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Synthesis, antioxidant and antiproliferative activities of 1,3,4-thiadiazoles derived from phenolic acids

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ABSTRACT

Two 2-amino-1,3,4-thiadiazoles containing phenolic hydroxyl groups were combined with different carboxylic acid chlorides giving sixteen amide derivatives with good antioxidant and antiproliferative potential. The compound **3′c** with an adamantane ring displayed excellent DPPH radical scavenging activity and good cytotoxic activity against human acute promyelocytic leukemia HL-60 cells, while 1,3,4-thiadiazole **3′h** with 4-chlorophenyl moiety was found to be the most effective in inhibition of survival of lung carcinoma A549 cells. All examined thiadiazoles except **3a** and **3′a** exerted higher cytotoxic activities on A549 and HL-60 cancer cells when compared with normal fibroblasts MRC-5, pointing to selectivity in their antiproliferative action. Some of the most active novel compounds **3c**, **3′c**, **3′g** and **3′h** induced significant increase in the percentage of HL-60 cells in the subG1 cell cycle phase in comparison with the control cells. The induction of cell death in HL-60 cells by these compounds was at least partially dependent on activation of caspase-3 and caspase-8. The compounds **3c** and **3′c** exerted strong antiangiogenic activity. Furthermore, compounds **3c**, **3′c**, **3′g** and **3′h** showed the ability to down-regulate the *MMP2* and *VEGFA* expression levels in the treated HL-60 cells when compared with the control cell samples.

Antioxidants are compounds that, in low concentration, are able to delay or prevent the oxidation of biomolecules (proteins, nucleic acids, lipids, sugars) and inhibit oxidative stress, DNA mutations, malignant changes and other forms of cell damage.[1] Phenolic compounds are a large group of substances that have recently received much attention due to their antioxidant properties. Numerous investigations relating to their radical-scavenging activity include structure-activity-relationship studies, reaction kinetics of polyphenols with radicals, substituent influence, number and arrangement of phenolic hydroxyl groups in the molecule and solvent effects.[2–5] Among phenolic compounds, the antioxidant activity of phenolic acids has attracted more attention because of their ubiquitous occurrence in nature and as potential models for the synthesis of new primary radical scavengers.[6] One of the most important phenolic acids, protocatechuic acid (3,4-dihydroxybenzoic acid), found in edible plants, vegetables and fruits, is known to exhibit potent antioxidant activity demonstrating the preventive effect on malignant diseases that are associated with radical species.[4,7–9]

Also, 2,3-dihydroxybenzoic acid, as a potent iron chelator, exerts an important protective effect against the cytotoxic action of H_2O_2 significantly increasing cell survival.[10] Moreover, the stable antioxidant molecules neutralize reactive oxygen species (ROS) by an electron transfer mechanism and diminish their DNA damaging ability and cancer formation. Thus, compounds exhibiting both antioxidant and antiproliferative potential are of great importance in discovery of new anticancer agents.

One of the well-known pharmacophores is 1,3,4-thiadiazole heterocyclic scaffold incorporated in many heterocyclic compounds with various grades of antiproliferative activity.[11–13] A series of 5-(2,5-dimethoxyphenyl)-1,3,4-thiadiazole-2-amino derivatives has been synthesized and screened for cytotoxic activity against HT-29 and MDA-MB-231 cancer cells.[14] New 2-arylamino-5-aryl-1,3,4-thiadiazoles displayed potent anticancer potential against several cell lines with IC_{50} values from 4.3 to 9.2 μM.[15] Revelant et al. prepared novel 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles and tested them against a panel of six cancer cell lines with IC_{50} values from <10 μM in some experiments.[16] Finally, X.-H. Yang. et al. presented a series of 1,3,4-thiadiazole-2-amide derivatives as potential anticancer agents with good potential in inhibition of MCF-7 and B16-F10 cell

