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RESEARCH ARTICLE

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# 2-Phenylsubstituted-3-Hydroxyquinolin-4(1*H*)-one-Carboxamides: Structure-Cytotoxic Activity Relationship Study

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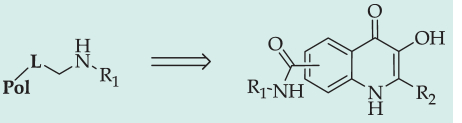
Supporting Information

**ABSTRACT:** A structure−activity relationship of some derivatives of 2-phenylsubstituted-3-hydroxyquinolin-4(1*H*)-one-7-carboxamides was systematically studied using combinatorial solid-phase synthesis and in vitro cytotoxic activity screening on representative cancer lines. The effect of substituent type in position 2 as well as of the carboxamide group was investigated via synthesis of generic libraries constructed with respect to polarity and bulkiness of appropriate substituents. The process of development afforded a set of compounds with significant cytotoxic activity. Subsequently, corresponding 2-phenylsubstituted-3-hydroxyquinolin-4(1*H*)-one-6-carboxamides and 2-phenylsubstituted-3-hydroxyquinolin-4(1*H*)-one-8-carboxamides were prepared to evaluate the influence of the carboxamide group position on the resulting biological activity.




**KEYWORDS:** hydroxyquinolinones, solid-phase synthesis, combinatorial chemistry, cytotoxicity, structure−activity relationship

## INTRODUCTION

Compounds derived from the 3-hydroxyquinolin-4(1*H*)-one skeleton (hydroxyquinolinones hereafter) represent a novel class of compounds with interesting biological properties.[1] Among their currently known effects are antiprotozoal, immunosuppressive, and anticancer activities, the latter of which have been studied the most frequently (Figure 1).

Although a relatively large number of variously substituted hydroxyquinolinones have been synthesized using solution-phase synthesis and introduced as biologically promising substances,[2−6] such compounds have never been systematically studied in terms of their optimal structure. Bearing this in mind we recently focused our attention on the application of solid-phase combinatorial chemistry which allows efficient preparation of sets of derivatives (chemical libraries) and subsequent systematic structure−activity relationship (SAR) studies. So far we have introduced two methodologies for solid-phase combinatorial synthesis of hydroxyquinolinone derivatives using the split-and-split concept.[7,8] In this study, we describe the subsequent process of biological testing and development of 2-substituted-3-hydroxyquinolin-4(1*H*)-one-7-carboxamides[7] (Scheme 1) focused on determination of their in vitro cytotoxicity toward selected cancer cell lines followed by fine-tuning the activity through a substitution of the carboxamide group.

Our general concept for the creation of chemical libraries is based on focused synthesis of limited sets of compounds (so-called "generation libraries") followed by evaluation of their biological properties and subsequent step-by-step structure/activity optimization (Scheme 2), rather than on routine preparation of sizable libraries with multiple similar substituents

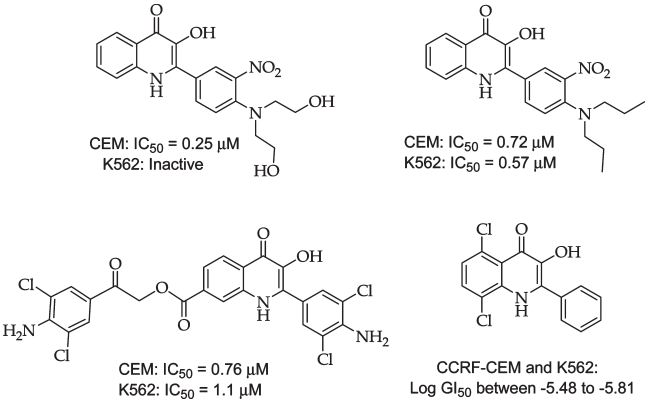



**Figure 1.** Examples of already known hydroxyquinolinones with anticancer activities.

with minimum chemical diversity. Such a sensitive procedure furnishes several advantages: (i) it is timesaving; (ii) it is economical (especially when commercially poorly available building blocks are needed); (iii) the process excludes useless preparation, purification, and screening often of a large number of derivatives with unsuitable biological properties.

Since there are only a limited number of substances to be synthesized and tested according to the above-described concept, the generation libraries are not scheduled to furnish series of derivatives with full combination of used building blocks.

