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Original article

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

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ABSTRACT

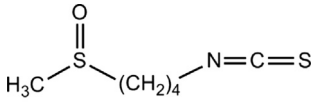
Three pairs of enantiomers of the unknown sulforaphane analogs bearing organofluorine substituents bonded to the sulfinyl sulfur atom and having different number of methylene groups in the central carbon chain were synthesized and fully characterized, including determination of their absolute configurations. 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.

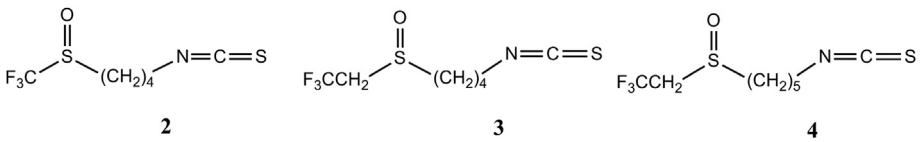
The commercially available substrate, 1-bromo-4-N-phtalimidobutane **5**, was treated either with sodium hydrogen sulfide or with thiourea, followed by buffer (pH 7.5), to give 4-N-phthalimidobutane-1-thiol **6**. In the latter case application of the buffer instead of sodium hydroxide, which is commonly 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

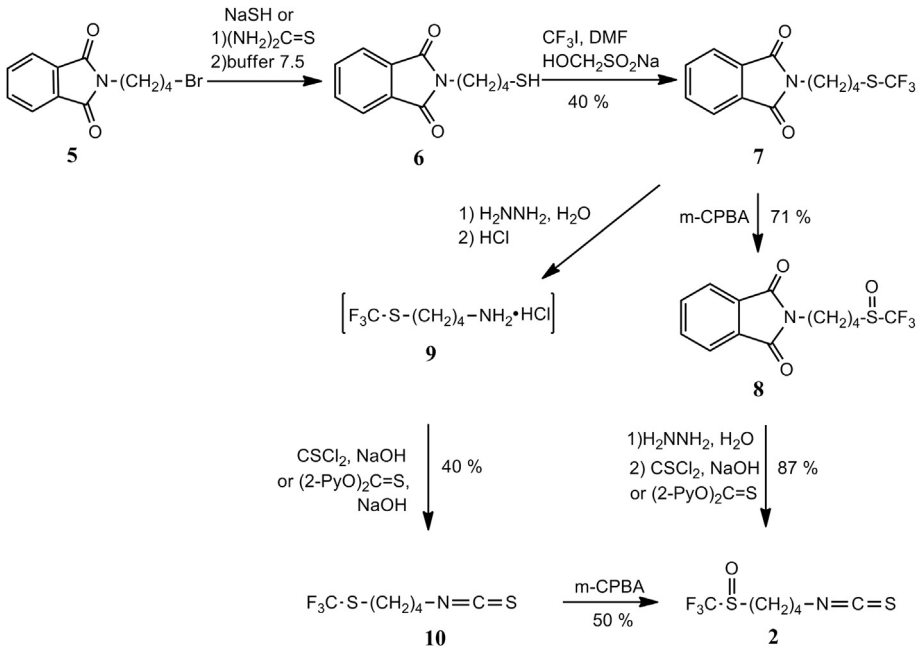
All the synthetic procedures presented above led to the formation of the targeted products in the racemic form. Several attempts were made to obtain enantiomers of each derivative via asymmetric oxidation of the appropriate sulfides: **7**, **9**, **10**, **12**–**15**, **17** and **18**. Thus, application 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



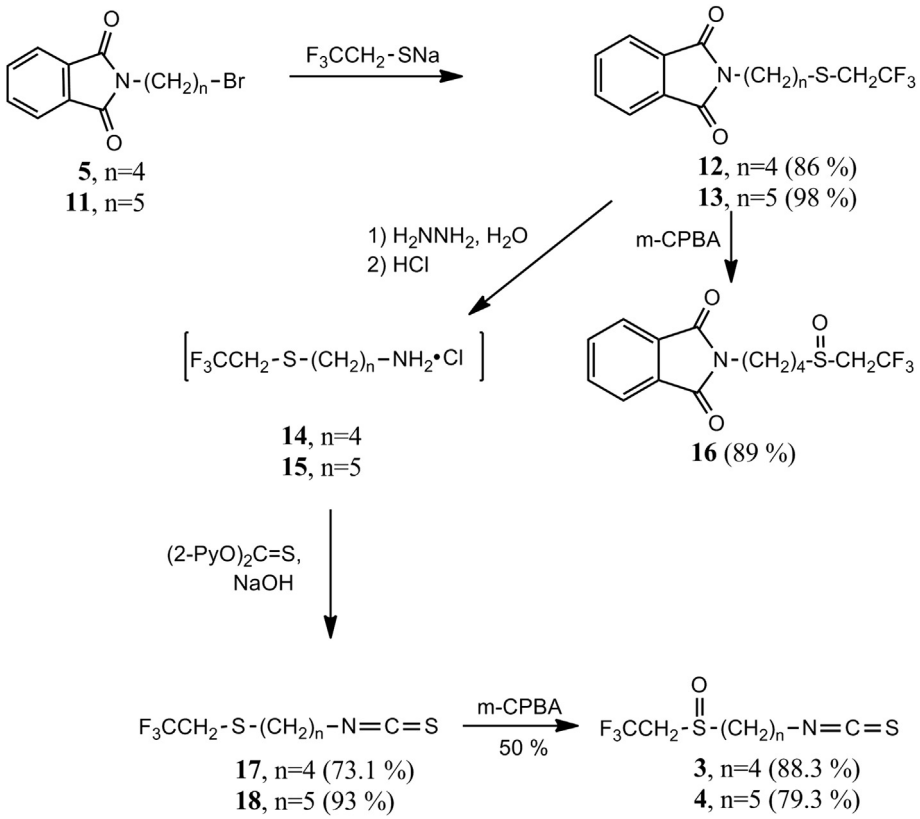
2 **3** **4**

Scheme 1. Our targeted fluoro analogs of sulforaphane.



Scheme 2. Synthesis of 4-isothiocyanato-1-butyl trifluoromethyl sulfoxide **2**.

isolated as oils, their absolute configurations were determined as (−)-(*S*)-**2** and (+)-(*R*)-**2** by chemical correlation, via the transformation of the (−)-(*S*) enantiomer of compound **8**, which is shown in Scheme 2. On the other hand, the crystals of **4** proved unsuitable for X-ray analysis and the absolute configuration of these compounds was established on the basis of the comparison of their CD spectra with those of compound **3** (Fig. 1). It turned out that the CD curves of (+)-(*R*)-**3** and the dextrorotatory **4** were of the same shape and exhibited the same sign of the Cotton effect. The same was valid for (−)-(*S*)-**3** and the levorotatory **4** (Fig. 1). Since



Scheme 3. Synthesis of 4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide **3** and 5-isothiocyanato-1-pentyl 2′,2′,2′-trifluoroethyl sulfoxide **4**.

Table 1
Enantiomeric products.

