1557, 1460, 1396, 1294, 1267, 1248, 1175, 1032, 872, 831, 737, 621, 575, 444 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.87 (d, 1H, $J_H3,_F$ = 6.8 Hz, H^3), 6.48 (d, 1H, $J_H6,_F$ = 9.5 Hz, H^6), 4.23 (br s, 2H, NH_2); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 162.4 (d, $J_C5,_F$ = 244.0 Hz, C^5), 148.2 (d, $J_C1,_F$ = 10.2 Hz, C^1), 146.5 (d, $J_C3,_F$ = 3.0 Hz, C^3), 101.2 (d, $J_C6,_F$ = 28.2 Hz, C^6), 77.9 (d, $J_C2,_F$ = 2.8 Hz, C^2), 66.1 (d, $J_C4,_F$ = 27.0 Hz, C^4); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −96.2 (dd, 1F, $J_F,_H6$ = 9.5; $J_F,_H3$ = 6.8 Hz, F^5); Anal. calcd. for $C_6H_4I_2FN$: C, 19.86; H, 1.11; N 3.86. Found: C, 19.90; H, 1.25; N, 3.86; HRMS (EI) calcd. for $C_6H_4I_2FN$ (M^+) 362.8412, found 362.9441. (Compound **2g** was also prepared in 55% isolated yield (1.8 g) via the same procedure by action on **4** (1.0 g, 9 mmol) in H_2O (50 mL) of fine-ground I_2 (5.1 g, 20 mmol) and $NaHCO_3$ (3.3 g, 32 mmol) at room temperature for 1 h.)

From the third fraction 1.4 g (74%) of **6** (R_f 0.23, hexane/ethyl acetate, 5:1) was isolated, colorless solid material, m.p. 67.3–67.7 °C (67.0–68.5 °C [21]) from hexane. IR (KBr): ν 3427, 3319, 3209, 2955, 2855, 1634, 1597, 1578, 1483, 1439, 1313, 1240, 1173, 1142, 1069, 1022, 959, 845, 797, 741, 588, 548, 444 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.38 (dd, 1H, $J_H3,_H2$ = 8.5; $J_H3,_F$ = 7.2 Hz, H^3), 6.40 (dd, 1H, $J_H6,_F$ = 9.9; $J_H6,_H2$ = 2.6 Hz, H^6), 6.25 (dd, 1H, $J_H2,_H3$ = 8.5; $J_H2,_H6$ = 2.6 Hz, H^2), 3.80 (br s, 2H, NH_2); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 162.2 (d, $J_C5,_F$ = 242.8 Hz, C^5), 148.5 (d, $J_C1,_F$ = 10.1 Hz, C^1), 139.0 (d, $J_C3,_F$ = 3.4 Hz, C^3), 112.8 (d, $J_C2,_F$ = 2.5 Hz, C^2), 102.4 (d, $J_C6,_F$ = 27.1 Hz, C^6), 65.5 (d, $J_C4,_F$ = 25.9 Hz, C^4); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −95.5 (dd, 1F, $J_F,_H6$ = 9.9; $J_F,_H3$ = 7.2 Hz, F^5); Anal. calcd. for C_6H_5IFN: C, 5.91; H, 2.13; N 5.91. Found: C, 30.39; H, 2.25; N, 5.90. HRMS (EI) calcd. for C_6H_5IFN (M^+) 236.9445, found 236.9451.

4.1.2. 4-(2-Amino-4-fluorophenyl)-2-methylbut-3-yn-2-ol (3a)

$Pd(PPh_3)_2Cl_2$ (28 mg, 0.04 mmol), CuI (17 mg, 0.09 mmol) and Et_3N (3 mL) were added to a stirred solution of iodoaniline **2a** (237 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (168 mg, 2 mmol) in dry MeCN (10 mL) at room temperature under an argon atmosphere. The mixture was heated at 50 °C for 1.5 h with stirring. The reaction mixture was allowed to cool down to room temperature, and CH_2Cl_2 (10 mL) was added. The mixture was poured into H_2O (20 mL) and extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were washed with H_2O (20 mL) and dried ($MgSO_4$). After the evaporation of the solvent in vacuo, the crude product **3a** was purified by TLC (Merck precoated plates, R_f 0.29, hexane/ethyl acetate, 5:1, three times) to afford the title compound (133 mg, 69%) as an oil. IR (liquid film): ν 3360, 2982, 2934, 2870, 2222, 1622, 1589, 1503, 1443, 1364, 1281, 1254, 1207, 1167, 978, 961, 899, 843, 793, 554, 513, 495 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$): δ = 7.15 (m*, 1H, $J_H3,_H4$ = 8.6; $J_H3,_F$ = 6.3; $J_H3,_H6$ = 0.3 Hz, H^3), 6.34 (m*, 1H, $J_H6,_F$ = 10.6; $J_H6,_H4$ = 2.5 Hz, H^6), 6.32 (m*, 1H, $J_H4,_H3$ = 8.6; $J_H4,_F$ = 8.5; $J_H4,_H6$ = 2.5 Hz, H^4), 4.27 (br s, 2H, NH_2), 2.20 (br s, 1H, $C(CH_3)_2O\underline{H}$), 1.61 (s, 6H, $C(C\underline{H}_3)_2OH$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 163.5 (d, $J_C5,_F$ = 247.0 Hz, C^5), 149.2 (d, $J_C1,_F$ = 11.7 Hz, C^1), 133.5 (d, $J_C3,_F$ = 10.4 Hz, C^3), 103.1 (d, $J_C2,_F$ = 2.5 Hz, C^2), 104.9 (d, $J_C4,_F$ = 22.6 Hz, C^4), 100.8 (d, $J_C6,_F$ = 25.4 Hz, C^6), 99.8 (s, C|$\underline{C}$–C(CH_3)$_2$OH), 77.6 (s, $\underline{C}$|C–C(CH_3)$_2$OH), 65.6 (s, C|C–$\underline{C}$(CH_3)$_2$OH),