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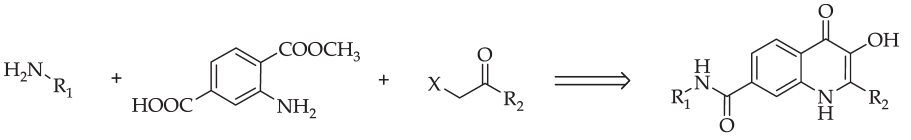
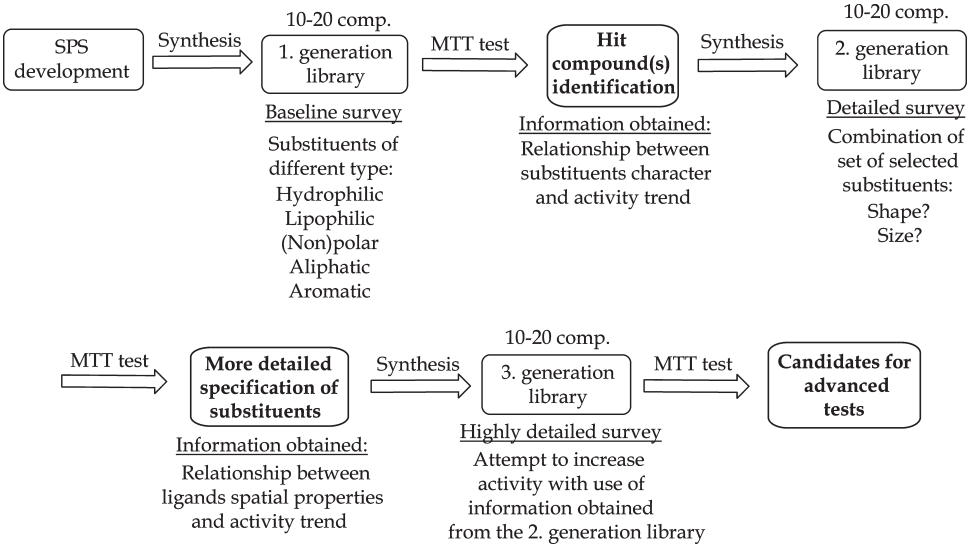
**Scheme 1. Building Blocks and General Structure of 2-Substituted-3-hydroxyquinolin-4(1*H*)-one-7-carboxamides with Two Diversity Positions**[7]




1 2 3 4

**Scheme 2. Step-by-Step Procedure for Research and Development of Compounds with Cytotoxic Activity**

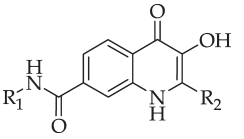



Their goal is rather a trend analysis of each diverse substitution. An example of this approach for development of biologically active compounds is given in this article.

## RESULTS AND DISCUSSION

With respect to the general concept (Scheme 2) the construction of the first-generation library was focused on the trend analysis of substituents in terms of their polar/nonpolar or hydrophilic/lipophilic properties. For these purposes the carboxamide group was substituted with two hydrophilic ligands (hydroxyethyl, hydroxypropyl), two strictly lipophilic ligands (4-methylbenzyl, propyl), two heterocyclic ligands of different polarity (imidazol-*N*-propyl, piperidine-*N*-ethyl) and also unsubstituted carboxamide was included in the set. To allow the individual trend analysis of the 7-carboxamide *N*-substitution effect, the mentioned ligands were used in combination with a randomly selected single substituent at position 2 of the hydroxyquinolinone moiety (4-methylphenyl in this case). A similar procedure was used to study the 2 position of the hydroxyquinolinone moiety: variously substituted phenyls were incorporated into the structure. As in the previous case, the ligands at position 2 were combined with randomly selected single 7-carboxamide *N*-substitution (propyl in this case). The outcome of the first generation library cytotoxicity screening is summarized in Table 1.

**Table 1. Summary of the First-Generation Library Cytotoxicity Screening (Relative $IC_{50}$, $\mu$M)**[a]




| Cmp. | $R_1$ | $R_2$ | CEM | K562 | HCT116 | BJ | PBMC |
|---|---|---|---|---|---|---|---|
| 5 | H | 4-Me-Ph | 12.9 | 10.4 | 9.1 | 31.8 | >100 |
| 6 | Propyl | 4-Me-Ph | 2.8 | 2.2 | 3.1 | NT | NT |
| 7 |  | 4-Me-Ph | 182.5 | 99.2 | 205.5 | NT | NT |
| 8 |  | 4-Me-Ph | 151.6 | 55.1 | 145.7 | NT | NT |
| 9 | 4-MeBn | 4-Me-Ph | 2.5 | 2.4 | 2.2 | NT | NT |
| 10 |  | 4-Me-Ph | 52.2 | 168.9 | 157.9 | NT | NT |
| 11 |  | 4-Me-Ph | 11.9 | 79.1 | 33.6 | NT | NT |
| 12 | Propyl | 4-MeO-Ph | 3.1 | 2.9 | 3.2 | NT | NT |
| 13 | Propyl | 3-Br-Ph | 2.5 | 2.4 | 2.7 | NT | NT |
| 14 | Propyl | 3-$NO_2$-4-Cl-Ph | 2.5 | 3.4 | 4.0 | NT | NT |
| 15 | Propyl | 4-F-Ph | 5.7 | 2.8 | 4.0 | NT | NT |
| 16 | Propyl | 3,5-Cl-4-$NH_2$-Ph | 0.98 | 9.6 | 7.9 | 16.1 | 70.4 |
| 17 | Propyl | 3-$NO_2$-4-N(Pr)$_2$-Ph | 2.3 | 7.6 | 10.8 | NT | NT |