Comp.	HPLC conditions	Enantiomers					
		$[\alpha]_D$ AcOEt	ee [%]	Absolute configuration	$[\alpha]_D$ AcOEt	ee [%]	Absolute configuration
2	Chiralcel OJ Hexane:*i*-PrOH 2:3	−49.5	95.4	*S*	+51.9	100	*R*
3	Chirobiotic V2 Hexane:*i*-PrOH 1:1	−44.7	99.9	*S*	+43.2	93.9	*R*
4	Chirobiotic V2 Hexane:*i*-PrOH 3:1	−40.6	99.8	*S*	+40.7	99.9	*R*
8	Chiralcel OJ Hexane:*i*-PrOH 2:3	−35.0	91	*S*	+38.6	100	*R*

both compounds are closely related and differ only by one methylene group in the alkyl chain, it seems very reasonable to ascribe the absolute configurations as (−)-(*S*)-**4** and (+)-(*R*)-**4**.

All the enantiomer structures are shown in Scheme 4. X-ray molecular structures are presented in Figs. 2–7 and discussed below while the corresponding data are collected in the Experimental Section and in the Supplementary Materials.

A different system in interaction between CF_3 groups is observed in the crystal lattices of compounds **8** and **3**. Compound **8** has its CF_3 and S(=O) groups directly bonded, while in compound **3** there is one CH_2 group between these groups. In the crystal lattice of **8**, the molecules A and B, that are present in the asymmetric unit (see Figs. 2 and 3), basically form dimers. In each dimer, the CF_3 groups of both monomers A and B are facing each other. The distances between fluorine atoms of the CF_3(A)–CF_3(B) groups, that are shown in orange color (in web version) in Fig. 6, are 2.95 and 3.03 Å. The packing of these dimers in the crystal is supported by the parallel stacking of the planar ring systems (see Fig. 6). In compound **3**, there is no sign of dimerization, however, the packing of monomeric molecules (see Figs. 4 and 5) in the crystal lattice is quite tight. In the crystal lattice of **3**, the CF_3 groups also interact between each other, but in a different way than in the crystal lattice of **8**. These interactions in **3** form characteristic pathway that extends through the entire crystal, and is marked schematically with orange (in web version) solid lines in Fig. 7. The distances between fluorine atoms that are shown in Fig. 6 in orange (in web version), are 3.09 Å. They are only slightly longer then the distances between CF_3 groups in **8** that are marked with orange (in web version) solid lines in Fig. 6.

2.2. Biological testing

The ability to inhibit the cells viability (compound cytotoxicity) was evaluated for the new sulforaphane analogs **2**, **3** and **4**, (both *R* and *S* enantiomers). (*R*)- and (*S*)-sulforaphanes were used as reference compounds. To evaluate overall properties of the synthesized compounds, the cytotoxicity was determined simultaneously in cancer and normal cell lines. A malignant melanoma Malme-3M and normal skin fibroblast Malme-3 cell lines were chosen. Both cell lines have been isolated from the same patient and provide tumor and normal counterparts for comparative *in vitro* melanoma studies.

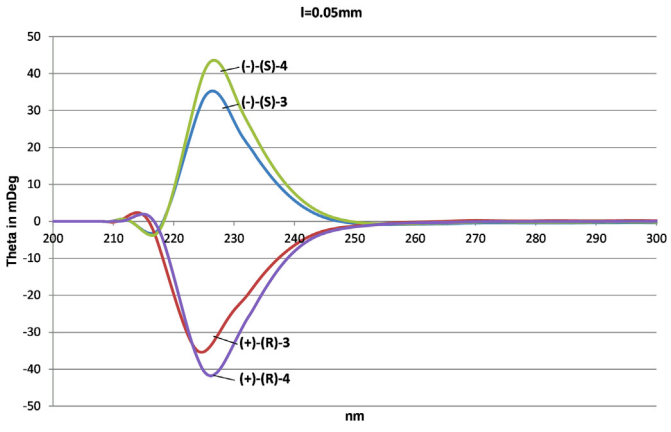


Fig. 1. Comparison of CD spectra of the enantiomers of **3** and **4**.

The popular colorimetric MTT test measuring the mitochondrial dehydrogenases activity was applied. Damaged or dead cells have low or zero dehydrogenase activity. This test was used therefore for quantification of viable cells in culture [28].

In order to compare quantitatively the cytotoxicity of the studied compounds, the IC_{50} values (a concentration causing 50% inhibition of the untreated cells viability) were calculated. The lower IC_{50} value the stronger the cytotoxic effect of the compound.

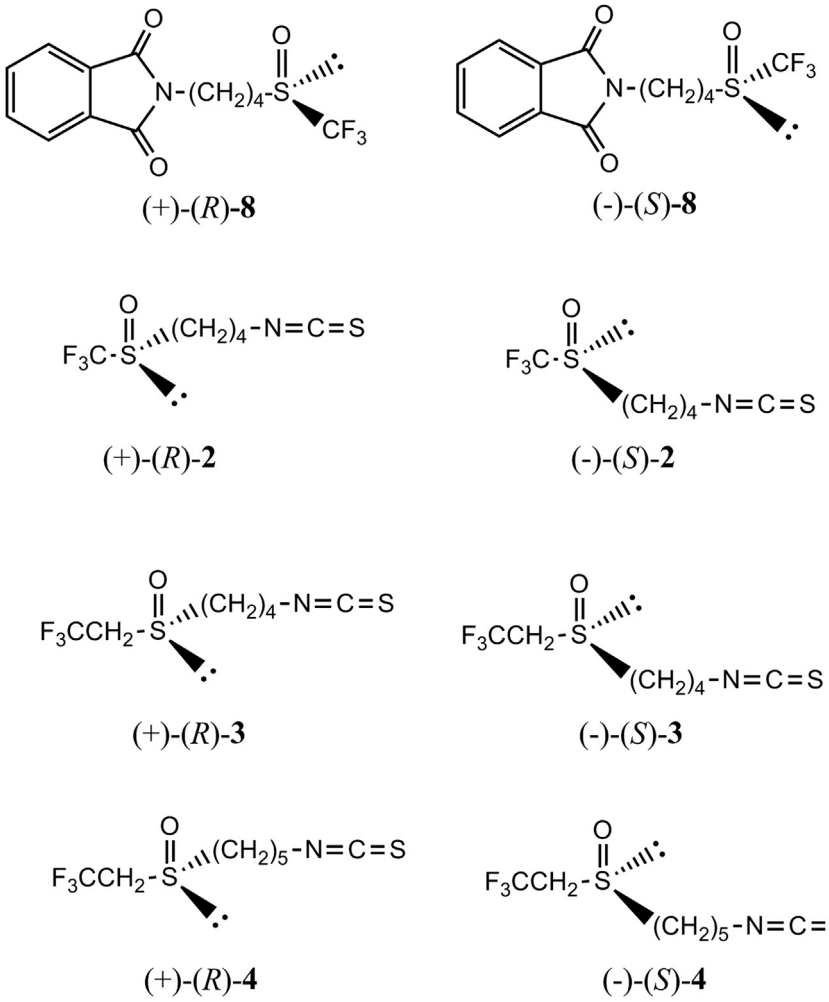
All the newly synthesized compounds after 48 h of incubation inhibited the cancer cell growth noticeably stronger ($p < 0.05$) than (*R*)-sulforaphane (*R*)-**1**. The fluorine analogs IC_{50} values were almost twice as low as for the (*R*)-sulforaphane (Fig. 8, Table 2). At the same time the (*S*)-sulforaphane (*S*)-**1** exhibited no cytotoxicity, which is in agreement with previous reports of the superiority of (*R*)-sulforaphane as compared to its *S* enantiomer [29].