* XSIM.LINUX software (version 93.02.01) was used to modeling of these signals to determine the coupling constants.

31.4 (s, C|C–C(CH$_3$)$_2$OH); ^{19}F NMR (282.37 MHz, CDCl$_3$): δ = −108.4 (m*, 1F, $J_{F,H}6$ = 10.6; $J_{F,H}4$ = 8.5; $J_{F,H}3$ = 6.3 Hz, F^5); HRMS (EI) calcd. for $C_{11}H_{12}FNO$ (M^+) 193.0897, found 193.0903.

4.1.3. 4,4′-(4-Amino-6-fluoro-1,3-phenylene)bis(2-methylbut-3-yn-2-ol) (**3g**)

Pd(PPh$_3$)$_2$Cl$_2$ (77 mg, 0.11 mmol), CuI (47 mg, 0.25 mmol) and Et$_3$N (3 mL) were added to a stirred solution of 2,4-diiodoaniline **2g** (500 mg, 1.4 mmol) and 2-methylbut-3-yn-2-ol (350 mg, 4.1 mmol) in dry MeCN (10 mL) at room temperature under an argon atmosphere. The mixture was heated at 70 °C for 3 h with stirring. The reaction mixture was allowed to cool down to room temperature, and CH_2Cl_2 (10 mL) was added. The mixture was poured into H_2O (20 mL) and extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were washed with H_2O (20 mL) and dried ($MgSO_4$). After the evaporation of the solvent in vacuo, the crude product **3g** was purified by column chromatography (hexane/EtOH, 1:1). The fractions containing **3g** (R_f 0.35, hexane/ethyl acetate, 1:1) were combined and evaporated to give the title compound as yellow viscous oil, yield 331 mg (86%). IR (liquid film): ν 3356, 2982, 2934, 2872, 2224, 1705, 1626, 1570, 1506, 1429, 1364, 1308, 1261, 1238, 1161, 1119, 961, 910, 843, 735, 554, 449 cm^{-1}; ^{1}H NMR (400.13 MHz, CDCl$_3$): δ = 7.19 (d, 1H, $J_H3,_F$ = 7.8 Hz, H^3), 6.31 (d, 1H, $J_H6,_F$ = 11.0 Hz, H^6), 4.56 (br s, 2H, NH$_2$), 3.32 (br s, 1H, C(CH$_3$)$_2$OH), 3.08 (br s, 1H, C(CH$_3$)$_2$OH), 1.56 (s, 6H, C(CH$_3$)$_2$OH), 1.54 (s, 6H, C(CH$_3$)$_2$OH); ^{13}C NMR (100.62 MHz, CDCl$_3$): δ = 163.3 (d, $J_C5,_F$ = 251.3 Hz, C^5), 149.6 (d, $J_C1,_F$ = 11.4 Hz, C^1), 137.1 (s, C^3), 103.7 (s, C^2), 100.9 (d, $J_C6,_F$ = 25.4 Hz, C^6), 100.3 (d, $J_C4,_F$ = 17.1 Hz, C^4), 99.3 (s, C|C–C(CH$_3$)$_2$OH), 96.7 (s, C|C–C(CH$_3$)$_2$OH), 77.0 (s, C|C–C(CH$_3$)$_2$OH), 75.4 (s, C|C–C(CH$_3$)$_2$OH), 65.7 (s, C|C–C(CH$_3$)$_2$OH), 31.6 (s, C|C–C(CH$_3$)$_2$OH), 31.4 (s, C|C–C(CH$_3$)$_2$OH); ^{19}F NMR (282.37 MHz, CDCl$_3$): δ = −105.9 (dd, 1F, $J_{F,H}^6$ = 11.0; $J_{F,H}^3$ = 7.8 Hz, F^5); HRMS (EI) calcd. for $C_{16}H_{18}FNO_2$ (M^+) 275.1316, found 275.1321; Anal. calcd. for $C_{16}H_{18}FNO_2$: C, 69.80; H, 6.59; N 5.09. Found: C, 70.18; H, 6.41; N, 5.12.