[a] Average values of $IC_{50}$ from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values. NT: not tested in nonmalignant cells, the $IC_{50}$ value in chemosensitive CEM leukemia cells was >1 $\mu$M. For cell line characteristics and methodological details please refer to our previous publications.[9−11]

The cytotoxic activity was analyzed on the human cancer cell lines (CEM, acute lymphoblastic leukemia; K562, acute myeloid leukemia; HCT116, colorectal carcinoma). The most potent compounds, with $IC_{50} \leq 1$ $\mu$M for the most chemosensitive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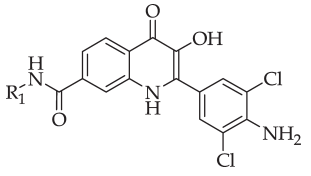
CEM cell line, were also tested on the normal human cells (BJ line of normal cycling fibroblasts and PBMC − human peripheral blood mononuclear cells) to analyze the therapeutic index (TI) which is based on the ratio between the $IC_{50}$ for normal human cells and cancer cell lines. The SAR ought to be evaluated for individual cell lines independently because it may vary due to different tissue origin and potential molecular targets in particular cell lines. However, in our case, the trend of activity was very similar for all tested cell lines indicating similar or identical target(s), and thus we will discuss SAR generally. The results from the first-generation library screening show the following dependence:

(i) Trend in *the N-carboxamide substitution versus cytotoxic effect* (entries **5**−**11**): (a) If the carboxamide is *N*-unsubstituted the activity is rather low (relative $IC_{50}$ = 9.1−12.9 $\mu$M); (b) Derivatives with lipophilic carboxamide *N*-substitution (such as propyl, 4-methylbenzyl) exhibit promising cytotoxicity (relative $IC_{50}$ = 2.2−3.1 $\mu$M); (c) If the carboxamide is substituted with hydrophilic ligands (hydroxypropyl, hydroxyethyl) there is no activity (except very low cytotoxicity of compound **8** against K562: $IC_{50}$ = 55.1); (d) If the carboxamide is substituted with the two named heterocyclic ligands the activity is medium or very low (relative $IC_{50}$ = 11.9−168.9 $\mu$M).

(ii) Trend in *the position 2 substitution versus cytotoxic effect* (entries **6**, **12**−**17**): When variously substituted aromatic ligands are incorporated, the cytotoxicity test furnishes approximately similar results, that is, no significant trend among the substituents used is observed. However, in a more detailed comparison the derivatives **16** and **17** (bearing aminogroup or dipropylaminogroup in position 4) exhibit lower activity against K562 and HCT116. In con-

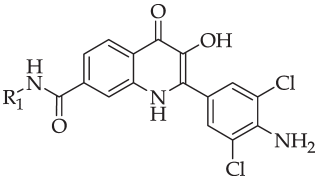
**Table 2. Summary of the Second-Generation Library Cytotoxicity Screening (Relative $IC_{50}$, $\mu$M)[a]**



| Cmp. | $R_1$ | CEM | K562 | HCT116 | BJ | PBMC |
|---|---|---|---|---|---|---|
| **18** | cyclopropyl | 3.7 | 16.9 | 15.1 | NT | NT |
| **19** | cyclobutyl | 1.2 | 13.6 | 10.6 | NT | NT |
| **20** | cyclopentyl | 1.1 | 15.3 | 12.9 | NT | NT |
| **21** | cyclohexyl | 1.1 | 12.6 | 9.4 | NT | NT |
| **22** | cycloheptyl | 1.0 | 9.5 | 10.9 | NT | NT |
| **23** | methyl | 3.9 | 25.8 | 32.1 | NT | NT |
| **24** | isobutyl | 1.8 | 11.6 | 8.1 | NT | NT |
| **25** | pentyl | 1.0 | 7.5 | 5.6 | NT | NT |
| **26** | benzyl | 0.94 | 6.5 | 6.6 | 9.6 | 96.9 |
| **27** | 2-Me-Bn | 0.96 | 7.6 | 7.9 | 16.7 | 75.9 |
| **28** | 3-Me-Bn | 0.75 | 3.1 | 4.1 | 5.0 | 43.2 |
| **29** | 4-Me-Bn | 0.87 | 3.4 | 7.4 | 32.7 | 57.6 |

