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Graphical Abstract

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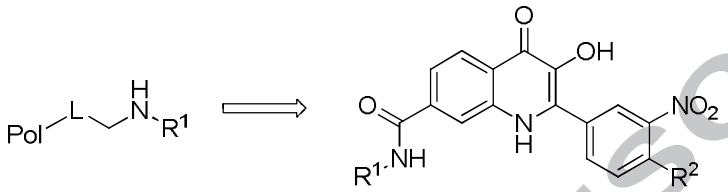

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Synthesis, cytotoxic activity and fluorescence properties of a set of novel 3-hydroxyquinolin-4(1*H*)-ones

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ABSTRACT

The targeted solid-phase synthesis of 3-hydroxyquinolin-4(1*H*)-one derivatives is described. Primary and secondary amines, 3-amino-4-(methoxycarbonyl)benzoic acid and 2-bromo-1-(4-chloro-3-nitrophenyl)ethanone were used as starting materials. The structures of the final compounds were designed in accordance with previous information obtained from structure-activity relationship studies of similar cytotoxic derivatives. Representative prepared compounds were subjected to *in vitro* screening of cytotoxic activity against various cancer cell lines; the results obtained are discussed. Fluorescence properties of selected compounds were also studied to compare the data with those obtained in analogous derivatives.

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Compounds based on the 3-hydroxyquinolin-4(1*H*)-one scaffold (3HQs) represent a new class of biologically promising compounds.[1] For this reason, synthetic approaches to such derivatives have often been studied and simple methods for their preparation have been developed.[2] 2-Phenyl-3-hydroxyquinolin-4(1*H*)-ones were identified as strong anticancer agents with significant *in vitro* cytotoxicity against various cancer cell lines.[3-6] Aside from this, some derivatives exhibit high antiprotozoal and immunosupressive activity.[6] With respect to their limited solubility, current research in this field is focused particularly on the improvement of the bioavailability of selected compounds.[7,8] In addition to the biological properties, 3-hydroxyquinolin-4(1*H*)-ones have been studied for their interesting fluorescence effects. Dual emission spectra and promising quantum yields of some derivatives have led to several studies on their possible use as fluorescence labels.[9-13] Alongside our recent focus on pharmacokinetic studies of selected compounds, we have also been interested in the development of novel structural analogues to obtain substances with improved biological properties, particularly higher activity, low toxicity and better selectivity. In this article, we describe the preparation and study of derivatives that have been designed as a combination of the structural motifs found in the most active compounds. In the past, compounds bearing a carboxamide group on the benzene ring of the quinolinone scaffold were synthesized using solid-phase synthesis.[14] With the use of combinatorial chemistry and structure-cytotoxicity relationship studies, substitution of the carboxamide group was targeted to specific ligands.[5] Similarly, 3HQs bearing specific 3-nitro-4-amino substitution at the 2-phenyl group were described as potent anticancer and antiprotozoal agents.[6] Some of the most active compounds are displayed in Figure 1.

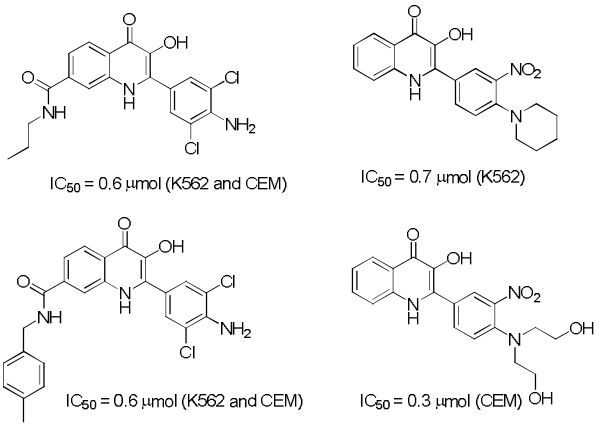

Figure 1: Some of the most active representatives of 3HQs

Based on previous information, target derivatives **III** were designed as a combination of the most active compounds **I** and **II** (Figure 2). For the preparation of the desired derivatives, polystyrene-supported chemistry was chosen to enable the

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preparation of a small chemical library of the target quinolinones. The detailed structure-activity relationships of these targets could then be studied.