4.1.4. 7-Fluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (**1a**)

PTSA (142 mg, 0.7 mmol) was added to the solution of **3a** (73 mg, 0.5 mmol) in 10 mL EtOH. The mixture was heated under reflux with stirring for 10 h, cooled to room temperature, diluted with CH_2Cl_2 (10 mL), poured into H_2O (20 mL) and extracted with CH_2Cl_2 (2× 50 mL). The combined organic layers were washed with H_2O (20 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo to give the crude product, which was purified by TLC (Merck precoated plates, R_f 0.21, hexane/ethyl acetate, 10:1) to afford the title compound **1a** (86 mg, 89%) as a yellow solid, m.p. 135.0–135.5 °C from hexane. IR (KBr): ν 3426, 3317, 3069, 2965, 2932, 1657, 1626, 1585, 1522, 1464, 1366, 1310, 1273, 1231, 1161, 1101, 1003, 851, 791, 702, 646, 563, 474 cm^{-1}; ^{1}H NMR (300.13 MHz, CDCl$_3$): δ = 7.80 (dd, 1H, $J_H5,_H6$ = 8.8; $J_H5,_F7$ = 6.6 Hz, H^5), 6.37 (ddd, 1H, $J_H6,_H5$ = 8.8; $J_H6,_F7$ = 8.4; $J_H6,_H8$ = 2.3 Hz, H^6), 6.24 (dd, 1H, $J_H8,_F7$ = 10.5; $J_H8,_H6$ = 2.3 Hz, H^8), 4.24 (br s, 1H, NH), 2.55 (s, 2H, CH$_2$), 1.30 (s, 6H, CH$_3$); ^{13}C NMR (100.62 MHz, CDCl$_3$): δ = 192.4 (s, COCH$_2$), 167.5 (d, $J_C7,_F7$ = 253.2 Hz, C^7), 151.5 (d, $J_C8a,_F7$ = 12.5 Hz, C^{8a}), 130.1 (d, $J_C5,_F7$ = 11.8 Hz, C^5), 114.8 (s, C^{4a}), 105.7 (d, $J_C6,_F7$ = 23.1 Hz, C^6), 101.1 (d, $J_C8,_F7$ = 24.8 Hz, C^8), 53.7 (s, C(CH$_3$)$_2$), 50.2 (s, CH$_2$), 27.6 (s, CH$_3$); ^{19}F NMR (282.37 MHz, CDCl$_3$): δ = −101.5 (ddd, 1F, $J_F7,_H8$ = 10.5; $J_F7,_H6$ = 8.4; $J_F7,_H5$ = 6.6 Hz, F^7); HRMS (EI) calcd. for $C_{11}H_{12}FON$ (M^+): 193.0897, found 193.0898. Anal. calcd. for $C_{11}H_{12}FON$: C, 68.38; H, 6.26; N 7.25. Found: C, 68.52; H, 6.20; N, 7.28.

4.1.5. 7-Fluoro-2,2-dimethyl-6-(3-methylbut-2-enoyl)-2,3-dihydroquinolin-4(1H)-one (**1g**)

PTSA (76 mg, 0.4 mmol) was added to the solution of **3g** (27 mg, 0.1 mmol) in 10 mL EtOH and the mixture was heated under reflux with stirring for 15 h. The mixture was cooled to room temperature, diluted with CH_2Cl_2 (10 mL), poured into H_2O (20 mL) and extracted with CH_2Cl_2 (2× 50 mL). The combined organic layers were washed with H_2O (20 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo. The crude product **1g** was purified by crystallization from hexane to afford the title compound (22 mg, 83%) as a colorless solid, m.p. 148.7–150.5 °C from hexane. IR (KBr): ν 3449, 3277, 3146, 2968, 2939, 1688, 1649, 1616, 1572, 1517, 1429, 1360, 1321, 1236, 1171, 1151, 1043, 1020, 847, 708, 646, 617, 548, 440 cm^{-1}; ^{1}H NMR (400.13 MHz, CDCl$_3$): δ = 8.31 (d, 1H, $J_H5,_F7$ = 8.5 Hz, H^5), 6.53 (m, 1H, COCH = C(CH$_3$)$_2$), 6.25 (d, 1H, $J_H8,_F7$ = 12.5 Hz, H^8), 4.68 (br s, 1H, NH), 2.58 (s, 2H, CH$_2$), 2.16 (d, 3H, $J_{H,H} \approx$ 1.0 Hz, COCH = C(CH$_3$)$_2$), 1.95 (d, 3H, $J_{H,H} \approx$ 1.0 Hz, COCH = C(CH$_3$)$_2$), 1.33 (s, 6H, CH$_3$); ^{13}C NMR (400.13 MHz, CDCl$_3$): δ = 191.7 (s, COCH$_2$), 187.4 (d, $J_C,_F$ = 3.2 Hz, COCH = C(CH$_3$)$_2$), 165.9 (d, $J_C7,_F7$ = 262.0 Hz, C^7), 156.0 (s, COCH = C(CH$_3$)$_2$), 153.1 (d, $J_C8a,_F7$ = 13.5 Hz, C^{8a}), 132.5 (d, $J_C5,_F7$ = 5.9 Hz, C^5), 123.7 (d, $J_C,_F$ = 4.7 Hz, COCH = C(CH$_3$)$_2$), 119.0 (d, $J_C6,_F7$ = 13.4 Hz, C^6), 114.1 (s, C^{4a}), 101.5 (d, $J_C8,_F7$ = 27.0 Hz, C^8), 53.8 (s, C(CH$_3$)$_2$), 50.0 (s, CH$_2$), 27.8 (s, COCH = C(CH$_3$)$_2$), 27.6 (s, CH$_3$), 21.1 (s, COCH = C(CH$_3$)$_2$); ^{19}F NMR (282.37 MHz, CDCl$_3$): δ = −103.8 (ddd, 1F, $J_F7,_H8$ = 12.5; $J_F7,_H5$ = 8.5; $J_F7,_H$ = 3.0 Hz, F^7); HRMS (EI) calcd. for $C_{16}H_{17}FNO_2$ ([M–H$^+$]$^-$): 274.1238, found 274.1236. Anal. calcd. for $C_{16}H_{18}FNO_2$: C, 69.80; H, 6.59; N 5.09. Found: C, 69.40; H, 6.57; N, 5.33.