[a] Average values of $IC_{50}$ from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values. NT: not tested in non-malignant cells, the $IC_{50}$ value in chemosensitive CEM leukemia cells was >1 $\mu$M. For cell line characteristics and methodological details please refer to our previous publications.[9−11]

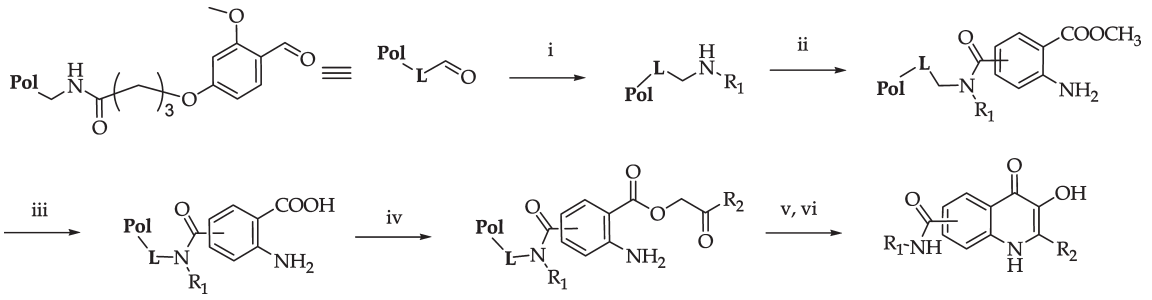
**Table 3. Summary of the Third-Generation Library Cytotoxicity Screening (Relative $IC_{50}$, $\mu$M)[a]**



| Cmp. | $R_1$ | CEM | K562 | HCT116 | BJ | PBMC |
|---|---|---|---|---|---|---|
| **30** | octyl | 1.0 | 4.6 | 9.2 | NT | NT |
| **31** | decyl | 1.1 | 4.4 | 11.4 | NT | NT |
| **32** | dodecyl | 0.70 | 3. 9 | 10.4 | 7.6 | 10.6 |
| **33** | cyclooctyl | 0.98 | 4.8 | 7.0 | 9.9 | 21.1 |
| **34** | cyclododecyl | 0.45 | 2.1 | 4.1 | 71.3 | 74.9 |
| **35** | 2-norbornyl | 1.13 | 6.8 | 9.1 | NT | NT |
| **36** | 2-ethylBn | 7.5 | 6.1 | 10.5 | NT | NT |
| **37** | indan-2-yl | 0.93 | 3.2 | 6.9 | 9.3 | 94.6 |
| **38** | naphthyl-1-Me | 0.69 | 2.4 | 2.4 | 52.2 | 19.1 |
| **39** | 2,2-diphenylEt | 0.74 | 2.5 | 5.3 | 6.9 | 2.5 |

[a] Average values of $IC_{50}$ from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values. NT: not tested in non-malignant cells, the $IC_{50}$ value in chemosensitive CEM leukemia cells was >1 $\mu$M. For cell line characteristics and methodological details please refer to our previous publications.[9−11]

**Scheme 3. Preparation of 6- and 8-Carboxamides[a]**



[a] Reagents: (i) amine, 10% HAc/DMF, overnight, $NaBH(OAc)_3$, 5 h; (ii) 4-amino-3-(methoxycarbonyl)benzoic acid or 2-amino-3-(methoxycarbonyl)-benzoic acid, DIC, HOBt, DCM, DMF, rt, overnight; (iii) potassium trimethylsilanolate, THF, rt, 7 h; haloketone, TEA, DMF, rt, 2 h; (v) 50% TFA, DCM, 30 min; (vi) TFA, 80 °C, 2 h.


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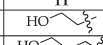
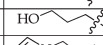
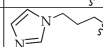
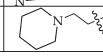
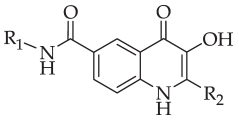
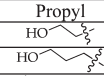
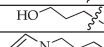
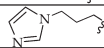
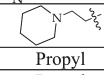
**Table 4. Completion of the 7-Carboxamides Library (Relative $IC_{50}$, $\mu$M)[a]**

| Cmp. | $R_1$ | CEM | K562 | HCT116 |
|---|---|---|---|---|
| **40** | H | 250.0 | 250.0 | 250.0 |
| **41** |  | 12.0 | 10.1 | 10.9 |
| **42** |  | 10.9 | 24.0 | 19.9 |
| **43** |  | 21.1 | 156.2 | 111.7 |
| **44** |  | 13.1 | 11.2 | 10.1 |

[a] Average values of $IC_{50}$ from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values. For cell line characteristics and methodological details please refer to our previous publications.[9,10]