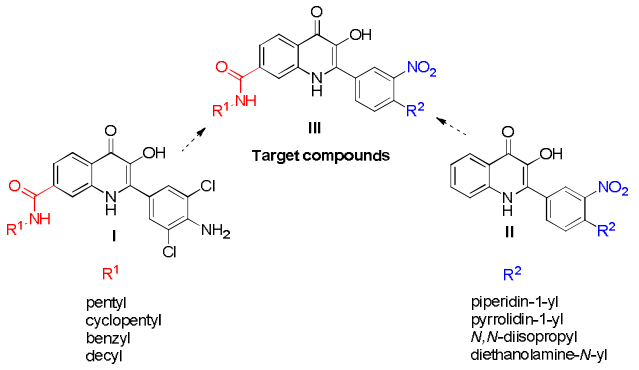
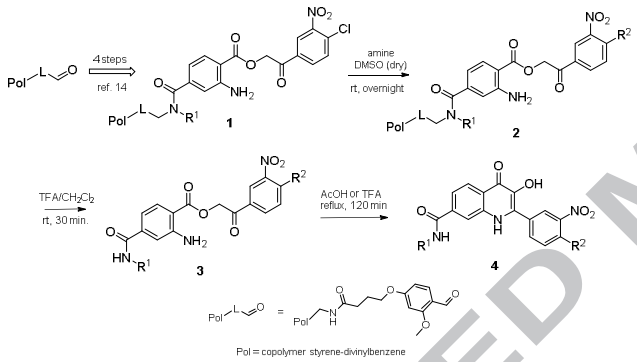


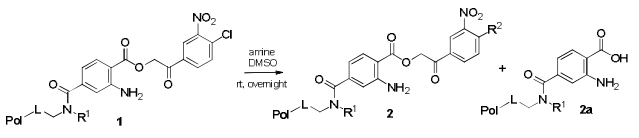
Figure 2: Design of target compounds and ligand selection

The key intermediates **1** were prepared according to the known procedure (Scheme 1). [14] The results achieved were the same as those previously published, and compounds **1** were obtained with crude purities of more than 95% (calculated from LC-UV traces).



Scheme 1: Preparation of the target compounds

However, the subsequent reaction with amines did not lead to the pure intermediates **2**. We observed unexpected cleavage of the ester bond and side products **2a** were detected (Scheme 2). The quantities of side products **2a** were too high (40-60%, calculated from LC-UV traces), to allow for continuation of this route under these conditions. It was clear that the ester cleavage had to occur during the reaction, but not during the substitution; after analysis of the cleaved samples, we did not detect any other compounds except derivatives **3** and their carboxylic analogues. On the other hand, formation of **2a** was surprising as aminolysis of the ester should afford an amide instead of the free carboxylic group. To suppress the unwanted hydrolysis, we used anhydrous reagents (dry DMSO and amines). Under such conditions the side product was still detected, but in limited quantities (5-10% for piperidine and pyrrolidine, 15-20% for diethanolamine and dipropylamine).



Scheme 2: Formation of side products during the nucleophilic substitution step

After cleavage from the polymer support the final cyclization of intermediates **3** was performed by heating in the presence of an acid. Two different methods were tested: reflux in TFA or AcOH. The latter gave significantly better results in terms of the crude purity of the final compounds **4**. Despite this, the cyclization step afforded variable results and the crude purity of the final products was limited in some cases (Table 1).