4.2. 6-Acetyl-7-fluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (**1h**)

PTSA (608 mg, 3.2 mmol) was added to the solution of **3g** (220 mg, 0.8 mmol) in 15 mL EtOH. The mixture was heated under reflux with stirring for 25 h, cooled to room temperature, diluted with CH_2Cl_2 (10 mL), poured into H_2O (30 mL) and extracted with CH_2Cl_2 (2× 50 mL). The combined organic layers were washed with H_2O (50 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo to give the crude product, which was purified by TLC (Sorbfil plates, R_f 0.31, hexane/ethyl acetate, 2:1, six times) to afford the title compound (126 mg, 67%) as a colorless solid, m.p. 182.7–183.2 °C from hexane. IR (KBr): ν 3344, 2976, 1680, 1603, 1516, 1418, 1362, 1319, 1294, 1256, 1223, 1171, 1151, 982, 939, 847, 577, 546, 438 cm^{-1}; ^{1}H NMR (400.13 MHz, CDCl$_3$): δ = 8.42 (d, 1H, $J_H5,_F7$ = 8.6 Hz, H^5), 6.26 (d, 1H, $J_H8,_F7$ = 12.8 Hz, H^8), 4.83 (br s, 1H, NH), 2.59 (s, 2H, CH$_2$), 2.52 (d, 3H, $J_{H,F}7$ = 4.3 Hz, COCH$_3$), 1.33 (s, 6H, CH$_3$); ^{13}C NMR (100.62 MHz, CDCl$_3$): δ = 193.8 (d, $J_C,_F$ = 3.4 Hz, COCH$_3$), 191.7 (s, CO), 166.7 (d, $J_C7,_F7$ = 262.0 Hz, C^7), 153.8 (d, $J_C8a,_F7$ = 13.7 Hz, C^{8a}), 132.9 (d, $J_C5,_F7$ = 5.8 Hz, C^5), 116.6 (d, $J_C6,_F7$ = 13.9 Hz, C^6), 114.4 (s, C^{4a}), 101.4 (d, $J_C8,_F7$ = 27.4 Hz, C^8), 53.9 (s, C(CH$_3$)$_2$), 50.1 (s, CH$_2$), 30.5 (d, $J_C,_F$ = 6.2 Hz, COCH$_3$), 27.8 (s, CH$_3$); ^{19}F NMR (282.37 MHz, CDCl$_3$): δ = −102.6 (m, 1F, $J_F7,_H8$ = 12.8; $J_F7,_H5$ = 8.6; $J_F7,_H$ = 4.3 Hz, F^7); HRMS (EI) calcd. for $C_{13}H_{14}FNO_2$ (M^+): 235.1003, found 235.1007. Anal. calcd. for $C_{13}H_{14}FNO_2$: C, 66.37; H, 6.00; N 5.95. Found: C, 66.28; H, 5.89; N, 5.94.

4.3. General procedure for synthesis of 2,2-dimethyl-2,3-dihydro-1H-quinolin-4-ones 1(b–f), 5

Pd(PPh$_3$)$_2$Cl$_2$ (28 mg, 0.04 mmol), CuI (17 mg, 0.09 mmol) and Et$_3$N (3 mL) were added to a stirred solution of iodoaniline **2**(**b**–**f**) or 2-iodoaniline (**7**) (1 mmol), 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol) in dry MeCN (10 mL) at room temperature under an argon atmosphere. The mixture was heated at 50 °C for 2 h with stirring. The reaction mixture was allowed to cool down to room temperature, and CH_2Cl_2 (10 mL) was added. The mixture was poured into H_2O (40 mL) and extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were washed with H_2O

(40 mL) and dried ($MgSO_4$). The evaporation of the solvent in vacuo gave the crude product **3**(**b**–**f**) or 4-(2-aminophenyl)-2-methylbut-3-yn-2-ol (**8**) that was used without further purification (the ^1H and ^{19}F NMR spectra agreed with the literature data [11,12]). PTSA was added to the solution of crude **3**(**b**–**f**) or **8** in AlkOH (25 mL), and the mixture was heated under reflux with stirring. The mixture was cooled to room temperature, diluted with CH_2Cl_2 (10 mL), poured into H_2O (20 mL) and extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were washed with H_2O (40 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue was purified by the TL or column chromatography.

