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Efficient Synthesis and Cytotoxic Activity of Some Symmetrical Disulfides Derived from the Quinolin-4(1*H*)-one Skeleton

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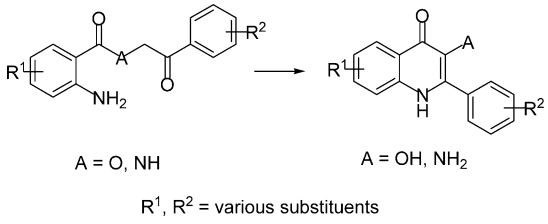
The preparation of novel organic disulfides containing the 2-(substituted phenyl)quinolin-4(1*H*)-one ring is described. The synthesis starts from thioanthranilic acid esterified with various bromoacetophenones. Cyclization of the resulting phenacyl thioanthranilates in trifluoroacetic acid afforded a mixture of 2-(substituted phenyl)-3-sulfanylquinolin-4(1*H*)-ones and 3,3′-disulfanediylbis[2-(substituted phenyl)quinolin-4(1*H*)-ones]. Heating of the mixture in *o*-xylene gave 3,3′-disulfanediylbis[2-(substituted phenyl)quinolin-4(1*H*)-ones] of high purity. The disulfides exhibited a significant in vitro cytotoxicity against various cancer cell lines including poly-resistant subclones. The data obtained are reported and discussed.

Introduction

Flavones and flavonols represent a well-known group of natural compounds that exhibit interesting biological activities of various kinds.[1] In contrast, their aza analogues, i.e derivatives containing the 3-hydroxyquinolin-4(1*H*)-one skeleton (termed "hydroxyquinolinones" hereafter), have not been studied so extensively due to their relative synthetic unavailability. Our department developed a new synthetic approach that allows the versatile and simple preparation of hydroxyquinolinones[2–4] as well as their 3-amino analogues[5] by taking advantage of the rearrangement of anthranilic acid derivatives (Scheme 1).



Scheme 1. General preparation of hydroxyquinolinones and aminoquinolinones.

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The compounds prepared according to this method were screened for their cytotoxicity by using the in vitro MTT test, and a significant activity of the hydroxyquinolinones[6–8] was detected, whereas 2-substituted 3-aminoquinolin-4(1*H*)-ones were inactive.

In the present study, our main goal was to extend our knowledge of the described rearrangement applicability. Considering the course of the cyclization of the anthranilic acid esters/amides we decided to test whether the same kind of rearrangement would take place for thioanthranilic acid esters. Theoretically, the products of such cyclizations should be derivatives of 2-phenyl-3-sulfanylquinolin-4(1*H*)-one (termed "sulfanylquinolinones" hereafter) that would give, after comparison with analogous hydroxyquinolinones and aminoquinolinones, useful information about structure/activity relationship in terms of position 3 of the 4(1*H*)-quinolinone moiety.