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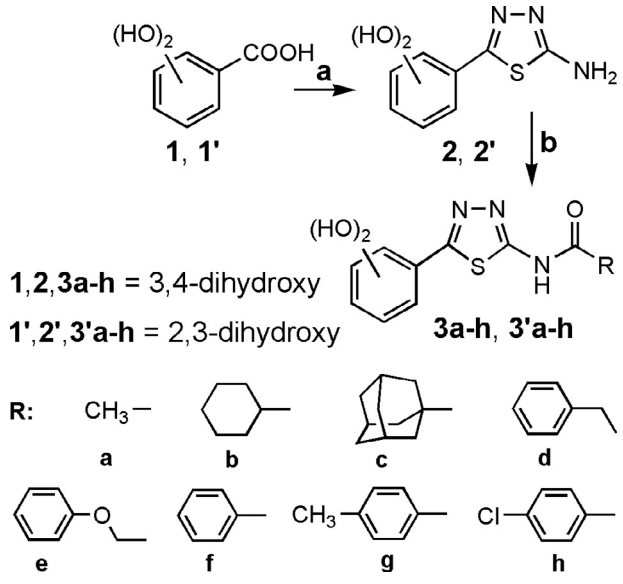
proliferation.[17] These points prompted us to combine the bioactive functions of 1,3,4-thiadiazole with those of a phenol acid moiety with the intention of synthesizing novel conjugates possessing antioxidant and antiproliferative properties.

The title molecules were synthesized in two steps as shown in Scheme 1. Heterocyclic 1,3,4-thiadiazole precursors **2** and **2′** were obtained by reacting 3,4-dihydroxybenzoic **1** and 2,3-dihydroxybenzoic acid **1′** with thiosemicarbazide in the presence of phosphoryl chloride.[17] In the next step, a coupling reaction between 5-substituted-1,3,4-thiadiazol-2-amines **2** and **2′** and different carboxylic acid chlorides was performed in tetrahydrofuran or dioxane to give the final amide derivatives **3a–h** and **3′a–h**. The solid sodium hydrogencarbonate was used for neutralization of liberated hydrogen chloride. In some cases, the reaction of **2** and **2′** with RCOCl requires a long period of time (even at reflux conditions) and an excess of carboxylic acid chlorides was necessary for its completion. Except **3f** and **3′f**, all other compounds were prepared for the first time with satisfactory analytical and spectroscopic data (Supplementary Material).

To confirm whether the protocatechuic acid shows better scavenging activity than its derivatives, a series of esters[18] and amides[19] were synthesized and screened for their antioxidant potential. The obtained results suggest that the slow DPPH scavenging activity of the protocatechuic acid compared with its derivatives is due to the dissociation of the carboxyl group since it decreases the electron-withdrawing property of the substituent; this leads to low susceptibility of the formed quinone toward a nucleophilic attack by a solvent molecule.[20] Better radical scavenging activity of phenolic acid derivatives containing 1,2,4-triazole[21] and 1,3,4-oxadiazole[22] in comparison with parent acids was recently determined and attributed to the participation of the heterocyclic scaffold in resonance stabilization of the formed radical after homolytic cleavage of the O—H and N—H bonds by the DPPH radical, as it was previously demonstrated by DFT calculations for 1,2,4-triazole derivatives.[21] Similarly, antioxidant capacity of 1,3,4-thiadiazoles derived from phenolic acids is related to their ability to release hydrogen atoms, either from nitrogen or oxygen; this leads to resonance stabilization of the obtained radical. The resulting phenoxyl or nitrogen radical can be highly stabilized through resonance since the unpaired electron may be additionally delocalized across 1,3,4-thiadiazole ring (Scheme 2).



Scheme 1. Reagents and conditions: a) $POCl_3$, thiosemicarbazide, 1 h, reflux; b) RCOCl, THF or dioxane, 24 h r.t. or 12 h reflux.