After extension of the incubation time up to 72 h toxicity of the new compounds remained nearly at the same level. At the same time cytotoxicity of both sulforaphane enantiomers increased substantially and reached the level of fluorine analogs (it may be due to the Malme-3M doubling time reported to be up to 50 h [30,31]). This result may suggest that the introduction of fluorine atom into the sulforaphane molecule may change the mechanism of cell proliferation inhibition. This is also supported by the fact that, contrary to sulforaphane, the absolute configuration of the fluorine analogs is not a key factor determining their cytotoxic activity. However, to fully elucidate the relevance of the obtained results further experiments clarifying the mechanism of cell growth inhibition and *in vivo* toxicity are necessary.

The above results clearly show that the fluorine-containing sulforaphane analogs may be considered as potential anti-melanoma agents. The replacement of hydrogen atoms in sulforaphane methyl group with fluorine atoms increased cytotoxicity of the resulted analogs (*R*)-**2** and (*S*)-**2** against cancer cells. Insertion of a methylene group at any side of the sulfoxide moiety (compounds **3** and **4**) resulted in an additional increase of cytotoxicity against cancer cells. Such an influence of the CH_2 group addition on isothiocyanate cytotoxicity has already been described for different isothiocyanates derivatives [32–34]. This structure–cytotoxicity relationship was also observed in normal cells. From among all analogs the most promising cytotoxicity profile was exhibited by both enantiomers of compound **3** being less cytotoxic against normal cells than against cancer cells at all incubation times.

3. Conclusions

The three newly synthesized pairs of enantiomers of the sulforaphane analogs bearing organofluorine substituents bonded to the sulfinyl sulfur atom and having different number of methylene groups in the central carbon chain were tested for their cytotoxicity



Scheme 4. Enantiomeric products.

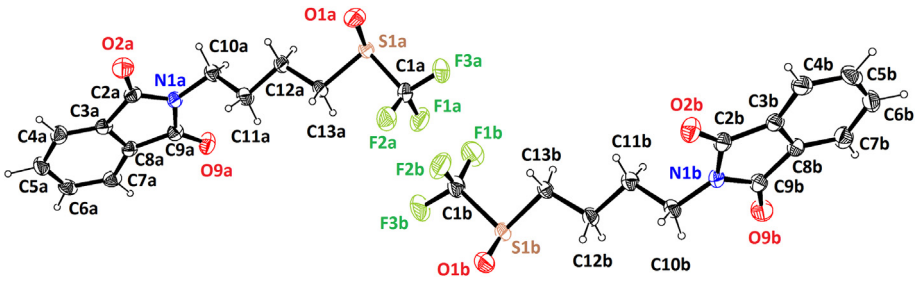


Fig. 2. Molecular view of (+)-(*R*)-**8**.

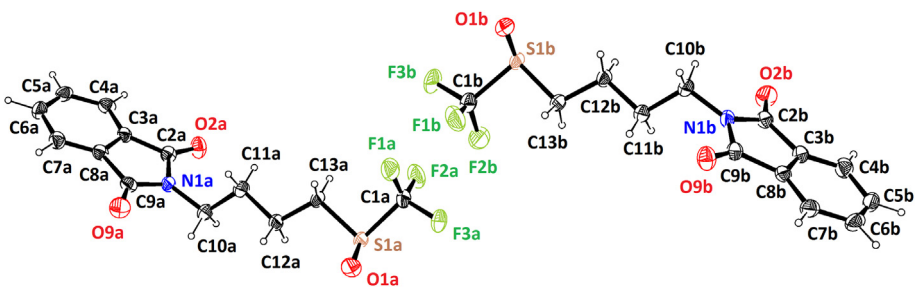


Fig. 3. Molecular view of (−)-(*S*)-**8**.

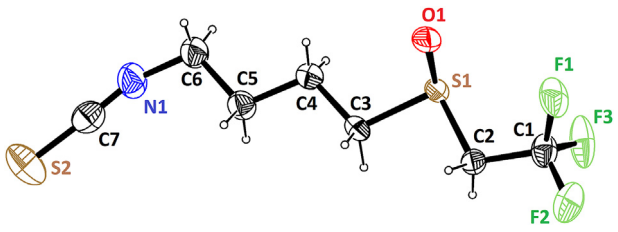


Fig. 4. Molecular view of (+)-(*R*)-**3**.

against melanoma cells. After 48 h of incubation they exhibited higher cytotoxicity in comparison with the original sulforaphane. After 72 h of incubation the cytotoxicity of new derivatives and sulforaphane were comparable. The results could suggest that the mechanism of sulforaphane and its analogs cytotoxicity was different. That could also explain why for fluorinated analogs the cytotoxicity was almost independent on the absolute configuration of the particular enantiomers. Among all the compounds tested one, i.e. (*S*)-4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide, proved to be reasonably efficient. The results allow to draw a conclusion that the replacement of hydrogen atoms with fluorine atoms in the sulforaphane molecule may positively change its cytotoxicity against melanoma cells and that further investigations directed towards the use of various organofluorine substituents, both alkyl and aryl, would be desirable. The studies will be continued.

4. Experimental section

4.1. Chemistry

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker instrument at 200 MHz with $CDCl_3$ as solvent. Mass spectra including HR–MS were measured on a Finnigan MAT instrument. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter (*c* as indicated below). Column chromatography was carried out using Merck 60 silica gel. TLC was performed on Merck 60 F254 silica gel plates. The enantiomeric excess (ee) values were determined by chiral HPLC (Varian Pro Star 210, Chiralpak AS, Chiralcel OD, Chirobiotic V) and the preparative enantiomer resolution by Chiralcel OJ and Chirobiotic V2 using Recycling Preparative HPLC instrument LC-9101, manufactured by Japan Analytical Industry Co., Ltd.

4.1.1. Preparation of 4-N-phthalimido-1-butyl trifluoromethyl sulfide **7** (Scheme 2)

A glass ampoule was filled with 4-N-phthalimidobutane-1-thiol **6** (1.2 g, 5.1 mmol), trisodium phosphate dodecahydrate (1.3 g, 5.1 mmol), DMF (9.8 mL), sodium hydroxymethanesulfinate ("Rongalite", $HOCH_2SO_2Na$), (2.35 g, 15.3 mmol) and gaseous iodotrifluoromethane (2.0 g, 10.2 mmol). The mixture was shaken for 20 h, then poured to water (50 mL) and extracted with chloroform. The organic layer was separated and dried with $MgSO_4$.

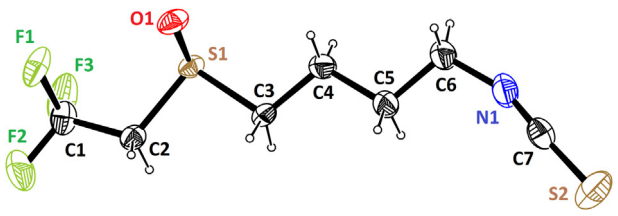


Fig. 5. Molecular view of (−)-(*S*)-**3**.