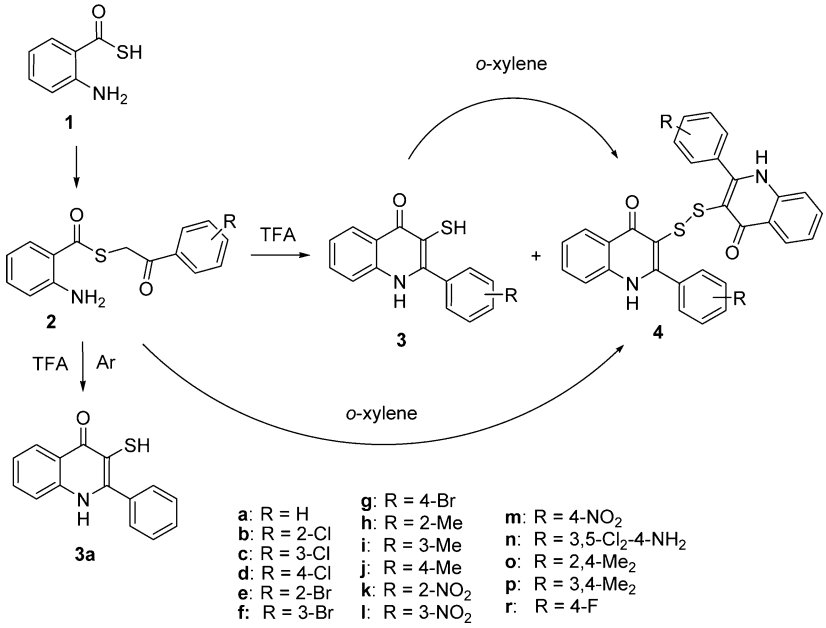
Results and Discussion

Synthesis

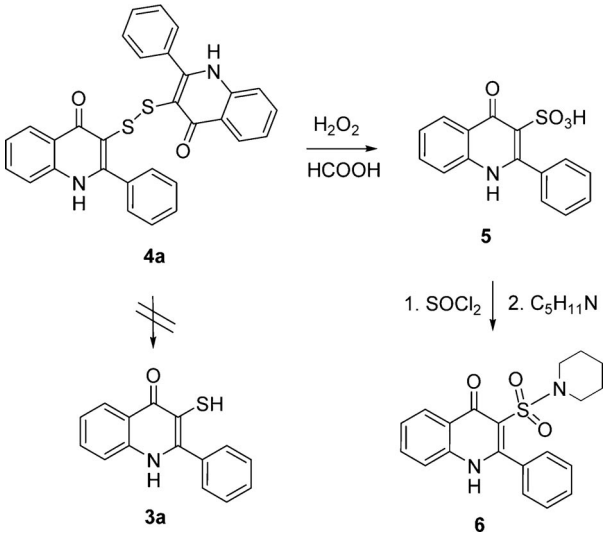
Thioanthranilic acid (**1**) was synthesized from isatoic anhydride and hydrogen disulfide according to a published protocol.[9] We observed that it was not necessary to run this reaction for 24 h as described by those authors; 2 h were sufficient for the conversion of isatoic anhydride to thioanthranilic acid, and the continuous addition of hydrogen disulfide could be stopped. The isolated thioanthranilic acid was esterified with a set of bromoacetophenones containing different substituents in various positions. The corresponding phenacyl thioanthranilates **2** were obtained. When refluxed in trifluoroacetic acid, they cyclized after a

short time to the expected sulfanylquinolinones **3**. However, the products were always accompanied by 40–50% of the corresponding 3,3′-disulfanediylbis[2-(substituted phenyl)-quinolin-4(1*H*)-ones] (**4**) (termed "disulfides" hereafter). We tried to suppress this unwanted oxidation reaction by using different cyclizing agents (such as formic acid and polyphosphoric acid) and by carrying out the reaction in degassed TFA under argon, but formation of disulfides persisted in each case. The only exception was the unsubstituted phenacyl thioanthranilate (**2a**) which, after cyclization under the inert gas, afforded the pure sulfanylquinolinone **3a**. Interestingly, the resulting ratio of disulfides in the mixtures obtained was not significantly increased when the cyclization in TFA was carried out for a longer period. The quantitative oxidation to disulfides **4** was then performed by briefly refluxing the mixtures in *o*-xylene after previous evaporation of trifluoroacetic acid. After cooling to room temperature, the disulfides **4** precipitated from *o*-xylene in excellent purity without the need of further purification. Starting from isatoic anhydride, the reaction sequence described represents a quite simple 4-step route to disulfides **4**, which can be accomplished within 1 d. We also found that it was possible to shorten the sequence by cyclizing phenacyl anthranilates **2** to disulfides **4** directly by heating in *o*-xylene, but using this procedure the yields were substantially lower than precyclization in trifluoroacetic acid. Unfortunately, the latter alternative also could not be applied for the preparation of sulfanylquinolinones **3**, because using degassed *o*-xylene and the inert gas led only to the corresponding disulfides **4** again (Scheme 2). The above described results indicate that the formation of disulfides was not influenced only by the presence of oxygen in the reaction mixtures but might be caused by harsh conditions needed for the rearrangement. When disulfides **4b**, **4e**, **4h**, **4k**, **4o** were analyzed by ^{1}H NMR spectroscopy, we observed an interesting anisochronity of the hydrogen atoms of the individual quinolinone skeletons. Since the effect was observed only for the derivatives with *ortho* substitution of the 2-phenyl moiety, we suppose this fact to be caused by the hindered S–S bond rotation resulting in atropisomerism.

