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Original article

Synthesis and anticancer activity of *N*-bis(trifluoromethyl)alkyl-*N*′-thiazolyl and *N*-bis(trifluoromethyl)alkyl-*N*′-benzothiazolyl ureas

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ABSTRACT

A number of *N*-bis(trifluoromethyl)alkyl-*N*′-thiazolyl and -benzothiazolyl ureas have been synthesized and evaluated for their *in vitro* antiproliferative activities against the human cancer cell lines at the National Cancer Institute (NCI, USA). The activity was shown for compounds **8a**,**c** and **9a–c**. The most sensitive cell lines relative to the tested compounds are: **8c** PC-3 (prostate cancer, log GI_{50} −7.10), **9c** SNB-75 (CNS cancer, log GI_{50} −5.84), **9b** UO-31 (renal cancer, log GI_{50} −5.66), and SR (leukemia, log GI_{50} −5.44) human cancer cells.

1. Introduction

Fluorinated organic molecules are known to perform a wide range of biological functions [1–9] and fluorinated anticancer agents have become a focus in the development of new therapies for cancer. An increasing number of fluorinated antitumor agents have now become available for cancer treatment [1,2,10,11]. Fluorine has become an invaluable tool for medicinal chemists because of the properties it confers on molecules that contain it, and the biological activity it can create as a result. Fluorine is the most electronegative element, so incorporating it into a potential drug molecule alters the electronic effects, and this can extend to altering the properties of neighboring functional groups, too. It can also have steric effects, changing the shape of the molecule. Furthermore, because of its rarity in nature, it is less likely to be degraded by naturally occurring enzymes, so it can help to improve the drug metabolic stability. Fluorine can have direct effects on the drug binding to the target site in the body, as it is able to form strong interactions with hydrogen bond donors and lipophilic side-chains, including aromatic groups [7–9]. The trifluoromethyl group, having three fluorine atoms and not just one, is one of the most lipophilic functional groups known. Its electronegative nature also gives it a much more dramatic effect on the drug molecule electronic character, with little more bulk than a normal methyl group [12,13].

A recent review on anticancer fluorine agents [11] reports about CF_3-containing anti-estrogens, protein kinase inhibitors, non-steroidal anti-inflammatory/analgesia compounds in cancer treatment. Already developed trifluoromethylated drugs such as flutamide (**I**), hydroxyflutamide (**II**), bicalutamide (**III**) (Fig. 1) and nilutamide (**IV**) (Fig. 2) are non-steroidal anti-androgens which are widely used for the treatment of metastatic prostate cancer [11,14,15]. Compounds (**I–III**) are amides (Fig. 1). However, according to Pomper [16–19] ureas possess better inhibition properties as compared with amides due to (enzymatic) hydrolysis resistance. This adds considerable support in favor of urea as a core moiety of new potential anticancer compounds. The best known cyclic fluorinated urea is 5-fluorouracil (**V**, also known as 5-FU, Adrucil, Efudex, Fluoroplex, NCS 19893), an NCI standard, which has been used as an anticancer drug for more than 40 years [20–23]. Synthetic methods for the preparation of several mono-, bis- and tris- trifluoromethyl containing physiologically active compounds, including the well known carcinolytic 5-trifluoromethyl uracil **VI** (another cyclic fluorinated urea) (Fig. 2), and their precursors were elaborated by us starting from perfluoroisobutene (**1**) [24–28], a toxic by-product of tetrafluoroethene and hexafluoropropene manufacture [29,30].