**Table 5. Summary of the 6-Carboxamides Library Cytotoxicity Screening (Relative $IC_{50}$, $\mu$M)[a]**



| Cmp. | $R_1$ | $R_2$ | CEM | K562 | HCT116 | BJ | PBMC |
|---|---|---|---|---|---|---|---|
| **45** | H | 3,5-Cl-4-$NH_2$-Ph | 44.1 | 145.3 | 139.4 | 82.2 | >100 |
| **46** | Propyl | 3,5-Cl-4-$NH_2$-Ph | 3.4 | 2.6 | 2.8 | NT | NT |
| **47** |  | 3,5-Cl-4-$NH_2$-Ph | 13.7 | 36.9 | 37.1 | NT | NT |
| **48** |  | 3,5-Cl-4-$NH_2$-Ph | 10.5 | 42.7 | 52.9 | NT | NT |
| **49** |  | 3,5-Cl-4-$NH_2$-Ph | 11.4 | 54.8 | 54.4 | NT | NT |
| **50** |  | 3,5-Cl-4-$NH_2$-Ph | 8.0 | 10.0 | 9.0 | NT | NT |
| **51** | Propyl | 4-MeO-Ph | 3.5 | 12.7 | 5.2 | NT | NT |
| **52** | Propyl | 3-Br-Ph | 188.8 | 187.0 | 178.5 | NT | NT |
| **53** | Propyl | 3-$NO_2$-4-Cl-Ph | 28.7 | 27.0 | 17.1 | NT | NT |
| **54** | Propyl | 4-F-Ph | 9.9 | 10.2 | 8.9 | NT | NT |
| **55** | Cyclopropyl | 3,5-Cl-4-$NH_2$-Ph | 1.7 | 2.6 | 2.8 | NT | NT |
| **56** | Cyclobutyl | 3,5-Cl-4-$NH_2$-Ph | 1.2 | 2.1 | 2.3 | NT | NT |
| **57** | Cyclopentyl | 3,5-Cl-4-$NH_2$-Ph | 2.0 | 2.5 | 2.2 | NT | NT |
| **58** | Cyclohexyl | 3,5-Cl-4-$NH_2$-Ph | 2.4 | 1.7 | 1.8 | NT | NT |
| **59** | Cycloheptyl | 3,5-Cl-4-$NH_2$-Ph | 0.77 | 0.79 | 0.59 | 5.2 | 24.8 |
| **60** | Methyl | 3,5-Cl-4-$NH_2$-Ph | 2.8 | 2.9 | 2.9 | NT | NT |
| **61** | Isobutyl | 3,5-Cl-4-$NH_2$-Ph | 4.6 | 3.0 | 2.2 | NT | NT |
| **62** | Pentyl | 3,5-Cl-4-$NH_2$-Ph | 3.1 | 2.3 | 1.8 | NT | NT |
| **63** | Benzyl | 3,5-Cl-4-$NH_2$-Ph | 3.1 | 2.8 | 2.5 | NT | NT |
| **64** | 2-Me-Bn | 3,5-Cl-4-$NH_2$-Ph | 2.5 | 2.4 | 1.7 | NT | NT |
| **65** | 3-Me-Bn | 3,5-Cl-4-$NH_2$-Ph | 2.6 | 2.6 | 2.3 | NT | NT |
| **66** | 4-Me-Bn | 3,5-Cl-4-$NH_2$-Ph | 2.8 | 2.6 | 2.8 | NT | NT |
| **67** | Octyl | 3,5-Cl-4-$NH_2$-Ph | 0.62 | 0.99 | 0.55 | 4.5 | 6.6 |
| **68** | Decyl | 3,5-Cl-4-$NH_2$-Ph | 3.5 | 0.42 | 0.24 | NT | NT |
| **69** | Dodecyl | 3,5-Cl-4-$NH_2$-Ph | 0.25 | 0.37 | 0.56 | 90.1 | 98.3 |
| **70** | Cyclooctyl | 3,5-Cl-4-$NH_2$-Ph | 0.75 | 0.62 | 0.75 | 12.4 | 27.0 |
| **71** | Cyclododecyl | 3,5-Cl-4-$NH_2$-Ph | 0.26 | 0.77 | 0.69 | 36.2 | 56.5 |
| **72** | 2-Norbornyl | 3,5-Cl-4-$NH_2$-Ph | 3.2 | 3.0 | 2.4 | NT | NT |
| **73** | Indan-2-yl | 3,5-Cl-4-$NH_2$-Ph | 2.6 | 2.7 | 2.7 | NT | NT |
| **74** | Naphthyl-1-Me | 3,5-Cl-4-$NH_2$-Ph | 1.0 | 0.76 | 0.70 | NT | NT |
| **75** | 2,2-DiphenylEt | 3,5-Cl-4-$NH_2$-Ph | 0.84 | 0.80 | 1.4 | 15.6 | 6.4 |

[a] Average values of $IC_{50}$ from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values. NT: not tested in non-malignant cells, the $IC_{50}$ value in chemosensitive CEM leukemia cells was >1 $\mu$M. For cell line characteristics and methodological details please refer to our previous publications.[9−11]

trast, the derivative **16** shows the best cytotoxicity against CEM among all the first-generation library members. Therapeutic index for the most active compounds was indentified within the range of 2−70.