As the cyclization was not generally usable for the preparation of sulfanylquinolinones **3** but for effective synthesis of disulfides **4**, we turned our attention to a possible reduction of the disulfidic bond, which would afford the required sulfanyl derivatives **3**. We chose derivative **4a** as a starting material and the only available pure sulfanylquinolinone **3a** as a reference sample for the reduction evaluation. After an unsuccessful attempt with sodium borohydride in dimethylformamide, the reduction with zinc in acetic acid was tested. In this case the reduction took place, but we observed the absence of the NMR signal for the sulfanyl group in the ^{1}H NMR spectrum of sulfanylquinolinone **3a** (δ = 5.52 ppm) indicating probable formation of a stable Zn^{2+} complex. Unfortunately, the attempt to liberate the sulfanyl group by acidic hydrolysis with degassed TFA caused partial back-oxidation to disulfide **4a**. As another possible synthetic use of the disulfides the oxidative destruction of the disulfidic bond was tested. Using unsubstituted disulfide **4a** and oxidation with hydrogen peroxide in formic acid we smoothly prepared 4-oxo-2-phenyl-1,4-dihydroquinoline-3-sulfonic acid (**5**), which was subsequently transformed via its chloride into the model sulfonamide derivative **6**. This example represents a possible extension of the disulfide chemistry to another group of novel 4-quinolinone derivatives with potential biological activity (Scheme 3).



Scheme 2. Preparation and cyclization reactions of phenacyl thioanthranilates.



Scheme 3. Chemical transformations of disulfide **4a**.

Biological Activity

All synthesized phenacyl thioanthranilates **2** and disulfides **4** were tested for their in vitro cytotoxic activity against various human malignant cell lines by using the MTT test.[10] Phenacyl thioanthranilates **2** did not exhibit any significant activity ($IC_{50} > 36$ μM) except derivative **2n** with medium cytotoxicity against CEM ($IC_{50} = 11.3$ μM). The general cytotoxicity of disulfides **4** was substantially higher, in the range $IC_{50} = 0.73$–33.1 μM (Table 1). In most cases, except for the diametrically different cytotoxicity of derivatives **4k** and **4n**, the tested substituents as well as their position on the 2-phenyl moiety did not dramatically influence the activity/selectivity. Although comparison of the cytotoxicity results for derivatives **2** and **4** generally shows the manifest necessity of the presence of the quinolinone moiety, one exception was detected: derivatives **2k** and **4k** exhibited the same cytotoxicity against CEM. Derivatives **2n** and **4n** were evaluated as the most active ones from each group. This confirms our previous promising experience with such substituted hydroxyquinolinones.[6] Derivative **4n** exhibited even submicromolar activity except against the chemotherapy-resistant subclones CEM-DNRB and K562-Tax.[11]

Table 1. Cytotoxicity results for compounds **4** (IC_{50} [μM]).[a]

	CEM	CEM-DNRB	K562	K562-Tax	A549
4a	2.6	3.4	2.3	8.2	2.7
4b	8.2	8.8	3.5	11.5	3.2
4c	2.9	6.2	2.7	9.3	5.4
4d	3.8	5.3	4.5	10.9	4.7
4e	4.4	8.5	3.5	11.4	3.1
4f	2.8	11.1	2.9	18.8	5.2
4g	3.5	8.4	4.1	18.8	5.2
4h	2.6	6.7	3.2	7.8	3.0
4i	2.9	7.1	3.3	11.5	3.2
4j	8.2	9.9	5.3	14.3	2.7
4k	42.7	24.7	12.4	33.1	3.1
4l	9.9	7.6	7.9	12.4	4.7
4m	7.8	7.4	9.1	17.2	6.1
4n	0.73	3.54	0.73	2.4	0.95
4o	2.6	9.2	3.6	14.7	3.0
4p	8.8	10.2	11.2	31.7	4.0
4r	2.9	9.7	3.3	11.1	3.3
4s	4.4	5.0	3.1	9.9	4.4