The versatile and synthetically accessible 2-(benzo)thiazolyl scaffold has provided the inspiration for the discovery of a number of new antitumor agents with unusual mechanisms of action in recent years [31–35]. A novel series of benzothiazole urea and

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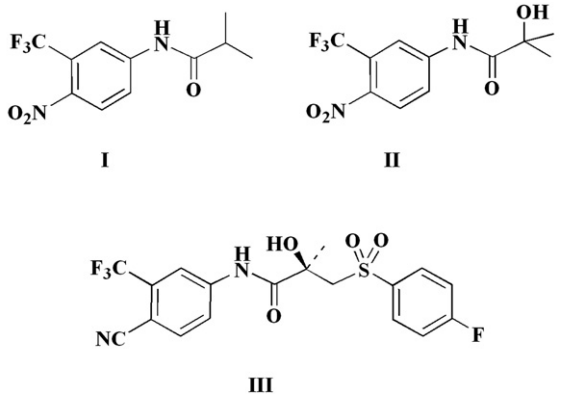


Fig. 1. Trifluoromethyl-containing amides, anticancer agents.

thiourea derivatives was synthesized and evaluated for its *in vitro* cytotoxicity against MCF7 breast cancer cells. Among those the *N*-(benzothiazol-2-yl)-*N*′-morpholinourea **VII** (Fig. 3) displayed the highest cytotoxic activity in this series [32]. Thiazolyl ureas **VIII** (Fig. 3) were synthesized and evaluated *in vivo* against leukemia P-388 tumor system in mice and showed moderate inhibitor activity [33]. A series of *N*-aryl-*N*′-heteroaryl or *N*,*N*′-diheteroaryl sulfonylurea derivatives showed a broad spectrum of antitumor activity as well as selective activity towards individual cell lines. In that series compound **IX** (Fig. 3) showed activities against RPMI-8226 leukemia, EKVX non-small cell lung cancer, PC-3 prostate cancer, OVCAR-4 ovarian cancer, CAKI-1 renal cancer, MDA-MB-435, and T-47D breast cancer [34].

As is seen from Fig. 3, most (benzo)thiazolyl ureas **VIII** and **IX**, which demonstrated anticancer activity, contain an electron withdrawing group (EWG) at another nitrogen atom, while *N*-methyl-*N*′-phenyl urea (**X**, Defenuron, NSC 10989), without EWG, demonstrated low anticancer activity [36]. It is notable that bis-trifluoromethylated 2-(3,3,3-trifluoro-2-trifluoromethyl-propenyl)-benzothiazole (**XI**, NSC 168629), in which a middle urea fragment is absent, revealed no anticancer activity [37]. We suggest that an EWG can change chemical properties of such ureas due to a possible (bio)chemical *O*-modification (alkylation, acylation, phosphorylation) [38–40]. Such modification is facilitated in substituted ureas with EWG due to urea–isourea tautomerism [41], which we have monitored in polar solvents by proton NMR spectroscopy for *N*-(bis)trifluoromethylalkyl-*N*′-(benzo)thiazolyl ureas, where the EWG is a bis-trifluoromethylated alkyl (see Experimental part, it is well seen for compound **9a**)Fig. 4.

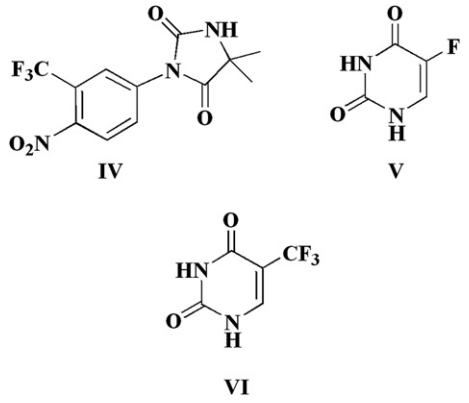


Fig. 2. Carcinolytic trifluoromethyl-containing ureas.