*4.3.1. 6,7-Difluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one(**1b**)*

The reaction of **2b** (255 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol), carried out according to the above general procedure, afforded the crude compound **3b** (the ^1H and ^{19}F NMR spectra agreed with the literature data [12]). Then PTSA (190 mg, 1 mmol) was added to the solution of crude **3b** in EtOH (25 mL) and the reaction mixture was heated under reflux for 8.5 h with stirring. The crude product **1b** was purified by TLC (Sorbfil plates, R_f 0.45, hexane/ethyl acetate, 7:1) to afford the title compound (148 mg, 70%) as a yellow solid, m.p. 134.8–135.0 °C from hexane. IR (KBr): ν 3337, 2984, 2966, 2932, 2878, 1657, 1639, 1593, 1510, 1462, 1369, 1310, 1285, 1256, 1163, 1119, 1032, 945, 885, 841, 770, 663, 625, 548, 500, 457, 436 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.54 (dd, 1H, $J_H5,_F6$ = 10.5; $J_H5,_F7$ = 9.0 Hz, H^5), 6.38 (dd, 1H, $J_H8,_F7$ = 11.4; $J_H8,_F6$ = 6.2 Hz, H^8), 4.30 (br s, 1H, NH), 2.53 (s, 2H, CH_2), 1.28 (s, 6H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 192.4 (s, CO), 155.8 (dd, $J_C7,_F7$ = 256.0; $J_C7,_F6$ = 15.0 Hz, C^7), 147.6 (d, $J_C8a,_F7$ = 10.4 Hz, C^{8a}), 143.8 (dd, $J_C6,_F6$ = 240.5; $J_C6,_F7$ = 14.0 Hz, C^6), 115.0 (dd, $J_C5,_F6$ = 17.7; $J_C5,_F7$ = 3.2 Hz, C^5), 113.8 (dd, $J_C4a,_F6$ = 3.7; $J_C4a,_F7$ = 1.9 Hz, C^{4a}), 103.7 (d, $J_C8,_F7$ = 20.6 Hz, C^8), 54.1 (s, $\underline{C}(CH_3)_2$), 50.1 (s, CH_2), 27.7 (s, CH_3); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −124.8 (ddd, 1F, $J_F7,_H6$ = 22.2; $J_F7,_H8$ = 11.4; $J_F7,_H5$ = 9.0 Hz, F^7), −149.5 (ddd, 1F, $J_F6,_F7$ = 22.2; $J_F6,_H5$ = 10.5; $J_F6,_H8$ = 6.2 Hz, F^6); HRMS (EI) calcd. for $C_{11}H_{11}F_2NO$ (M^+): 211.0803, found 211.0800. Anal. calcd. for $C_{11}H_{11}F_2NO$: C, 62.55; H, 5.25; N 6.63. Found: C, 62.62; H, 5.00; N, 6.89.

*4.3.2. 6,8-Difluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (**1c**)*

The reaction of **2c** (255 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol) according to the general procedure gave the crude compound **3c** (the ^1H and ^{19}F NMR spectra agreed with the literature data [12]). PTSA (380 mg, 2 mmol) was added to the solution of crude **3c** in MeOH (25 mL) and the mixture was heated under reflux for 15 h with stirring. The crude product **1c** was purified by TLC (Merck precoated plates, R_f 0.65 hexane/ethyl acetate, 10:1, twice) to afford the title compound (80 mg, 38%) as a yellow solid, m.p. 75–81 °C after sublimation. IR (KBr): ν 3325, 3325, 3080, 2972, 2932, 1661, 1591, 1518, 1476, 1373, 1310, 1290, 1263, 1177, 1146, 1113, 1092, 997, 922, 864, 827, 731, 669, 579, 461, 430 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.26 (ddd, 1H, $J_H5,_F6$ = 8.8; $J_H5,_H7$ = 2.9; $J_H5,_F8$ = 1.7 Hz, H^5), 6.91 (ddd, 1H, $J_H7,_F8$ = 10.7; $J_H7,_F6$ = 8.0; $J_H7,_H5$ = 2.9 Hz, H^7), 4.21 (br s, 1H, NH), 2.58 (s, 2H, CH_2), 1.30 (s, 6H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 192.2 (s, CO), 153.3 (dd, $J_C6,_F6$ = 240.0; $J_C6,_F8$ = 10.4 Hz, C^6), 150.8 (dd, $J_C8,_F8$ = 245.0; $J_C8,_F6$ = 10.7 Hz, C^8), 135.9 (d, $J_C8a,_F8$ = 12.9 Hz, C^{8a}), 119.1 (s, C^{4a}), 109.4 (dd, $J_C7,_F6$, $J_C7,_F8$ = 28.0, 22.0 Hz, C^7), 107.3 (dd, $J_C5,_F^6$ = 22.0; $J_C5,_F8$ = 3.0 Hz, C^5), 53.9 (s, $\underline{C}(CH_3)_2$), 50.5 (s, CH_2), 27.5 (s, CH_3); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −126.4 (ddd, 1F, $J_F6,_H5$ = 8.6; $J_F6,_H7$ = 8.0; $J_F6,_F8$ = 1.3 Hz, F^6), −133.3 (ddd, 1F, $J_F8,_H7$ = 10.7; $J_F8,_H5$ = 1.7; $J_F8,_F6$ = 1.3 Hz, F^8); HRMS (EI) calcd. for $C_{11}H_{11}F_2NO$ (M^+): 211.0803, found 211.0806. Anal. calcd. for $C_{11}H_{11}F_2NO$: C, 62.55; H, 5.25; N 6.63. Found: C, 62.57; H, 5.20; N, 6.32.