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

The cytotoxicity of the sulfonamide **6** and of the single successfully prepared sulfanylquinoline **3a** has also been demonstrated. Comparison of **3a** with the corresponding disulfide **4a** shows a 2.5–15 times lower cytotoxicity against all tested cell lines (Table 2).

Table 2. Cytotoxicity results for compounds **3a** and **6** (IC_{50} [μM]).[a]

	CEM	CEM-DNRb	K562	K562-Tax	A549
3a	11.0	9.7	8.2	13.2	3.8
6	201.5	115.2	154.5	134.0	190.0

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

Conclusions

Our general synthesis of sulfanylquinolinones using the rearrangement of phenacyl thioanthranilates was unsuccessful. However, the disulfides obtained as unwanted reaction products were identified as an interesting group of derivatives with significant activity against human malignant cell lines including chemotherapy-resistant subclones. One example allows the prediction that the disulfides may exert an even higher cytotoxicity compared to the sulfanylquinolinones that were the original target of the research. In addition, the prepared disulfides can serve as a starting material for the synthesis of various novel derivatives of 4(1*H*)-quinolinone, as an example a synthetic route leading to sulfonamides was demonstrated. The disulfides were active against childhood acute T-lymphoblastic leukemia cells and thus form a new group of lead compounds for further studies as potential agents in this orphan disease.

Experimental Section

Chemical Synthesis

General Procedure for the Preparation of Phenacyl Thioanthranilates 2: Thioanthranilic acid (2 g, 13 mmol) was dissolved in DMF (10 mL), and triethylamine (1.4 mL, 13 mmol) was added. After stirring at room temperature for 10 min, bromoacetophenone (13 mmol) was added, and the reaction mixture was stirred for another 60 min. Then ice-cold water was poured into the mixture. The precipitated material was collected by suction and washed with water. If the precipitation did not occur, the product was extracted with diethyl ether, which was subsequently evaporated in vacuo. Crude products were crystallized from a mixture of ethanol/diethyl ether to give the corresponding anthranilates as light yellow crystals.

General Procedure for the Preparation of Disulfides 4. Method A (Used for the Synthesis): Phenacyl thioanthranilate **2** (100 mg) was

refluxed in trifluoroacetic acid (1 mL) for 60 min. Then the trifluoroacetic acid was evaporated in vacuo, and *o*-xylene (1 mL) was added. The reaction mixture was refluxed for 30 min and subsequently cooled to room temperature. The precipitated material was collected by suction, washed with *o*-xylene, ethanol and dried. **Method B:** Phenacyl thioanthranilate **2** (100 mg) was refluxed in *o*-xylene (2 mL) for 120 min. After cooling to room temperature, the precipitated material was collected by suction, washed with *o*-xylene, ethanol and dried.

Preparation of 2-Phenyl-3-sulfanylquinolin-4(1*H*)-one (3a): Phenacyl thioanthranilate **2a** (100 mg) was refluxed in degassed trifluoroacetic acid (1 mL) under argon for 120 min. Then the reaction mixture was poured into ice-cold water, and the precipitated material was collected by suction, washed with water and dried. An analytical sample was crystallized from ethanol.

Preparation of 4-Oxo-2-phenyl-1,4-dihydroquinoline-3-sulfonic Acid (5): Disulfide **4a** (0.66 g, 1.3 mmol) was dissolved in formic acid (5 mL), and 25% hydrogen peroxide (1.8 mL, 13 mmol) was added. After 30 min of stirring at room temperature, the precipitated white solid was collected by suction and washed with water.