Table 1: Purities/yields of synthesized compounds after precipitation

[a]Purity calculated from LC-UV traces (220 nm), NI=not isolated
[b]Overall yields after the whole reaction sequence and purification

Product	R^1	R^2	Purity [a] (%)	Yield [b] (%)
4a	cyclopentyl	piperidin-1-yl	45	NI
4b	cyclopentyl	pyrrolidin-1-yl	32	NI
4c	cyclopentyl	*N,N*-dipropylamino	60	NI
4d	Bn	piperidin-1-yl	93	20
4e	Bn	pyrrolidin-1-yl	95	23
4f	Bn	*N,N*-dipropylamino	55	20
4g	pentyl	piperidin-1-yl	98	39
4h	pentyl	pyrrolidin-1-yl	97	38
4i	pentyl	*N,N*-dipropylamino	95	20
4j	decyl	piperidin-1-yl	98	21
4k	decyl	pyrrolidin-1-yl	92	20
4l	decyl	*N,N*-dipropylamino	85	10

Separation of side products **2a** proved simple; after evaporation of acetic acid, the crude material was suspended in diethyl ether. The quinolinones **4** precipitated while side products **2a** remained dissolved. With simple filtration it was possible to separate some of the products in excellent purity. On the other hand, some compounds **4** were accompanied by numerous side products and purification by precipitation or crystallization was not successful. For compounds synthesized from diethanolamine, the cyclization was not applicable due to partial acetylation of both hydroxyl groups. Subsequent attempts to hydrolyze the acetates failed due to total decomposition of the molecule.

Compounds that were isolated in sufficient quantity and high purity were subjected to MTT colorimetric assay and their cytotoxicity against various cancer cell lines was evaluated. To compare the results with the cytotoxicities of previously studied compounds **I** and **II**, similar cell lines were selected: human myeloid leukemia (K562), human myeloid leukemia resistant to paclitaxel (K562-tax), T-lymphoblastic leukemia (CEM), T-lymphoblastic leukemia resistant to doxorubicine (CEM-DNR-bulk) and lung adenocarcinoma (A549). Results of the MTT testing are summarized in Table 2.

Table 2: Results of biological screening of representative compounds (IC_{50}, μM)[a]

Product	CEM	CEM-DNR-bulk	K562	K562-tax	A549
4i	182.9	150.2	152.6	150.7	168.8
4g	3.0	9.3	2.7	11.1	5.2
4h	3.0	9.9	4.1	4.7	2.4
4d	9.1	41.0	39.5	62.6	10.9
4e	6.7	41.7	11.4	67.6	8.7
4k	2.9	9.8	7.2	9.4	2.9

[a]Average IC_{50} values from 3-4 independent experiments with SDs ranging from 10-25% of the average values.

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Some interesting structure-activity relationships were observed in compounds **4g**, **4h** and **4i**. It can be concluded that the presence of the five-membered ring (pyrrolidine) or the six-membered ring (piperidine) gives the same results, while the aliphatic analogue (dipropylamine) does not exhibit any activity. As well as the presence of R^2 substituents effecting the activity, varying the R^1 ligand also caused a difference in the biological properties. This is shown by the comparison of **4h** and **4e** in which the locked pyrrolidine scaffold at position R^2 gave 14 times higher activity for R^1=pentylamine compared to R^1=benzylamine (K562 cell line). However, from the overall results, it is evident that the combination of selected ligands does not lead to an improvement in the anticancer activity. No single compound from the analyzed set exhibited a lower IC_{50} value than that reported for model compounds **I** and **II**.[5,6]

The fluorescence properties of representative synthesized compounds **4g-i** were investigated to compare the results with previously studied analogues of type **I** and **II** (Figure 3).[9,13] The excitation spectra of **4g-i** showed mostly several relatively narrow distinctive local maxima at wavelengths around 290, 322 and 360 nm (Figure 3, Table 3).