Our results predetermined derivatives with lipophilic substituents bearing a carboxamide group (and almost any aromatic ligand at position 2 of the hydroxyquinolinone skeleton) for a more detailed survey. Because the activity is almost independent of the substitution of phenyl in position 2, the 4-amino-3, 5-dichloro substitution, which is submicromolar for CEM cells, was fixed for a detailed study of the carboxamide substitution. Instead of the propyl, the carboxamide group was substituted with a set of various lipophilic ligands of aliphatic, cycloaliphatic, or aromatic structure of different shape/size. The results of the second-generation library cytotoxicity screening are summarized in Table 2.

Whereas the first-generation library results proved the existence of a carboxamide ligands polarity-activity relationship, the outcome of the second-generation library screening indicates also the existence of the shape/size-activity relationship in terms of the carboxamide lipophilic substitution. For instance, elongation of the aliphatic chain slightly increases activity against all lines (methyl→pentyl, compare entries **23**−**25**) and expanding the carbocycle moderately increases activity against CEM (cyclopropyl→cyclopentyl, compare entries **18**−**20**). To further explore this possible relationship we decided to synthesize and screen a third-generation library focused on the survey of larger cyclic hydrocarbons (such as cyclooctane, cyclododecane, norbornan, indan, naphthalene, or 2,2-diphenylethan) and longer aliphatic chains. The outcome of the third-generation library cytotoxicity screening is summarized in Table 3. The results indicate that the presence of longer carbon chains as well as larger carbocycles does not further significantly increase the cytotoxicity of the substrates.

For the comparative study of carboxamide structural isomers described in the second part of this paper, five more derivatives (entries **40**−**44**) were prepared and screened (Table 4). The results obtained independently confirm the basic trend in *N*-carboxamide substitution versus cytotoxicity effect discussed earlier in this paper (the first-generation library results).

**Carboxamide Group Location versus Cytotoxic Activity Relationship.** Our next goal was to investigate the effect of the carboxamide group location on the resulting cytotoxicity. For this purpose we synthesized a set of analogous 2-phenylsubstituted-3-hydroxyquinolin-4(1*H*)-one-6-carboxamides and 2-phenylsubstituted-3-hydroxyquinolin-4(1*H*)-one-8-carboxamides. Synthesis was performed according to Scheme 3 using the method previously described.[7]

As in the case of 7-carboxamides,[7] both 6-carboxamides and 8-carboxamides were obtained in an excellent yield (85% on average) and crude purity (above 95% in each case, LC/MS traces) without need for further purification. Unfortunately, the method could not be applied for the preparation of 5-carboxamides: When 3-amino-2-(methoxycarbonyl)benzoic acid was used to acylate immobilized amines (step ii), surprisingly no reaction was observed. For this reason 2-substituted-3-hydroxyquinolin-4(1*H*)-one-5-carboxamides are not discussed in this paper. To carry out the SAR study (in terms of the carboxamide location) as detailed as possible we did not use the step-by-step development strategy but complete sets of 6- and 8-carboxamides were prepared and screened. The results obtained from MTT cytotoxicity tests are summarized in Tables 5 and 6.

From the whole library screening results it is evident that a lot of common features exist for 6-, 7- and 8-carboxamides when appropriate structural isomers are compared. For instance, unsubstituted carboxamides (compare entries **40**, **45**, and **76**) or carboxamides with hydrophilic ligands (compare entries **41**, **42** to **47**, **48** and **78**, **79**) exhibit rather medium or weak cytotoxicity, whereas generally the best results are again obtained for