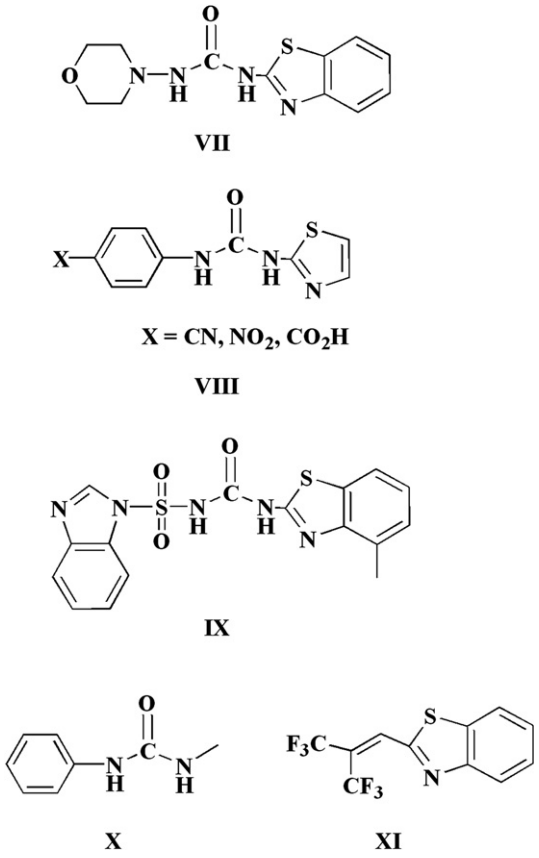


Fig. 3. (Benzo)thiazolyl ureas that revealed anticancer activity (**VII**–**IX**), as well as low active defenuron (**X**) and non-active bis(trifluoromethyl)ethenyl-benzothiazole **XI**.

Having taken the above data into account it was expected that a combination of trifluoromethylalkyl and (benzo)thiazole substituents in one molecule of urea could be interesting as compounds with potential anticancer activity. To the best of our knowledge such structures have never been tested for physiological activity. From this time on we have started to fill the gap aiming to explore a new class of compounds. For this purpose, here we report the synthesis and *in vitro* anticancer activity against human cancer cell lines of a series of fluorinated ureas of 2-aminothiazole and 2-aminobenzothiazole.

2. Results and discussion

2.1. Chemistry

The preparation of *N*-fluoroalkyl-*N*′-thiazolyl **8a**–**c** and *N*-fluoroalkyl-*N*′-benzothiazolyl **9a**–**c** ureas has been carried out by addition of 2-aminothiazole **6** and 2-aminobenzothiazole **7** to fluoroalkyl isocyanates **5a** and **5b**,**c** (Scheme 1). Isocyanates **5** have been prepared starting from perfluoroisobutene **1**. Hydrolysis of

