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Original article

New enantiomeric fluorine-containing derivatives of sulforaphane: Synthesis, absolute configurations and biological activity

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ABSTRACT

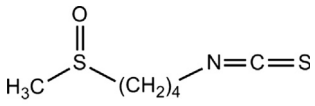
Three pairs of enantiomers of the unknown sulforaphane analogs bearing organofluorine substituents bonded to the sulfinyl sulfur atom and having different number of methylene groups in the central carbon chain were synthesized and fully characterized, including determination of their absolute configurations. All the new compounds were tested *in vitro* for their cytotoxicity against melanoma cells to show increased activity in comparison with the natural sulforaphane. The influence of the particular structural changes in the molecule on the cytotoxicity is discussed.

1. Introduction

In spite of great medical progress, cancer continues to be one of the most deadly diseases. Hence, the search for new anticancer drugs is still an important subject of interest for various kinds of researchers.

It has been found that the consumption of cruciferous vegetables, in particular broccoli, may suppress the chemical carcinogenesis. This suppression is considered to arise from the presence of the organic isothiocyanates, which are formed by enzymatic transformation of certain phytochemicals, glucosinolates, naturally occurring in those types of vegetables. Isothiocyanates are strong inducers of phase II enzymes and inhibitors of phase I enzymes of xenobiotic transformation and therefore are considered as cancer chemopreventing and chemotherapeutic agents. Recently several isothiocyanates have been shown to induce apoptosis in many cell lines, like in prostate cancer, T-lymphocytes, T-leukemia or colon carcinoma [1–4].

Sulforaphane **1** is a naturally occurring isothiocyanate which is especially abundant in broccoli. It is considered to be one of the most promising anticancer agents and has attracted significant attention since its identification in 1992 [5]. Thus, sulforaphane itself and various types of its analogs and derivatives have been a subject of intense investigations in recent years, including synthesis [6–9] and evaluation of their anticancer properties [1–4]. For example, previous studies have revealed that sulforaphane **1**, its homolog alyssin and its sulfur-free analog, 2-oxohexyl isothiocyanate induce apoptosis in human melanoma and murine leukemia cell lines [10,11] and that sulforaphane blocks the cell cycle in lymphoblastoid cells bearing various inherited BRCA1 mutations [12,13].



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On the basis of our experience in the preparation of chiral sulfur derivatives [14–16], we envisaged to synthesize new, so far unknown, analogs of sulforaphane, in which the S-methyl group will be replaced by fluorinated alkyl groups and to obtain them in the form of pure enantiomers. By introducing the fluorine atom into the sulforaphane molecule we expected to properly change its original chemical and biological properties and to enhance its anticancer activity. This hypothesis is based on literature reports indicating that the introduction of fluorine atoms into organic molecules has a great impact on their biological activity [17–20], which is visible in the fact that a great number of modern medicines contain this element as a substituent in various parts of the molecule.

Among various types of cancer, melanoma is the most dangerous form of skin cancer causing a high death rate (mortality) of the patients. Since sulforaphane was found to exhibit certain activity against melanoma, we decided to check whether its newly prepared fluorine-containing derivatives would exhibit an enhanced anti-melanoma properties. To establish a possible dependence of the activity on the stereochemistry of the compounds, biological activity of each enantiomer of the new derivatives was determined.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis

The aim of this part of the work was to elaborate the methods of synthesis of new biologically active analogs of sulforaphane in which the methyl group that is bound to the central sulfur atom would be replaced by various organofluorine substituents R_F. Three derivatives have been chosen as typical examples of these kinds of compounds: 4-isothio-cyanato-1-butyl trifluoromethyl sulfoxide **2**, 4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide **3** and its homolog, 5-isothiocyanato-1-pentyl 2′,2′,2′-trifluoroethyl sulfoxide **4**. They are depicted in Scheme 1.

They have been prepared in 3–4 steps from commercially available fluoro substrates. Two routes of the formation of the important sulfur – R_F bond, have been applied depending on the commercial availability of the organofluorine substrates. As can be seen, compound **2** differs from compounds **3** and **4** by the presence of a direct bond between sulfur and the carbon bearing three fluorine atoms. Formation of this crucial bond, which is known to be troublesome, requires special conditions. The synthesis of compound **2** is shown in Scheme 2.