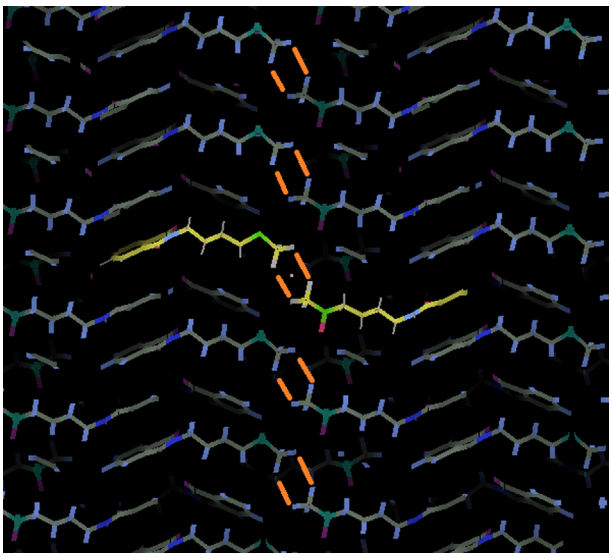


Fig. 6. The packing of the CF_3 groups in the crystal lattice of (+)-(*R*)-**8**.

Evaporation of the solvent gave the crude product (1.6 g), which was purified by liquid chromatography on silica gel using chloroform as eluent. The pure product was obtained as an oil (612 mg, 40%). ^{1}H NMR ($CDCl_3$): δ 1.70–1.87 (m, 4H, CH_2CH_2), 2.91 (t, 2H, $J = 6.66$ Hz, CH_2S), 3.70 (t, 2H, $J = 6.60$ Hz, CH_2N), 7.68–7.85 (m, 4H, arom). ^{19}F NMR ($CDCl_3$): δ − 40.51. ^{13}C NMR ($CDCl_3$): δ 26.93, 27.59, 29.47, 37.24, 123.47, 131.24 (q, J = 306 Hz, CF_3), 132.19, 134.23, 168.55). MS (CI, isobutane): $m/z = 304.1$ $[M + H]^+$. HRMS: m/z calcd for $[M]^+$ $C_{13}H_{12}F_3NO_2S$ 303.053, found 303.054.

4.1.2. Synthesis of 4-N-phthalimido-1-butyl trifluoromethyl sulfoxide **8** (Scheme 2)

To a solution of sulfide **7** (400 mg, 1.32 mmol) in dichloromethane (10 mL) a solution of *m*-chloroperbenzoic acid, m-CPBA (269 mg, 1.32 mmol) on dichloromethane (5 mL) was added dropwise with stirring. After 3 days solid $NaHCO_3$ (170 mg, 2 mmol) was added and stirring was continued for 1 h. Water was added and the layers were separated. The organic layer was dried with $MgSO_4$

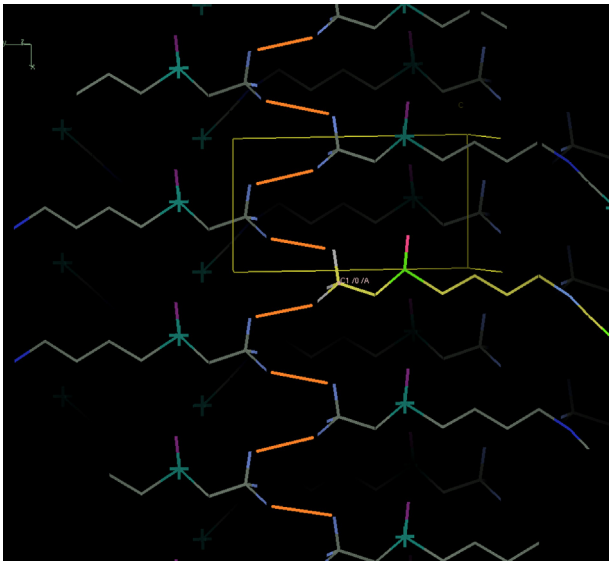


Fig. 7. The packing of the CF_3 groups in the crystal lattice of (+)-(*R*)-**3**.

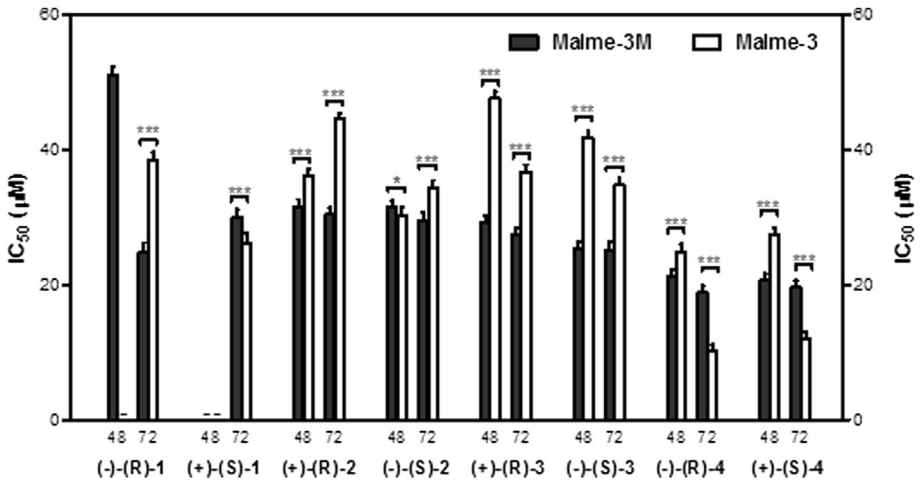


Fig. 8. The IC_{50} indexes in Malme-3M and Malme-3 cell lines after 48 and 72 h incubation with isothiocyanates. When "–" is used the IC_{50} was not determined due to lack of cytotoxicity. The graph presents mean values and a standard deviation ($n = 12$). $^{*}p < 0.05$, $^{***}p < 0.001$.

and the solvent was removed under vacuum to give the crude product (369 mg). Chromatographic purification (silica gel, hexane : acetone 3:2) gave pure product **8** as a solid (300 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 1.88 (m, 4H, CH_2CH_2), 2.89–3.08 (m, 2H, CH_2SO), 3.72 (t, 2H, $J = 6.12$ Hz, CH_2N), 7.66–7.81 (m, 4H, arom). ^{19}F NMR ($CDCl_3$): δ − 72.9. MS (CI, isobutane): $m/z = 320.1$ $[M + H]^+$.

The racemic mixture of **8** was resolved into enantiomers using a recycling HPLC instrument equipped with chiral column Chiralcel OJ with hexane : *i*-PrOH 2:3 as eluent.

$$(-)-(S)-8;\ [\alpha]_D - 35(\text{c } 1.3,\ \text{AcOEt}),\ \text{ee} = 91\%$$

$$(+)-(R)-8;\ [\alpha]_D + 38.6(\text{c } 4.0,\ \text{AcOEt}),\ \text{ee} = 100\%$$

The enantiomers were crystallized from methanol [(−)-(*S*)-**8]** or ethanol/water [(+)-(*R*)-**8]** and subjected to X-ray analysis to determine their absolute configurations.