*4.3.3. 5,6,8-Trifluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (**1d**)*

The reaction of **2d** (273 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol) gave the crude compound **3d** (the ^1H and ^{19}F NMR spectra agreed with the data reported [12]). Then PTSA (380 mg, 2 mmol) was added to the solution of crude **3d** in EtOH (25 mL) and the mixture was heated under reflux for 17 h with stirring. The crude product **1d** was purified by TLC (Sorbfil plates, R_f 0.27 hexane/ethyl acetate, 7:1, twice) to afford the title compound (98 mg, 43%) as a yellow solid, m.p. 161.1–161.6 °C from hexane. IR (KBr): ν 3335, 3088, 2972, 2934, 1668, 1524, 1474, 1393, 1310, 1269, 1227, 1190, 1155, 1109, 1020, 999, 957, 905, 878, 743, 679, 629, 582, 447 cm^{-1}; ^1H NMR (300.13 MHz, $CDCl_3$): δ = 7.02 (ddd, 1H, $J_H7,_F8$ = 10.4; $J_H7,_F6$ = 9.7; $J_H7,_F5$ = 6.8 Hz, H^7), 4.29 (br s, 1H, NH), 2.60 (s, 2H, CH_2), 1.33 (s, 6H, CH_3); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 190.3 (s, CO), 145.4 (ddd, $J_C5,_F5$ = 260.5; $J_C5,_F6$ = 13.0; $J_C5,_F8$ = 4.0 Hz, C^5), 144.9 (ddd, $J_C8,_F8$ = 240.8; $J_C8,_F6$ = 9.1; $J_C8,_F5$ = 4.5 Hz, C^8), 140.0 (ddd, $J_C6,_F6$ = 240.5; $J_C6,_F5$ = 14.1; $J_C6,_F8$ = 10.5 Hz C^6), 135.3 (ddd, $J_C8a,_F8$ = 13.9; $J_C8a,_F5$ = $J_C8a,_F6$ = 2.5 Hz, C^{8a}), 109.6 (ddd, $J_C7,_F6$ = $J_C7,_F8$ = 23.3; $J_C7,_F5$ = 1.8 Hz, C^7), 108.5 (dd, $J_C4a,_F5$ = 8.2; $J_C4a,_F$ = 2.8 Hz, C^{4a}), 53.5 (s, $\underline{C}(CH_3)_2$), 51.2 (s, CH_2), 27.2 (s, CH_3); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −139.2 (dddd, 1F, $J_F8,_F5$ = 17.0; $J_F8,_H7$ = 10.4; $J_F8,_F6$ = 3.0; $J_F8,_H1$ = 3.0 Hz, F^8), −146.5 (dddd, 1F, $J_F5,_F6$ = 20.2; $J_F5,_F8$ = 17.0; $J_F5,_H7$ = 6.8; $J_F5,_H1$ = 1.6 Hz, F^5), −152.9 (ddd, 1F, $J_F6,_F5$ = 20.2; $J_F6,_H7$ = 9.7; $J_F6,_F8$ = 3.0 Hz, F^6); HRMS (EI) calcd. for $C_{11}H_{10}F_3NO$ (M^+): 229.0709, found 229.0705. Anal. calcd. for $C_{11}H_{10}F_3NO$: C, 57.64; H, 4.40; N 6.11. Found: C, 57.86; H, 4.36; N, 6.15.

*4.3.4. 5,6,7,8-Tetrafluoro-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (**1e**)*