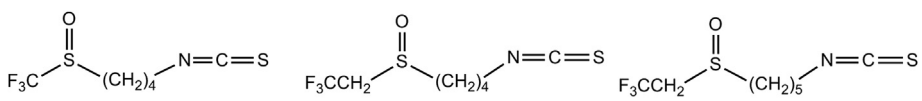
The commercially available substrate, 1-bromo-4-N-phtalimidobutane **5**, was treated either with sodium hydrogen sulfide or with thiourea, followed by buffer (pH 7.5), to give 4-N-phthalimidobutane-1-thiol **6**. In the latter case application of the buffer instead of sodium hydroxide, which is commonly used in this type of synthesis of thiols, was necessary to avoid opening of the phthalimido ring [21]. Preparation of thiol **6** proved essential for the synthesis of 4-N-phthalimido-1-butyl trifluoromethyl sulfide **7**, because iodotrifluoromethane is the only commercially accessible source of the trifluoromethyl group. Since it is known that the reaction of thiols with fluoroorganyl halides does not proceed according to a simple nucleophilic substitution mechanism, special conditions had to be applied which included the use of sodium hydroxymethanesulfinate ("Rongalite") in the presence of DMF and trisodium phosphate [22]. The desired sulfide **7** was obtained in moderate yield. Its further transformations comprised two paths. Removal of the phthalimido group by hydrazine, followed by hydrochloric acid, gave 4-amino-1-butyl trifluoromethyl sulfide hydrochloride **9**, which was *in situ* treated with thiophosgene (or its synthon, bis(O-2-pyridyl) thiocarbonate [23]) to yield 4-isothiocyanato-1-butyl trifluoromethyl sulfide **10**. Oxidation of **10** with *meta*-chloroperbenzoic acid (*m*-CPBA) resulted in the desired product, 4-isothiocyanato-1-butyl trifluoromethyl sulfoxide **2**. Alternatively, sulfide **7** was first oxidized to 4-N-phthalimido-1-butyl trifluoromethyl sulfoxide **8**, which, after similar treatment as that described above, led to sulfoxide **2**. It should be stressed that trifluoromethyl sulfides are strongly resistant to oxidation and, from among a variety of oxidizing agents used, only *m*-CPBA proved relatively efficient [24].

Synthesis of 4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide **3** and 5-isothiocyanato-1-pentyl 2′,2′,2′-trifluoroethyl sulfoxide **4** turned out to be relatively easier because of the commercial availability of 2,2,2-trifluoroethanethiol. Hence, its crucial reaction leading to sulfides **12** and **13** could be performed directly with the commercially accessible substrates **5** and **11**. The sulfides **12** and **13** were then treated in similar ways as the sulfide **7** which ultimately allowed to obtain the targeted products **3** and **4**. (Scheme 3). It is worth noting that the overall yields were in these cases higher than in the synthesis of product **2**, including the oxidation step of sulfides **12, 13, 17** and **18**. The latter can be explained in terms of the lack of a direct bond between sulfur and the trifluoroalkyl group.

2.1.2. Stereochemistry. Determination of absolute configurations

All the synthetic procedures presented above led to the formation of the targeted products in the racemic form. Several attempts were made to obtain enantiomers of each derivative via asymmetric oxidation of the appropriate sulfides: **7**, **9**, **10**, **12**–**15**, **17** and **18**. Thus, application of an oxidative enzyme, chloroperoxidase from *Caldariomyces fumago*, which is known for its highly efficient and stereoselective oxidation of alkyl aryl sulfides [25] and methionine derivatives [26] to the corresponding sulfoxides, failed. From among all the sulfides checked, only compounds **14** and **15** underwent slow oxidation to the corresponding sulfoxides. However, the reaction was completely non-stereoselective. Similarly, an attempt at the asymmetric synthesis via oxidation of sulfide **18** using Davis oxaziridines [27] gave racemic sulfoxide **3**.

Taking into account our original plans to synthesize all new derivatives in enantiomerically pure forms in order to check their biological activities, we decided to perform resolution of the racemic products obtained by preparative HPLC using various chiral columns and the recycling HPLC instrument. Four compounds, namely **2**, **3**, **4** and **8**, were resolved into enantiomers and their stereochemistry data are collected in Table 1. The enantiomerically pure products were obtained after additional crystallization and the appropriate crystals of compounds **3**, and **8** were subjected to X-ray analysis. On the basis of the molecular structures their absolute configurations were determined as (−)-(*S*)-**3** and (−)-(*S*)-**8** (see Table 3 for details). Since the enantiomers of compound **2** were



2 **3** **4**

Scheme 1. Our targeted fluoro analogs of sulforaphane.