4.1.3. Synthesis of 4-isothiocyanato-1-butyl trifluoromethyl sulfide *10* (Scheme 2)

To a solution of sulfide **7** (560 mg, 1.85 mmol) in ethanol (1.85 mL) hydrazine hydrate (120 mg, 2.30 mmol) was added under argon and the resulting solution was heated at 75 °C. Concentrated HCl_{aq} (740 μL) was added. The mixture was refluxed for 1 h and left overnight at room temperature. Water (9.5 mL) was added, a precipitate was filtered off and the solvents were removed under vacuum to yield crystalline 4-amino-1-butyl trifluoromethyl sulfide hydrochloride **9**, which was used in the ensuing steps without purification. To the sulfide **9**, dissolved in chloroform (7 mL), thiophosgene (230 mg, 2 mmol) was added followed by an aqueous solution of NaOH (240 mg, 6 mmol) and the mixture was stirred at room temperature for 20 h. Then it was diluted with water (3 mL) and extracted with chloroform. The organic extract was dried with $MgSO_4$ and the solvent was removed under vacuum to give the crude product **10** (356 mg). Purification by column chromatography (Lichroprep) furnished pure 4-isothiocyanato-1-butyl trifluoromethyl sulfide **10** as a yellow-brown oil (158 mg, 40% from sulfide **7**). ^{1}H NMR ($CDCl_3$): δ 1.70–1.87 (m, 4H, CH_2CH_2), 2.90 (t, 2H, $J = 6.6$ Hz, CH_2S), 3.60 (t, 2H, $J = 6.0$ Hz, CH_2N). ^{19}F NMR ($CDCl_3$): δ − 40.4. ^{13}C NMR ($CDCl_3$): δ 26.53, 28.55, 29.02, 40.41, 130.69 (NCS), 130.86 (q, $J = 306$ Hz, CF_3). MS (CI, isobutane): $m/z = 216$ $[M + H]^+$. HRMS: m/z calcd for $[M]^+$ $C_6H_8F_3NS_2$ 215.00527, found 215.00503.

4.1.4. Synthesis of 4-isothiocyanato-1-butyl trifluoromethyl sulfoxide *2*

To a solution of sulfide **10** (75 mg, 0.46 mmol) in dichloromethane (7 mL) *m*-chloroperbenzoic acid, m-CPBA (88 mg, 0.56 mmol) in dichloromethane (5 mL) was added portionwise with stirring. The reaction conversion was monitored by ^{19}F NMR. After evaporation of the solvent the products were separated by preparative TLC to give the recovered sulfide **10** (41 mg) and the targeted sulfoxide **2** (41 mg, 49%). ^{1}H NMR ($CDCl_3$): δ 1.88–2.09 (m, 4H, CH_2CH_2), 2.94–3.09 (m, 2H, CH_2SO), 3.60 (t, 2H, $J = 6.2$ Hz, CH_2N). ^{19}F NMR ($CDCl_3$): δ − 72.9. ^{13}C NMR ($CDCl_3$): δ 19.4, 29.7, 44.4, 47.5, 125.2 (q, $J = 329.5$ Hz, CF_3), 130.69 (NCS). MS (CI, isobutane): $m/z = 232$ $[M + H]^+$.

The racemic mixture of **2** was resolved into enantiomers using a recycling HPLC instrument equipped with chiral column Chiralcel OJ with hexane:*i*-PrOH 2:3 as eluent. Their absolute configurations were ascribed by chemical correlation, i.e. by comparison of their optical rotation signs with those of the samples obtained from sulfoxides **8**.

$$(-)-(S)-2;\ [\alpha]_D - 49.5(\text{c } 1.3,\ \text{AcOEt}),\ \text{ee} = 95\%$$

Table 2
In vitro data of sulforaphane and its analogs.

Compound	Incubation Time (h)	Malme-3M IC_{50} (μM)	Malme-3 IC_{50} (μM)
(−)-(*R*)-**1**	48	51	–
	72	25	38
(+)-(*S*)-**1**	48	–	–
	72	30	26
(+)-(*R*)-**2**	48	32	36
	72	30	44
(−)-(*S*)-**2**	48	31	30
	72	30	34
(+)-(*R*)-**3**	48	29	48
	72	27	37
(−)-(*S*)-**3**	48	25	42
	72	25	35
(+)-(*R*)-**4**	48	21	25
	72	19	12
(−)-(*S*)-**4**	48	21	27
	72	20	10

(+) − (*R*) − 2; $[\alpha]_D$ + 51.9(c 4.0, AcOEt), ee = 100%

4.1.5. (−)-(S)-4-isothiocyanato-1-butyl trifluoromethyl sulfoxide (−)-(S)-2 (from sulfoxide(−)-(S)-8)(Scheme 2)

To a solution of sulfoxide **(−)-(*S*)-8** ($[\alpha]_D$ − 35 (c 1.3, AcOEt), ee = 91%) (64 mg, 0.2 mmol) in ethanol (0.2 mL)hydrazine hydrate (13 mg, 0.26 mmol) was added under argon and the resulting solution was refluxed at 75 °C for 2 h. 30% H_2SO_4 (30 mg, 0.3 mmol) was added and refluxing was continued for 1 h. The mixture was left overnight at room temperature. Water (1.0 mL) was added, a precipitate was filtered off and the solvents were removed under reduced pressure to yield crystalline 4-trifluoromethylsulfinylbutylammonium sulfate (57 mg, 99%), which was dissolved in CH_2Cl_2 and treated with bis(O-2-pyridyl) thiocarbonate, followed by an aqueous solution of NaOH (8 mg, 0.2 mmol). The mixture was stirred for 4 h then diluted with H_2O (3 mL) and extracted with CH_2Cl_2 (5 × 3 mL). The combined organic solutions were dried over $MgSO_4$ and the solvent was removed under vacuum to give the crude product (43 mg, 90%). After column chromatography (SiO_2, CH_2Cl_2–MeOH, 95:5) pure **(−)-(*S*)-2** was obtained (40 mg, 87%). Chiral HPLC showed that its ee was only 55%.

4.1.6. Preparation of 4-N-phthalimido-1-butyl 2′,2′,2′-trifluoroethyl sulfide 12 (Scheme 3)

To a solution of sodium methoxide, prepared by dissolving sodium (0.4 g, 17.225 mmol) in methanol (15 mL), a solution of 2,2,2-trifluoroethanethiol (2 g, 17.225 mmol) in methanol (5 mL) was dropped under argon at 0 °C. After 15 min. the methanol was removed under vacuum to give sodium 2,2,2-trifluoroethanethiolate as a solid. It was dissolved in dimethoxyethane (15 mL), cooled to 0 °C and a solution of N-(4-bromobutyl) phthalimide (4.86 g, 17.225 mmol) was added under argon. The mixture was stirred overnight at room temperature. Water (50 mL) was added and the layers were separated. The organic layer was extracted with dichloromethane (4 × 25 mL) and the combined organic layers were washed with water and dried with $MgSO_4$. After removal of the solvents under vacuum, the crude product was purified by column chromatography (silica gel, dichloromethane as solvent) to give pure sulfide **12**, 5.25 g (86%). ^{1}H NMR ($CDCl_3$): δ 1.58–1.85 (m, 4H, CH_2CH_2), 2.69 (t, 2H, J = 7.07 Hz, CH_2S), 3.04 (q, 2H, J = 9.33 Hz, $\underline{CH_2}CF_3$), 3.69 (t, 2H, J = 6.81 Hz, CH_2N), 7.67–7.86 (m, 4H, arom). ^{19}F NMR ($CDCl_3$): δ − 65.82 (t, 3F, J_{H-F} = 9.85 Hz). MS (CI, isobutane): m/z = 318.1 $[M + H]^+$. HRMS: m/z calcd for $[M + H]^+$ $C_{14}H_{14}F_3NO_2S$ 318.07756, found 318.07742 (Scheme 4).