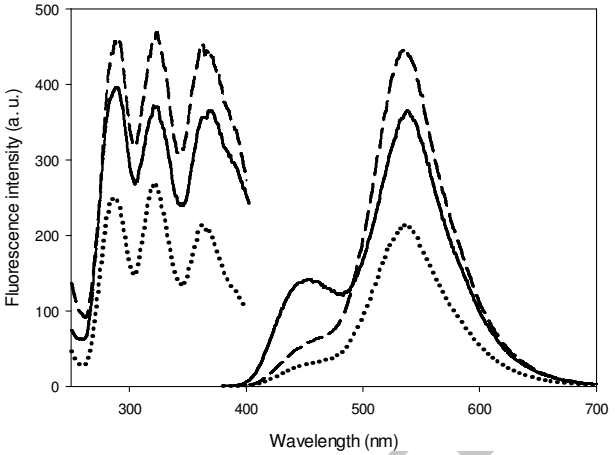

Figure 3: Excitation and emission spectra of compounds **4g-i** in methanol. (—) **4i**; (– –) **4h**; (•••) **4g**. (for excitation and emission wavelengths see Table 3; **4i** conc = 20 µmol.L^{-1}).

Table 3: Spectroscopic properties of **4g**, **4h** and **4i** in methanol.

Product	ε^a (mol^{-1} cm^{-1})	$\lambda_{ex}{}^b$ (nm)	$\lambda_{em,1}{}^c$ (nm)	$\lambda_{em,2}{}^d$ (nm)	φ^f (%)
4i	9808	370	451	555	4.85
4h	11294	360	450[e]	558	2.35
4g	6986	360	450[e]	522	5.06

[a] ε, molar extinction coefficient for λ_{ex}.
[b] λ_{ex}, excitation wavelength.
[c] $\lambda_{em,1}$, the fluorescence emission maximum at lower wavelengths.
[d] $\lambda_{em,2}$, the fluorescence emission maximum at higher wavelengths.
[e] indistinguishable local maximum, see Figure 1.
f φ, fluorescence quantum yield (determined with quinine sulfate in 0.5 M H_2SO_4 (φ = 0.577[15]) taken as a reference fluorescence standard).

The emission spectra had a dual behavior that is characteristic for 3-hydroxyquinolones resulting from the formation of two excited state tautomeric forms.[9-13] Interestingly, only in the case of **4i** was the lower wavelength local maximum of the emission spectrum sufficiently distinguished (Figure 3). Quantum yields were detected within the range of 2 to 5 percent. Such values are generally higher than in the case of 3HQs **I**[9] which shows that introduction of the carboxamide group increases the quantum yield values. On the other hand, the quantum yield values of analogous 3HQs **II** with different 2-phenyl substitution were higher.[10]

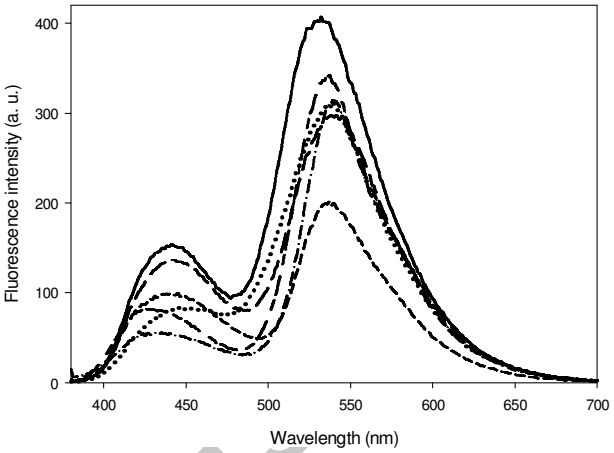

Figure 4: Emission spectra of **4i** in various solvents. (– -) EtOAc; (—) $CHCl_3$; (–•–) THF; (---) DMSO; (•••) MeOH; (– –) toluene. (Excitation wavelength for all solvents = 370 nm; **4i** conc = 20 µmol.L^{-1}).

Additionally, the influence of solvents and pH on the fluorescence properties of compound **4i** were studied. The effect of solvents on the dual emission spectrum was significant (Figure 4, compare the emission spectrum in dimethyl sulfoxide and chloroform), nevertheless, no obvious relationship between the solvent polarity and fluorescence was observed.

Also the pH (**4i** was dissolved in a phosphate buffer at a conc. of 100 mg.L^{-1}) significantly affected the shape of the emission spectrum (Figure 5). The fluorescence measured at a low wavelength maximum (451 nm) reached the maximum intensity at a pH of 3.26 and then gradually decreased. The fluorescence intensity measured at 555 nm decreased nearly linearly (R^2 = 0.9687) with increasing pH (Figure 5).