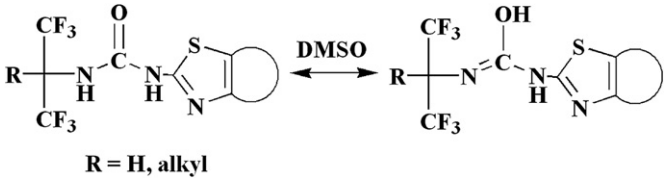
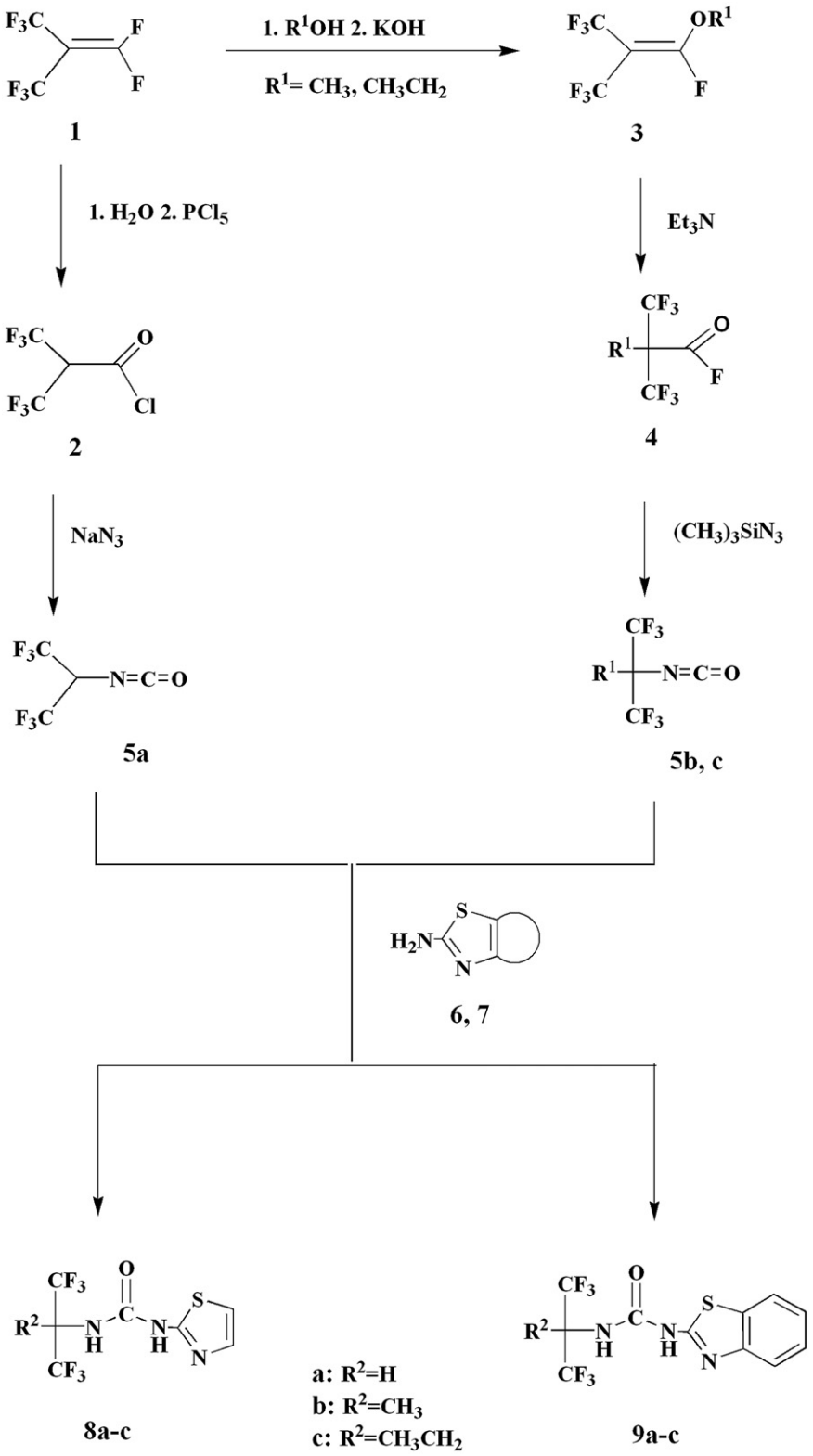


Fig. 4. Urea–isourea tautomerism of *N*-bis(trifluoromethyl)alkyl-*N*′-heteroaryl ureas.



Scheme 1. Synthesis of polyfluorinated ureas **8a–c** and **9a–c**.

perfluoroalkene **1** into hexaflouroisobutyric acid (not shown) [30] followed by treatment with phosphorus pentachloride leads to chloroanhydride **2** [42]. The reaction of acid chloride **2** with sodium azide under heating resulted in isocyanate **5a** [43]. Reaction of perfluoroisobutene **1** with methanol and ethanol followed by alkali treatment resulted in ethers **3** which in the presence of triethylamine were converted into fluoroanhydrides **4** [28–30,44]. Curtius rearrangement of acylazides (not shown) obtained from acid fluorides **4** under the heating with trimethylsilyl azide in xylenes resulted in isocyanates **5b**,**c** [28].

Table 1
In vitro anticancer activity – NCI's 3 cell line pre-test.[a]

Compound	Growth of cells (%)			
	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)	Result[b]
8a	22	35	20	Active
8b	49	59	74	Inactive
8c	6	20	40	Active
9a	−74	−10	−39	Active
9b	43	62	28	Active
9c	−93	−79	−94	Active

[a] Data were obtained from the NCI's in vitro anticancer 3 cell line pre-test. Negative numbers indicate cell kill. Each cell line was inoculated and preincubated on a microtiter plate. Test agents (10^{-4} M) were added and the culture incubated for 48 h. End-point determinations were made with alamar blue. Results for each test agent were reported as the percentage of growth of the treated cells when compared to untreated control cells.
[b] Compounds which reduce the growth of any one of the cell lines to 32% or less are called "active" and passed on for evaluation in the main test.