4.1.7. Synthesis of 5-N-phthalimido-1-pentyl 2′,2′,2′-trifluoroethyl sulfide 13 (Scheme 3)

To a solution of sodium methoxide, prepared by dissolving sodium (1.1 g, 47.82 mmol) in methanol (80 mL), a 2,2,2-trifluoroethanethiol (5 g, 43.0626 mmol) was added and left at room temperature for 1.5 h. N-(5-bromopentyl)phthalimide was added and the mixture was stirred at room temperature for 48 h. After removal of methanol the residue was dissolved in chloroform and the solution was washed with water. The organic layer was dried with $MgSO_4$ and the solvent was removed under vacuum. The crude product was purified by column chromatography (silica gel, dichloromethane as solvent) to give pure sulfide **13** as a solid, mp = 89 °C (13.88 g, 98%). ^{1}H NMR ($CDCl_3$): δ 1.41–1.50 (m, 2H), 1.58–1.77 (m, 4H), 2.65 (t, 2H, J = 7.20 Hz, CH_2S), 3.05 (q, 2H, J_{H-F} = 10.03 Hz, $\underline{CH_2}CF_3$), 3.69 (t, 2H, J = 7.07 Hz, CH_2N), 7.67–7.82 (m, 4H, arom). ^{19}F NMR ($CDCl_3$): δ − 65.8 (t, 3F, J_{H-F} = 10.30 Hz). ^{13}C NMR ($CDCl_3$): δ 25.79, 28.11, 28.56, 32.99, 34.31 (q, J_{C-F} = 32.31 Hz, $\underline{CH_2}CF_3$), 37.69, 123.24, 125.97 (q, J_{C-F} = 125.97 Hz, CF_3CH_2), 132.11, 133.97, 168.47. MS (CI, isobutane): m/z = 332.1 $[M + H]^+$. HRMS: m/z calcd for $[M + H]^+$ $C_{15}H_{17}F_3NO_4S$ 332.09321, found 332.09272.

4.1.8. Synthesis of 4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfide 17 (Scheme 3)

To a solution of sulfide **12** (2.024 g, 6.378 mmol) in ethanol (20 mL) hydrazine hydrate (0.414 g, 6.378 mmol) in ethanol (4 mL) was added under argon. The resulting solution was refluxed for 3 h and then left overnight at room temperature. ^{19}F NMR control showed complete conversion. Concentrated HCl_{aq} (1 mL) was added and the mixture was refluxed for 1 h. Water (40 mL) was added, a precipitate was filtered off and the solvents were removed under vacuum to yield crystalline 4-amino-1-butyl 2′,2′,2′-trifluoroethyl sulfide hydrochloride **14** (1.483 g), which was used in the ensuing steps without purification. To the sulfide **14**, dissolved in chloroform (25 mL), O,O-di(2-pyridyl) thiocarbonate (1.6 g, 6.8951 mmol) was added followed by an aqueous solution of NaOH (827 mg, 20.675 mmol) and the mixture was stirred at room temperature over weekend. Then it was diluted with water (10 mL) and extracted with chloroform (3 × 30 mL). The organic extract was dried with $MgSO_4$ and the solvent was removed under vacuum to give the crude product **17** (1.527 g). Purification by column chromatography (silica gel, dichloromethane) furnished pure 4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfide **17** as a yellow-brown oil (1.07 g, 73.1% from sulfide **12**). ^{1}H NMR ($CDCl_3$): δ 1.66–1.90 (m, 4H, CH_2CH_2), 2.72 (t, 2H, J = 6.55 Hz, CH_2S), 3.08 (q, J = 9.91 Hz, $\underline{CH_2}CF_3$), 3.56 (t, 2H, J = 6.05 Hz, CH_2N). ^{19}F NMR ($CDCl_3$): δ − 65.76 (t, 3F, J_{H-F} = 9.81 Hz). ^{13}C NMR ($CDCl_3$): δ 25.94, 28.69, 32.32, 34.35 (q, J_{C-F} = 32.89 Hz, $\underline{CH_2}CF_3$), 44.57, 125.81 (q, J_{C-F} = 276.15 Hz, $CH_2\underline{CF_3}$), 130.52 (NCS). MS (CI, isobutane): m/z = 230 $[M + H]$.

4.1.9. Synthesis of 5-isothiocyanato-1-pentyl 2′,2′,2′-trifluoroethyl sulfide 18 (Scheme 3)

In a similar way, sulfide **13** (9 g, 26.1616 mmol) in ethanol (50 mL) was treated with hydrazine hydrate (1.42 g, 26.1616 mmol) in ethanol (5 mL). Since a large amount of a precipitate was formed, additional amounts of ethanol (50 mL) and hydrazine hydrate (0.7 g, 13.13 mmol) were added. After complete conversion was reached (next 6 h at room temperature), the precipitate was filtered off and the solution was concentrated to the half of its volume. Concentrated HCl_{aq} (1.5 mL) was added and the mixture was refluxed for 1 h. The precipitate was filtered off and the solvents were removed under vacuum to yield crystalline 5-amino-1-pentyl 2′,2′,2′-trifluoroethyl sulfide hydrochloride **15** (4.091 g, 64%; ^{19}F NMR ($CDCl_3$): δ − 65.75, t, J_{H-F} = 10.11 Hz), which was used in the ensuing steps without purification.

To the sulfide **15** (2 g, 8.4136 mmol), dissolved in chloroform (30 mL), thiophosgene (0.8 mL, 1.3 eq., 10.9377 mmol) was added followed by an aqueous 5% solution of NaOH (11.779 mmol) at 0–5 °C and the mixture was stirred for 1 h. To keep the pH of the solution slightly above 7, additional amounts of aqueous 5% solution of NaOH were dropped in. The layers were separated and the aqueous layer was extracted with chloroform. The combined organic layers were washed with water and the dried with $MgSO_4$. Removal of the solvents under vacuum followed by purification by column chromatography (silica gel, dichloromethane – hexane 1:1) yielded pure sulfide **18** (1.9 g, 93%). ^{1}H NMR ($CDCl_3$): δ 1.52–1.79 (m, 6H), 2.69 (t, 2H, J = 6.87 Hz, CH_2S), 3.07 (q, 2H, J_{H-F} = 9.92 Hz, $\underline{CH_2}CF_3$), 3.53 (t, 2H, J = 6.38 Hz, CH_2N). ^{19}F NMR ($CDCl_3$): δ − 65.8 (t, 3F, J_{H-F} = 9.80 Hz). ^{13}C NMR ($CDCl_3$): δ 25.60, 28.31, 29.50, 32.95, 34.44 (q, J_{C-F} = 32.73 Hz, $\underline{CH_2}CF_3$), 44.90, 125.94 (q, J_{C-F} = 276.26 Hz, $CH_2\underline{CF_3}$), 129.95 (NCS). MS (CI, isobutane): m/z = 244 $(M + H)$.