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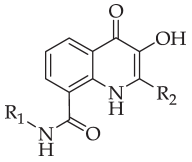
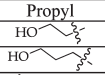
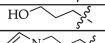
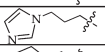
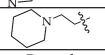
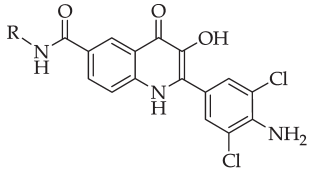
**Table 6. Summary of the 8-Carboxamides Library Cytotoxicity Screening (Relative $IC_{50}$, $\mu M$)[a]**



| Cmp. | $R_1$ | $R_2$ | CEM | K562 | HCT116 | BJ | PBMC |
|---|---|---|---|---|---|---|---|
| **76** | H | 3,5-Cl-4-$NH_2$-Ph | 58.3 | 104.7 | 66.7 | 85.4 | >100 |
| **77** | Propyl | 3,5-Cl-4-$NH_2$-Ph | 8.3 | 12.0 | 16.7 | NT | NT |
| **78** |  | 3,5-Cl-4-$NH_2$-Ph | 8.1 | 9.0 | 15.0 | NT | NT |
| **79** |  | 3,5-Cl-4-$NH_2$-Ph | 7.5 | 9.6 | 10.1 | NT | NT |
| **80** |  | 3,5-Cl-4-$NH_2$-Ph | 9.8 | 7.4 | 11.6 | NT | NT |
| **81** |  | 3,5-Cl-4-$NH_2$-Ph | 2.8 | 5.2 | 7.4 | NT | NT |
| **82** | Propyl | 4-MeO-Ph | 5.2 | 40.2 | 19.0 | NT | NT |
| **83** | Propyl | 3-Br-Ph | 5.0 | 10.0 | 9.9 | NT | NT |
| **84** | Propyl | 3-$NO_2$-4-Cl-Ph | 8.1 | 10.9 | 10.6 | NT | NT |
| **85** | Propyl | 4-F-Ph | 3.3 | 7.4 | 6.9 | NT | NT |
| **86** | Cyclopropyl | 3,5-Cl-4-$NH_2$-Ph | 4.7 | 5.1 | 33.1 | NT | NT |
| **87** | Cyclobutyl | 3,5-Cl-4-$NH_2$-Ph | 2.2 | 4.8 | 5.3 | NT | NT |
| **88** | Cyclopentyl | 3,5-Cl-4-$NH_2$-Ph | 8.7 | 9.0 | 9.7 | NT | NT |
| **89** | Cyclohexyl | 3,5-Cl-4-$NH_2$-Ph | 2.6 | 8.7 | 9.1 | NT | NT |
| **90** | Cycloheptyl | 3,5-Cl-4-$NH_2$-Ph | 3.9 | 9.7 | 10.9 | NT | NT |
| **91** | Methyl | 3,5-Cl-4-$NH_2$-Ph | 18.5 | 11.3 | 48.3 | NT | NT |
| **92** | Isobutyl | 3,5-Cl-4-$NH_2$-Ph | 8.9 | 9.7 | 10.8 | NT | NT |
| **93** | Pentyl | 3,5-Cl-4-$NH_2$-Ph | 2.5 | 5.3 | 6.8 | NT | NT |
| **94** | Benzyl | 3,5-Cl-4-$NH_2$-Ph | 4.9 | 5.5 | 14.6 | NT | NT |
| **95** | 2-Me-Bn | 3,5-Cl-4-$NH_2$-Ph | 2.1 | 2.6 | 37.6 | NT | NT |
| **96** | 3-Me-Bn | 3,5-Cl-4-$NH_2$-Ph | 2.5 | 2.9 | 52.8 | NT | NT |
| **97** | 4-Me-Bn | 3,5-Cl-4-$NH_2$-Ph | 6.2 | 5.9 | 9.3 | NT | NT |
| **98** | Octyl | 3,5-Cl-4-$NH_2$-Ph | 2.8 | 3.3 | 5.2 | NT | NT |
| **99** | Decyl | 3,5-Cl-4-$NH_2$-Ph | 2.3 | 2.8 | 3.0 | NT | NT |
| **100** | Dodecyl | 3,5-Cl-4-$NH_2$-Ph | 2.5 | 3.0 | 42.9 | NT | NT |
| **101** | Cyclooctyl | 3,5-Cl-4-$NH_2$-Ph | 2.3 | 6.3 | 31.7 | NT | NT |
| **102** | Cyclododecyl | 3,5-Cl-4-$NH_2$-Ph | 2.2 | 3.0 | 29.4 | NT | NT |
| **103** | 2-Norbornyl | 3,5-Cl-4-$NH_2$-Ph | 6.8 | 11.6 | 10.8 | NT | NT |
| **104** | Indan-2-yl | 3,5-Cl-4-$NH_2$-Ph | 2.4 | 4.8 | 11.4 | NT | NT |
| **105** | Naphthyl-1-Me | 3,5-Cl-4-$NH_2$-Ph | 1.5 | 1.9 | 53.8 | 78.1 | 25.2 |
| **106** | 2,2-DiphenylEt | 3,5-Cl-4-$NH_2$-Ph | 2.6 | 3.6 | 11.3 | NT | NT |