The reaction of **2e** (291 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol) gave the crude compound **3e** [12]. Then PTSA (190 mg, 1 mmol) was added to the solution of crude **3e** in MeOH (25 mL) and the mixture was heated under reflux for 40 h with stirring. The crude product **1e** was purified by TLC (Merck precoated plates, R_f 0.50, hexane/ethyl acetate, 10:1) to afford the title compound (141 mg, 57%) as a yellow solid, m.p. 128.3–128.5 °C from hexane. IR (KBr): ν 3337, 2974, 2938, 2895, 1676, 1659, 1531, 1504, 1474, 1416, 1373, 1310, 1283, 1234, 1205, 1165, 1119, 1088, 1026, 961, 922, 868, 758, 667, 598, 450, 438 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 4.29 (br s, 1H, NH), 2.60 (s, 2H, CH_2), 1.33 (s, 6H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 189.6 (s, CO), 147.0 (dm, $J_C5,_F5$ = 262.5; $J_C5,_F6$ = 10.5; $J_C5,_F7 \approx J_C5,_F8 \approx$ 4.3 Hz, C^5), 144.5 (dm, $J_C7,_F7$ = 257.5; $J_C7,_F6 \approx J_C7,_F8 \approx$ 13.8; $J_C7,_F5$ = 5.2 Hz, C^7), 135.5 (ddd, $J_C8,_F8$ = 241.0; $J_C8,_F7$ = 12.4; $J_C8,_F5$ = 1.6 Hz, C^8), 135.5 (m, $J_C8a,_F8$ = 10.5; $J_C8a,_F5 \approx J_C8a,_F7 \approx$ 4.2; $J_C8a,_F6$ = 2.1 Hz, C^{8a}), 132.2 (dm, $J_C6,_F6$ = 244.0; $J_C6,_F5$, $J_C6,_F7$ = 16.3, 13.1; $J_C6,_F8$ = 2.1 Hz, C^6), 103.8 (d, $J_C4a,_F5$ = 7.5 Hz, C^{4a}), 53.9 (s, $\underline{C}(CH_3)_2$), 51.3 (s, CH_2), 27.4 (s, CH_3); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −143.5 (dddd, 1F, $J_F5,_F6$ = 20.8; $J_F5,_F8$ = 12.6; $J_F5,_F7$ = 9.4; $J_F5,_H1$ = 1.6 Hz, F^5), −149.6 (ddd, 1F, $J_F7,_F6$ = 21.1; $J_F7,_F8$ = 20.4; $J_F7,_F5$ = 9.4 Hz, F^7), −164.5 (dddd, 1F, $J_F8,_F7$ = 20.4; $J_F8,_F5$ = 12.6; $J_F8,_F6$ = 6.1; $J_F8,_H1$ = 2.8 Hz, F^8), −175.5 (ddd, 1F, $J_F6,_F7$ = 21.1; $J_F6,_F5$ = 20.8; $J_F6,_F8$ = 6.1 Hz, F^6); HRMS (EI) calcd. for $C_{11}H_9F_4NO$ (M^+): 247.0615, found 247.0614. Anal. calcd. for $C_{11}H_9F_4NO$: C, 53.45; H, 3.67; N 5.67. Found: C, 53.79; H, 3.85; N, 5.68.

*4.3.5. 5,7,8-Trifluoro-2,2-dimethyl-6-(trifluoromethyl)-2,3-dihydroquinolin-4(1H)-one (**1f**)*

The reaction of **2f** (341 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol) gave the crude compound **3f** [12]. Then PTSA (380 mg, 2 mmol) was added to the solution of crude **3f** in *n*-BuOH (25 mL) and the reaction mixture was heated under reflux for 17.5 h with stirring. The crude product **1f** was purified by column chromatography (hexane/ethyl acetate, 10:1). After the

evaporation of the fractions that contained the pure title compound **1f** (R_f 0.25, hexane/ethyl acetate, 7:1), 163 mg (55%) of colorless solid material was obtained, m.p. 152–156 °C after sublimation. IR (KBr): ν 3327, 2974, 2934, 1683, 1655, 1599, 1538, 1477, 1346, 1319, 1263, 1250, 1221, 1175, 1117, 1074, 957, 945, 910, 843, 826, 762, 704, 609, 563, 532, 467, 413 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 5.00 (br s, 1H, NH), 2.61 (s, 2H, CH_2), 1.38 (s, 6H, CH_3); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 188.4 (s, CO), 156.0 (d, $J_C5,_F5$ = 273.0 Hz, C^5), 150.5 (ddd, $J_C7,_F7$ = 263.5; $J_C7,_F8$ = 13.4, $J_C7,_F5$ = 8.5 Hz, C^7), 142.0 (m, $J_C8a,_F8$ = 10.0; $J_C8a,_F5 \approx J_C8a,_F7 \approx$ 6.7 Hz, C^{8a}), 134.9 (ddd, $J_C8,_F8$ = 240.0; $J_C8,_F7$ = 14.8; $J_C8,_F5$ = 4.5 Hz, C^8), 121.5 (q, $J_C,_F$ = 273.0 Hz, CF_3), 104.0 (d, $J_C4a,_F5$ = 11.2 Hz, C^{4a}), 96.2 (m, $J_C6,_{CF3}$ = 34.4; $J_C6,_F5$, $J_C6,_F7 \approx$ 15.0, 13.0 Hz, C^6), 53.7 (s, $\underline{C}(CH_3)_2$), 50.6 (s, CH_2), 27.3 (s, CH_3); ^{19}F NMR (282.37 MHz, $CDCl_3$): δ = −56.2 (dd, 3F, $J_{CF3},_F5$ = 23.0; $J_{CF3},_F7$ = 21.2 Hz, CF_3), −116.1 (qdd, 1F, $J_F5,_{CF3}$ = 23.0; $J_F5,_F8$ = 14.1; $J_F5,_F7$ = 4.7 Hz, F^5), −131.6 (qdd, 1F, $J_F7,_{CF3}$ = 21.2; $J_F7,_F8$ = 20.0; $J_F7,_F5$ = 4.7 Hz, F^7), −165.8 (ddd, 1F, $J_F8,_F7$ = 20.0; $J_F8,_F5$ = 14.1; $J_F8,_H1$ = 3.0 Hz, F^8); HRMS (EI) calcd. for $C_{12}H_9F_6NO$ (M^+): 297.0583, found 297.0578. Anal. calcd. for $C_{12}H_9F_6NO$: C, 48.50; H, 3.05; N 4.71. Found: C, 48.84; H, 3.10; N, 4.71.