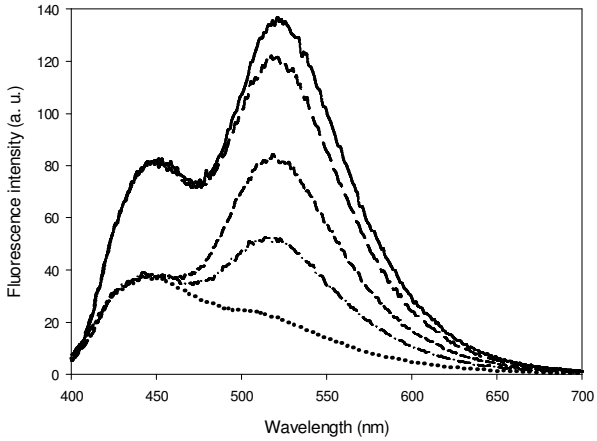

Figure 5: Emission spectra of **4i** depending on the pH (for better lucidity emission spectra for selected pH values are depicted, for complete data see Figure 6). (—) pH 2.86; (– –) pH 3.99; (---) pH 7.09; (– • –) pH 8.82; (•••) pH 10.23. (Excitation wavelength for all solvents = 370 nm; **4i** conc = 20 µmol.L^{-1}).

However, the dependence of I_2/I_1 on the pH was not linear, which limits the possible applicability of **4i** as a fluorescent pH

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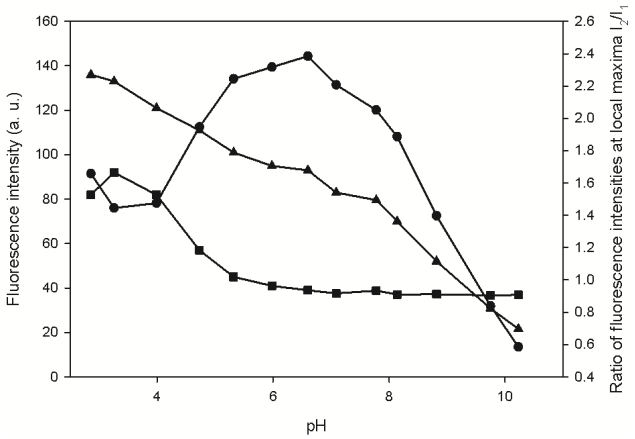
indicator with the dual emission spectrum. The fluorescence of dual fluorescence labels is not dependent on the concentration in the case when the ratio of the two band intensities can be applied as a signal.[9,13]

Figure 6: Fluorescence intensities at local maxima of the emission spectra and their ratio for **4i** depending on pH. ■ I_1 451 nm; ▲ I_2 555 nm; ● I_2/I_1. (excitation wavelength for all solvents = 370 nm; **4i** conc = 20 μmol.L^{-1}).



In conclusion, we have identified several new promising 3HQ derivatives. Although the studied compounds exhibited high anticancer activity and interesting fluorescence, both their emission properties and cytotoxicity were not significantly improved in comparison to analogues studied previously. Despite this fact, some surprising results have been obtained. Regardless of the structural similarity, compound **4i** showed quite different biological/spectral properties and other derivatives exhibited significantly different activities towards the same cell lines. Further, the linear dependence of fluorescence intensity measured at 555 nm and pH allows the possible application of compound **4i** as a pH indicator.

Acknowledgements

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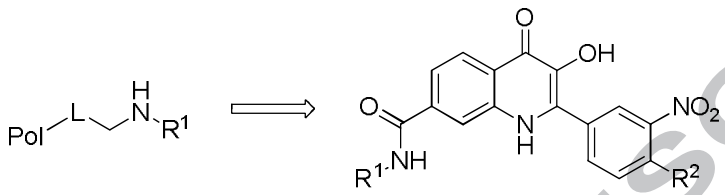

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