[a] Average values of $IC_{50}$ from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values. NT: not tested in non-malignant cells, the $IC_{50}$ value in chemosensitive CEM leukemia cells was >1 $\mu M$. For cell line characteristics and methodological details please refer to our previous publications.[9−11]

**Table 7. Best Compounds Resulting from the SAR Study**



R: Cyclooctyl, $IC_{50}$=0.62-0.75 μM, TI=17-43
R: Dodecyl, $IC_{50}$=0.25-0.56 μM, TI=160-393
R: Cyclododecyl, $IC_{50}$=0.26-0.77 μM, TI= 47-217

lipophilic carboxamide substitution. Also substitution of 6- and 7-isomers with heterocyclic ligands (compare entries **43**, **44** to **49**, **50**) gives similar results. Although it may appear that the activity does not depend on the position of the carboxamide group but rather on the overall polarity of compounds resulting from the carboxamide substitution, some specific exceptions can be found:

(i) For longer alkyl chains and larger cycloalkyls (C7−C12), 6-isomers give significantly better results for cell lines K562 and HCT116 in comparison to 7- and 8-isomers (compare entries **22**, **30**−**34** and **90**, **98**−**102** to **59**, **67**−**71**). Corresponding 7-carboxamides exhibit comparable activity only for CEM cells.

(ii) Brominated derivatives **13** and **83** exhibit similar (medium) cytotoxicity whereas the corresponding 6-isomer **52** is totally inactive.

In conclusion, we have synthesized and screened a library of 2-substituted-3-hydroxyquinolin-4(1*H*)-one-carboxamides and investigated the effect of both carboxamide substitution and its position on the resulting in vitro cytotoxicity. The results show that the activity depends mainly on the substitution of the carboxamide, but in specific cases also the position of the carboxamide on the quinolinone skeleton substantially changes the biological properties of the corresponding substrates. Fine-tuning the carboxamide substitution led to derivatives with submicromolar cytotoxic activity (relative $IC_{50}$ about 250 nmol) and some derivatives suitable for further biological research were identified with highly favorable TI ranging approximately from 17 to 400 (Table 7).

## ■ ASSOCIATED CONTENT

**Supporting Information.** Supporting Information contains details of the experimental synthetic and screening procedures and spectroscopic data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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## ■ REFERENCES

(1) Hradil, P.; Hlaváč, J.; Soural, M.; Hajdúch, M.; Kolář, M.; Večeřová, R. *Mini-Rev. Med. Chem.* **2009**, *9*, 696–702.

(2) Hradil, P.; Krejčí, P.; Hlaváč, J.; Wiedermannová, I.; Lyčka, A.; Bertolasi, V. *J. Heterocyclic Chem.* **2004**, *41*, 375–379.

(3) Krejčí, P.; Hradil, P.; Hlaváč, J.; Hajdúch, M. Patent WO 2008028427.

(4) Sui, Z. H.; Nguyen, V. N.; Altom, J.; Fernandez, J.; Hilliard, J. J.; Bernstein, J. I.; Barrett, J. F.; Ohemeng, K. A. *Eur. J. Med. Chem.* **1999**, *34*, 381–387.

(5) Sui, Z.; Jason, A.; Nguyen, V. N.; Fernandez, J.; Bernstein, J. I.; Hilliard, J. J.; Barrett, J. F.; Podlogar, B. L.; Ohemeng, K. A. *Bioorg. Med. Chem.* **1998**, *6*, 735–742.

(6) Iwanowicz, J. E.; Watterson, S. H.; Murali, T. G.; Pitts, W. J.; Gu, H. H. Patent WO 2001081340

(7) Soural, M.; Krchňák, V. *J. Comb. Chem.* **2007,** *9,* 793–796.
(8) Krupkova, S.; Soural, M.; Hlavac, J.; Hradil, P. *J. Comb. Chem.* **2009,** *11,* 951–955.
(9) Noskova, V.; Dzubak, P.; Kuzmina, G.; Ludkova, A.; Stehlik, D.; Trojanec, R.; Janostakova, A.; Korinkova, G.; Mihal, V.; Hajduch, M. *Neoplasma* **2002,** *49,* 418–425.
(10) Dzubak, P.; Hajduch, M.; Gazak, R.; Svobodova, A.; Psotova, J.; Walterova, D.; Sedmera, P.; Kren, V. *Bioorg. Med. Chem.* **2006,** *14,* 3793–3810.
(11) Sarek, J.; Klinot, J.; Dzubak, P.; Klinotová, E.; Nosková, V.; Krecek, V.; Korinkova, G.; Thomson, J. O.; Janostakova, A.; Wang, S.; Parsons, S.; Fisher, P. M.; Zhelev, N. Z.; Hajduch, M. *J. Med. Chem.* **2003,** *46,* 5402–5415.