4.3.6. 2,2-Dimethyl-2,3-dihydroquinolin-4(1H)-one (**5**)

The reaction of **7** (219 mg, 1 mmol) and 2-methylbut-3-yn-2-ol (126 mg, 1.5 mmol) afforded the crude compound **8** (the ^1H NMR spectra agreed with the literature data [11]). Then PTSA (285 mg, 1.5 mmol) was added to the solution of crude **8** in EtOH (25 mL) and the mixture was heated under reflux for 8 h with stirring. The crude product **5** was purified by column chromatography (eluant: hexane/ethyl acetate, 8:1). The evaporation of the fractions containing **5** (R_f 0.30, hexane/ethyl acetate, 7:1), gave 157 mg (90%) of the title product as yellow solid material, m.p. 82.7–82.9 °C from hexane (yellow oil [18]; 82–83 °C [22]). IR (KBr): ν 3325, 3065, 2984, 2961, 2928, 1661, 1616, 1512, 1483, 1462, 1431, 1348, 1315, 1267, 1231, 1155, 1126, 1111, 1024, 758, 681, 631, 571, 528, 473 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.78 (dd, 1H, $J_H5,_H6$ = 7.9; $J_H5,_H7$ = 1.5 Hz, H^5), 7.26 (ddd, 1H, $J_H7,_H8$ = 8.2; $J_H7,_H6$ = 7.0; $J_H7,_H5$ = 1.5 Hz, H^7), 7.67 (ddd, 1H, $J_H6,_H5$ = 7.9; $J_H6,_H7$ = 7.0; $J_H6,_H8$ = 0.9 Hz, H^6), 6.58 (dm, 1H, $J_H8,_H7$ = 8.2 Hz, H^8), 4.13 (br s, 1H, NH), 2.57 (s, 2H, CH_2), 1.30 (s, 6H, CH_3); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 194.0 (s, C^4), 149.8 (s, C^{8a}), 135.2 (s, C^7), 127.1 (s, C^5), 117.8 (s, C^{4a}), 117.2 (s, C^6), 115.6 (s, C^8), 53.5 (s, C^2), 50.4 (s, C^3), 27.6 (s, CH_3); HRMS (EI) calcd. for $C_{11}H_{13}NO$ (M^+): 175.0992, found 175.0995. Anal. calcd. for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N 7.99. Found: C, 75.66; H, 7.33; N, 7.98.

4.4. Cytotoxicity assays

Tumor cell lines from human myeloma RPMI 8226, human mammary adenocarcinoma MCF-7, mouse fibroblasts LMTK and primary mouse fibroblast cell line (PMF) (~2000 cells per well) were incubated for 24 h at 37 °C in IMDM or RPMI 1640 medium (5% CO_2) and then were treated with 2,2-dimethyl-2,3-dihydro-1*H*-quinolin-4-ones **1**(**a**–**h**) or **5**. After 72 h of cell incubation, the relative amount of live cells was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (a standard colorimetric MTT-test [23]) and the drug concentration that caused 50% cell growth inhibition (IC_{50}) was determined. The results are expressed as mean ± standard deviation of at least 3 independent experiments.

4.5. Determination of mutagenisity of compounds

In the Ames test, the histidine-dependent strain of *S. typhimurium* TA102 was used, which carries a mutation at the histidine operon [17]. The mutagenic activity of the samples was analyzed by the standard method without metabolic activation [17]. A liquid culture of TA102 was obtained by 16-h growth of cells from a frozen stock at 37 °C in LB medium with penicillin. Then cells were plated on minimal glucose agar, antibiotics and histidine at the density sufficient to obtain isolated colonies. A separate bacterial colony was inoculated into LB medium (5 mL) containing ampicillin (50 μg/mL) and tetracycline (2 μg/mL), and grown with shaking (130 rpm) for 15 h at 37 °C.

The Ames test was carried out using the double-layer method as described in [17]. The overnight culture of bacteria (100 μl) containing one of the tested compounds in different concentrations and, if required, 3 mM H_2O_2, were mixed at 42 °C with 2 mL of liquid 0.6% top agar. The mixture was poured onto plates with a minimal medium containing 0.2% glucose and 3% agar, taking care to distribute the mixture uniformly on the surface of the solid agar. The plates were incubated for 48 h at 37 °C, and the revertants were counted. The cells incubated with H_2O_2 in the absence of compounds analyzed were used as positive controls, and the cells grown in the absence of H_2O_2 and antioxidants served as negative controls for mutation induction. The results are expressed as mean ± standard deviation of at least 3 independent experiments.

Acknowledgments

The analytical and spectral measurements were performed by the Collective Service Center of the Siberian Branch of the Russian Academy of Science. The authors thank the Russian Foundation of Basic Research for financial support (grant 14-03-00108). The biological work was supported by the interdisciplinary grant 98 from the Siberian Branch of the Russian Academy of Science.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfluchem.2015.07.006.

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