4.1.10. Synthesis of 4-N-phthalimido-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide ***16***

To a solution of sulfide **12** (150 mg, 0.4727 mmol) in dichloromethane (5 mL)*m*-chloroperbenzoic acid, m-CPBA (82 mg, 0.4727 mmol) was added portionwise with stirring. After 10 min. TLC control revealed complete consumption of the substrate. The solution was washed with water. The organic layer was dried with $MgSO_4$ and the solvent was removed under vacuum to give the crude product consisting of two compounds. Chromatographic separation (silica gel, dichloromethane: methanol 30:1) gave pure 4-N-phthalimido-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide **16** (140 mg, 89%): ^{1}H NMR ($CDCl_3$): δ 1.64–2.11 (m, 4H, CH_2CH_2), 2.93 (t, 2H, $J = 6.78$ Hz, CH_2SO), 3.45 (q, 2H, $J = 10.10$ Hz, $\underline{CH_2}CF_3$, 3.76 (t, 2H, $J = 6.46$ Hz, CH_2N), 7.73–7.87 (m, 4H, arom). ^{19}F NMR ($CDCl_3$): δ − 60.18. MS (CI, isobutane): m/z = 334.1 $[M + H]^+$. HRMS: m/z calcd for $[M + H]^+$ $C_{14}H_{14}F_3NO_3S$ 334.072475, found 318.07742; and the corresponding sulfone: ^{1}H NMR ($CDCl_3$): δ 1.84–2.16 (m, 4H, CH_2CH_2), 3.25 (t, 2H, $J = 7.37$ Hz, CH_2SO_2), 3.71–3.87 (m, 4H, $\underline{CH_2}CF_3$ and CH_2N), 7.70–7.86 (m, 4H, arom). ^{19}F NMR ($CDCl_3$): δ − 60.18 (t, 3F, J_{H-F} = 9.85 Hz). MS (CI, isobutane): m/z = 350.1 $[M + H]^+$. HRMS: m/z calcd for $[M + H]^+$ $C_{14}H_{14}F_3NO_4S$ 350.06739, found 350.06753.

4.1.11. Synthesis of 4-isothiocyanato-1-butyl 2′,2′,2′-trifluoroethyl sulfoxide ***3***

a) To a solution of sulfide **17**(200 mg, 0.8734 mmol) in dichloromethane (7 mL)*m*-chloroperbenzoic acid, m-CPBA (150 mg, 0.8734 mmol) was added portionwise with stirring and external cooling. After 1 h TLC control revealed complete consumption of the substrate. The solvent was removed under vacuum and the crude product was purified by preparative TLC (CH_2Cl_2: methanol 60:1) to give pure sulfoxide **3** (189 mg, 88.3%).
b) To a solution of sulfide **17** (200 mg, 0.8734 mmol) in tetrachloroethane (5 mL) (+) or (−) Davis oxaziridine[26] (260 mg, 0.8734 mmol) in tetrachloroethane (2 mL) was added and the mixture was stirred for 12 days (TLC control). After similar work-up pure sulfoxide **3** was obtained (200 mg, 93.4%). ^{1}H NMR ($CDCl_3$): δ 1.80–2.07 (m, 4H, CH_2CH_2), 2.78–3.01 (m, 2H, CH_2SO), 3.47 (q, 2H, J = 10.07 Hz, $\underline{CH_2}CF_3$), 3.61 (t, 2H, J = 6.01 Hz, CH_2N). ^{19}F NMR ($CDCl_3$): δ − 60.08 (t, 3F, $J_{H-F} = 9.77$ Hz). ^{13}C NMR ($CDCl_3$): δ 19.67, 28.73, 44.49, 52.56, 55.93 (q, J = 28.84 Hz), 123.15 (q, J = 277.36 Hz), 131.09. MS (CI, isobutane): m/z = 246 $[M + H]^+$. HRMS: m/z calcd for $C_{14}H_{14}F_3NO_4S$ 246.023418 found 246.02342.

The racemic mixture of **3** was resolved into enantiomers using a recycling HPLC instrument equipped with chiral column Chirobiotic V2 with hexane:*i*-PrOH 1:1 as eluent.

$$(-)-(S)-3;[\alpha]_D - 44.7(c\ 0.84, i-PrOH),\ ee = 99.9\%$$

$$(+)-(R)-3;[\alpha]_D + 43.2(c\ 0.94,\ i-PrOH),\ ee = 93.9\%.$$

The enantiomers were crystallized from diethyl ether/hexane mixture and subjected to X-ray analysis to determine their absolute configurations.

4.1.12. Synthesis of 5-isothiocyanato-1-pentyl 2′,2′,2′-trifluoroethyl sulfoxide ***4***

To a solution of sulfide **18** (500 mg, 2.057 mmol) in dichloromethane (10 mL)*m*-chloroperbenzoic acid, m-CPBA (355 mg, 2.057 mmol) was added portionwise with stirring at 0 °C. after 15 min. TLC control revealed complete conversion. 5% Aqueous solution of $NaHCO_3$ was added and stirring was continued for 0.5 h. The layers were separated and the aqueous layer was extracted with dichloromethane (2 × 5 mL) The combined organic layers were dried with $MgSO_4$ and the solvent was removed under vacuum to give the crude product. Chromatographic purification (preparative TLC, dichloromethane: methanol 50:1) gave pure product **4** as a solid, 423 mg (79.3%), mp = 55 °C. %). ^{1}H NMR ($CDCl_3$): δ 1.61–1.71 (m, 2H, CH_2), 1.78 (*p*, 2H, $J = 7.03$ Hz), 1.84–1.97 (m, 2H) 2.81–2.96 (m, 2H, CH_2SO), 3.42–3.51 (m, 2H, $\underline{CH_2}CF_3$), 3.56 (t, 2H, $J = 6.38$ Hz, CH_2N). ^{19}F NMR ($CDCl_3$): δ − 60.11 (t, $J_{H-F} = 9.79$ Hz). ^{13}C NMR ($CDCl_3$): δ 21.64, 25.76, 29.58, 44.74, 53.43, 56.14 (q, J_{C-F} = 28.74 Hz, $\underline{CH_2}CF_3$), 123.24 (q, J_{C-F} = 277.01 Hz, CH_2CF_3), 130.55 (NCS). MS (CI, isobutane): m/z = 260 $[M + H]^+$. HRMS: m/z calcd for $C_8H_{13}F_3NOS_2$ 260.039068 found 260.03811.