Preparation of 2-Phenyl-3-(piperidin-1-ylsulfonyl)quinolin-4(1*H*)-one (6): Sulfonic acid **5** (200 mg. 0.6 mmol) was dissolved in pyridine (0.3 mL), and thionyl chloride (0.6 mL, 8 mmol) was added dropwise. The reaction mixture was stirred at 50 °C for 2 h and then concentrated to dryness. The resulting oily residue was suspended in pyridine (1 mL), and piperidin (0.75 mmol) was added. The reaction mixture was stirred at 50 °C for 2 h, and the solvents were evaporated to dryness. The resulting residue was suspended in cold water (10 mL) and collected by suction. The crude product was crystallized from ethanol.

Biological Activity

Cell Lines: All cells were purchased from the American Tissue Culture Collection (ATCC), unless otherwise indicated. The daunorubicin-resistant subline of CEM cells (CEM-DNR bulk) and paclitaxel-resistant subline K562-tax were selected in our laboratory by the cultivation of maternal cell lines in increasing concentrations of daunorubicine or paclitaxel, respectively.[11] The cells were maintained in Nunc/Corning 80 cm^2 plastic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum, and $NaHCO_3$).

Cytotoxic MTT Assay:[10,11] The cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2500–30000 cells/well based on cell growth characteristics). The cells were added by pipette (80 μL) into 96-well microtiter plates. The inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5% CO_2 for stabilisation. Four-fold dilutions, in 20-μL aliquots, of the intended test concentration were added to the microtiter plate wells at time zero. All test compound concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in 5% CO_2 at 100% humidity. At the end of the incubation period, the cells were assayed by using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period, the formazan produced was dissolved by the addition of 100 μL/well of 10% aq. SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumour cell survival (TCS) was calculated by using the following equation: $TCS = (OD_{drug\text{–}exposed\ well}/\text{mean}\ OD_{control\ wells}) \times 100\%$. The TCS_{50} value, the drug concentration lethal to 50% of the tumour cells, was calculated from appropriate dose-response curves.

Supporting Information (see footnote on the first page of this article): Analytical data of the compounds prepared.

Acknowledgments

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[1] G. Brahmachari, D. Gorai, *Curr. Org. Chem.* **2006**, *10*, 873–898.
[2] P. Hradil, J. Jirman, *Collect. Czech. Chem. Commun.* **1995**, *60*, 1357–1366.
[3] P. Hradil, J. Hlaváč, K. Lemr, *J. Heterocycl. Chem.* **1999**, *36*, 141.
[4] P. Hradil, L. Kvapil, J. Hlaváč, T. Weidlich, A. Lyčka, K. Lemr, *J. Heterocycl. Chem.* **2000**, *37*, 831.
[5] P. Hradil, M. Grepl, J. Hlaváč, M. Soural, M. Maloň, V. Bertolasi, *J. Org. Chem.* **2006**, *71*, 819.
[6] M. Soural, J. Hlaváč, P. Hradil, I. Fryšová, M. Hajdúch, V. Bertolasi, *Eur. J. Med. Chem.* **2006**, *41*, 467–474.
[7] P. Krejčí, P. Hradil, J. Hlaváč, M. Hajdúch, Patent WO2008028427, 2008.
[8] P. Hradil, P. Krejčí, J. Hlaváč, I. Wiedermannová, A. Lyčka, V. Bertolasi, *J. Heterocycl. Chem.* **2004**, *41*, 375–379.
[9] A. T. Fanning, G. R. Bickford, T. D. Roberts, *J. Am. Chem. Soc.* **1972**, *94*, 8505–8506.
[10] M. Hajdúch, V. Mihal, J. Minarik, E. Faber, M. Safarova, *Cytotechnology* **1996**, *19*, 243–245.
[11] V. Nosková, P. Džubák, G. Kuzmina, A. Ludková, D. Stehlík, R. Trojanec, A. Janošťáková, G. Kořínková, V. Mihál, M. Hajdúch, *Neoplasma* **2002**, *49*, 418–425.

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