The nature of the R-substituents, reasonably selected to cover electron-donating, electron-withdrawing and steric properties, strongly influenced DPPH scavenging activity. Electron-donating groups stabilize free radical intermediates formed after abstraction of a hydrogen atom from oxygen or nitrogen, increasing electron density on the radical center. Generally, all synthesized 1,3,4-thiadiazole derivatives showed moderate to excellent antioxidant capacity, with IC_{50} values in the range of 17.85–52.97 μM for 3,4-dihydroxybenzoic acid derivatives and 14.21–111.32 μM for 2,3-dihydroxybenzoic acid derivatives (Table 1).

It can be noticed that for both groups of compounds **3a–h** and **3′a–h**, thiadiazoles **3c** and **3′c** with the adamantyl group as a substituent (electron-donating group) showed the highest antioxidant capacity, with IC_{50} values significantly lower than IC_{50} values of reference standards, ascorbic acid and nordihydroguaiaretic acid (NDGA). Results of antioxidant activity for 2,3-derivatives are in agreement with substituent effects on resonance stabilization, with the exception of compound **3′a**, which showed lower antioxidant capacity than expected. Also, the order of activity of compounds **3′f** and **3′g** was disrupted, because compound **3′g** has an electron donating methyl group in its structure which influences resonance stabilization. It would be expected to have better scavenging activity than **3′f**. Results of antioxidant activity for thiadiazoles derived from 3,4-dihydroxybenzoic acid are not in good agreement with the substituent effects on resonance stabilization, probably as a consequence of other factors, like the influence of solvent or intermolecular hydrogen bonds.[20]

Cytotoxic activity of all synthesized 1,3,4-thiadiazole compounds was evaluated against three human malignant cell lines (HL-60, HeLa, and A549) and a normal, non-transformed MRC-5 cells using MTT cell survival test. The obtained IC_{50} values are shown in Table 2. Both series of thiadiazoles exerted the most pronounced activity against the human acute promyelocytic leukemia HL-60 cells, with adamantane containing derivatives **3c** and **3′c** as the most active ones showing IC_{50} values of 7.4 and 7.3 μM, respectively. Furthermore, these two compounds displayed the strongest DPPH radical scavenging activity. There is no significant difference between the influence of electron-donating and electron-withdrawing groups in **3g** and **3h** and especially in **3′g** and **3′h**, on the cytotoxic action of HL-60 and A549 cells. The derivatives containing a cyclohexyl group, **3b** and **3′b**, also exhibited high cytotoxic activity on HL-60 cells, while those with *p*-substituted phenyl group **3g**, **3′g** and **3′h** were the most active compounds containing an aromatic ring. The examined compounds with the exception of **3a** and **3′a** showed good cytotoxic activity against human lung carcinoma A549 cells, where **3c**, **3f**, **3g** and the compounds from series **3′b–h** showed similar or slightly better cytotoxicity comparing to cisplatin as a referent chemotherapeutic. In this case, the best activity was observed for the compound containing *p*-chlorophenyl moiety in the molecule structure, **3′h** (IC_{50} = 9.5 μM). This compound also exhibited good activity on human cervical adenocarcinoma HeLa cells, with IC_{50} value of 13.0 μM. Generally, 1,3,4-thiadiazoles bearing voluminous adamantyl and cyclohexyl substituents showed the best activity against HL-60 cells, while HeLa cells were the most sensitive to compounds with planar aromatic rings from **3′**-series. In addition, human cervical adenocarcinoma HeLa cells were the least sensitive to the cytotoxic activity of the examined thiadiazoles. The cytotoxicity against normal human lung fibroblasts MRC-5 cells was considerably lower for all of the tested compounds in comparison with the referent drug cisplatin. All examined 1,3,4-thiadiazoles from **3-** and **3′-** series except **3a** and **3′a** exerted higher cytotoxic activities on A549 and HL-60 malignant cell lines when compared with normal MRC-5 cells.

Since the compounds **3c**, **3′c**, **3′b** and **3′e** exerted the highest antioxidant activity, we decided to explore their possible