The racemic mixture of **4** was resolved into enantiomers using a recycling HPLC instrument equipped with chiral column Chirobiotic V2 with hexane:*i*-PrOH 3:1 as eluent.

$$(-)-(S)-4;[\alpha]_D - 40.6(c\ 1.09, i-PrOH),\ ee = 98.8\%;$$

$$(+)-(R)-4;[\alpha]_D + 40.7(c\ 0.95,\ i-PrOH),\ ee = 99.9\%.$$

4.2. Crystallographic data (Table 3 and Supporting Information)

All X-ray data were collected with Bruker APEX-II CCD diffractometer using CuKα radiation [35,36]. The crystal structures and absolute configurations were determined with SHELXL-97 [37]. Structures were solved by direct methods (with SHELXS-97) [37] and refined using full-matrix least-squares using F^2. In both structures, the H atoms were placed geometrically and refined freely, i.e. the positional and the isotropic thermal displacement parameters for all H atoms were refined with no restrictions. All nonhydrogen atoms in both structures were refined anisotropically. The correct absolute structure was proved by Flack parameter [38]. The *Coot*, *Mercury* and *Ortep3* programs were used for model building and structure visualization [39–41].

For (−)-(*S*)-**8,** the Flack parameter was $x = 0.01(1)$. For all data, the final wR^2 was 0.0609, R_1 = 0.0241, S = 1.011, max residual density = 0.22 eÅ^{-3} (see Table 3). The calculated parameters for inverted structure, i.e. with assumed opposite (incorrect) chirality, were: $x_{(inv)} = 0.99(2)$, $wR^2_{(inv)} = 0.1124$, and $R_{1(inv)} = 0.0416$.

For (+)-(*R*)-**8,** the Flack parameter was $x = 0.01(1)$. For all data, the final wR^2 was 0.0656, R_1 = 0.0250, S = 1.007, max residual density = 0.25 eÅ^{-3} (Table 3). The calculated parameters for inverted structure, i.e. with assumed opposite (incorrect) chirality, were: $x_{(inv)} = 0.98(2)$, $wR^2_{(inv)} = 0.1146$, and $R_{1(inv)} = 0.0422$.

For (−)-(*S*)-**3,** the Flack parameter was $x = 0.00(1)$. For all data, the final wR^2 was 0.0408, R_1 = 0.0160, S = 1.000, max residual density = 0.17 eÅ^{-3} (Table 3). The calculated parameters for inverted structure, i.e. with assumed opposite (incorrect) chirality, were: $x_{(inv)} = 1.01(2)$, $wR^2_{(inv)} = 0.0894$, and $R_{1(inv)} = 0.0354$. For (+)-(*R*)-**3,** the Flack parameter was $x = 0.05(2)$. For all data, the final wR^2 was 0.0605, R_1 = 0.0222, S = 1.024, max residual density = 0.31 eÅ^{-3} (Table 3). The calculated parameters for inverted structure, i.e. with assumed opposite (incorrect) chirality, were: $x_{(inv)} = 0.94(3)$, $wR^2_{(inv)} = 0.1003$, and $R_{1(inv)} = 0.0370$.

Crystallographic data for (−)-(*S*)-**8,** (+)-(*R*)-**8**, (−)-(*S*)-**3** and (+)-(*R*)-**3** have been deposited with the Cambridge Crystallographic Data Centre as entries CCDC 938869, CCDC 938846, CCDC 953779 and CCDC 939924, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 3
Crystal data and experimental details.

Compound	(−)-(*S*)-**8**	(+)-(*R*)-**8**	(−)-(*S*)-**3**	(+)-(*R*)-**3**
Molecular formula	$C_{13}H_{12}NO_3F_3S$	$C_{13}H_{12}NO_3F_3S$	$C_7H_{10}NOF_3S_2$	$C_7H_{10}NOF_3S_2$
Formula weight	319.3	319.3	245.3	245.3
Crystallographic system	Monoclinic	Monoclinic	Orthorhombic	Orthorhombic
Space group	$P2_1$	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a [Å]	13.0823(2)	13.0879(3)	5.1804(1)	5.1787(1)
b [Å]	7.2641(1)	7.2702(2)	9.7097(1)	9.7075(2)
c [Å]	14.7325(2)	14.7333(3)	21.5963(3)	21.6130(3)
α [°]	90	90	90	90
β [°]	104.200(1)	104.178(1)	90	90
γ [°]	90	90	90	90
V [Å^3]	1357.27(3)	1359.19(6)	1086.30(3)	1086.53(3)
Z	4	4	4	4
D_{calcd} [g/cm^3]	1.563	1.560	1.500	1.499
Data collection temperature [K]	100(2)	100(2)	100(2)	100(2)
F(000)	656	656	504	504
Absorption coefficient μ [mm^{-1}]	2.572	2.568	4.620	4.619
Reflections measured	15,143	14,929	12,370	12,164
Reflections unique	4922	4925	2145	2082
R_{int}	0.0262	0.0236	0.0198	0.0217
Parameters refined	476	476	168	167
Absolute structure flack × parameter	0.013(9)	0.011(10)	−0.002(11)	0.051(16)
wR^2 (no cutoff)	0.0609	0.0656	0.0408	0.0605
R_1 (no cutoff)	0.0241	0.0250	0.0160	0.0222
Goodness-of-fit	1.011	1.001	1.000	1.024
Residual density max [eÅ^{-3}]	0.221	0.251	0.169	0.314
Resolution max [Å]	0.8205	0.8205	0.8089	0.8097

4.3. The assessment of ITC cytotoxicity

The normal skin fibroblast Malme-3 and the malignant melanoma Malme-3M were purchased from ATCC (American Cell Culture Collection). Malme-3 was cultured in the McCoy's 5A Medium, supplemented with 15% heat-inactivated fetal bovine serum and Malme-3M was cultured in the Iscove's Modified Dulbecco's Medium, supplemented with 20% heat-inactivated fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2.

For cytotoxicity tests, the Malme-3 and Malme-3M were seeded in 96-wells plates at a density of 0.75×10^5 cells/mL and 1.25×10^5 cells/mL, respectively. Cells were incubated with increasing concentrations (0.2–60 μM) of ITC. The ITC solutions were prepared in DMSO and were diluted at the ratio 1:1000 (v/v) in growth media. Pure DMSO was added to control wells. After 48 or 72 h of incubation the cytotoxicity test was performed. It based on the ability of living cells mitochondrial dehydrogenases to convert water-soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to non-soluble formazan. The cells were washed with PBS and incubated for 3 h with MTT solution. The formed formazan crystals were dissolved in isopropyl alcohol and solution absorbance was measured in a Power Wavex microplate spectrophotometer (Biotek Instruments) at 570 nm and 690 nm [42].

Data analysis. In order to compare the cytotoxic effect the IC_{50} values (the concentrations causing death of 50% of the cells) were determined. The normalized MTT data were fitted to Equation (1):

$$V = \frac{100}{1 + 10^{(\log IC50 - c)\cdot h}} \quad (1)$$

where: V – cell viability, c – concentration of the ITC, h – Hill coefficient.

To evaluate the difference between compound IC_{50} in normal and cancer cell line the unpaired *t*-test with the significance level of $p < 0.05$ was performed. The analysis of variance (ANOVA) with Dunnet post-hoc test was applied to determine the statistical significance of differences in compounds activity in comparison to sulforaphane (*R*)-**1**. All calculations were performed using Prism 5 ver 5.03 (GraphPad Software, Inc).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.036.

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