2.2. Biological activity

All six synthesized polyfluorinated ureas **8a–c** and **9a–c** do not violate Lipinski's rules for new potential drug candidates [45]. They are also known as the "Rule of five" for perspective orally active drugs. Namely:

1) a molecular weight is under 500: $293.19 \leq MW_{\mathbf{8,9}} \leq 371.70$;
2) a limited lipophilicity (expressed by Log P < 5): $2.51 \leq$ ca. Log $P_{\mathbf{8,9}} \leq 4.56$ (calculated with ChemDraw™ Ultra, version 9.0);
3) maximum 5 H-bond donors (expressed as the sum of OHs and NHs): this value is 2 for compounds **8a–c** and **9a–c**;
4) maximum 10 H-bond acceptors (expressed as the sum of Os and Ns): this value equals 4 for compounds **8a–c** and **9a–c**.

The synthesized ureas were evaluated in the anticancer screen for human disease-oriented tumor cell line developed at the NCI [46–48]. This *in vitro* screen is subdivided into a pre-test and a main test. Within the one dose pre-test consisting of three tumor cell lines MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS) test agents were added at only one concentration (10^{-4} M) to each cell line inoculated and preincubated on a microtiter plate, and the culture was then incubated for 48 h. End-point determinations were made with alamar blue [49]. The results for each tested compound are reported as the percentage of growth of the treated cells in comparison with that of the untreated control cells (Table 1). Negative numbers indicate cell death. Compounds reducing the growth of any one of the three-cell lines by 32% or more are called "active" (**8a**,**c**, **9a–c**) and subsequently passed on for evaluation in the main test, this time consisting of approximately 60 cell lines over a 5-log dose range (10^{-4}–10^{-8} M). Urea **8b** did not reveal the threshold activity and was ruled out from the main test as an "inactive" one.

Within the main test the antitumor activity of a test compound is expressed by three different dose–response parameters for each of the 60 cell lines derived from nine different types of cancer, namely, leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast: GI_{50} (molar concentration required for half-growth inhibition), TGI (molar concentration leading to total growth inhibition), and LC_{50} (molar concentration required for 50% cell death). Additionally, a mean graph midpoint (MG_MID) is calculated for each of the above-mentioned parameters, which displays an averaged activity parameter over all cell lines, as well as the Delta parameter that is the difference between the highest and the average values [47]. Table 2 represents a general overview on these main parameters that characterize cytostatic activity of the tested compounds. The most sensitive cell lines (Table 3) relative to the tested compounds are: **8a** against HS 578T breast (log GI_{50} −4.65, log TGI −4.2); **8c** against PC-3 prostate (log GI_{50} −7.10, log TGI −4.52); **9a** against UO-31 renal (log GI_{50} −5.49, log TGI −4.94, log LC_{50} −4.34); **9b** against UO-31 renal (log GI_{50} −5.66, log TGI −5.11, log LC_{50} −4.47); **9c** against SNB-75 CNS (log GI_{50} −5.84, log TGI −4.71, log LC_{50} −4.28). As shown in Table 2, urea **9c** exhibited the highest average anticancer activity among the tested compounds. Moreover, compound **9c** demonstrated the cytostatic activity against almost all 57 cell lines tested with it (57, 56 and 53 for GI_{50}, TGI and LC_{50}, respectively, Tables 2–4).

Table 4 shows the antiproliferative data for polyfluoroalkyl (benzo)thiazolyl ureas **8a**,**c** and **9a–c** against 60 human cancer cell lines. The NCI standard anticancer agent 5-fluorouracil **V** [36], as well as structural analogs of compounds **8** and **9** bearing similar fragments, namely, nonfluorinated urea **X** [36] and non-urea bis-trifluoromethylated benzothiazole **XI** [37] were used as reference compounds. (The latter is not shown in Table 3 due to the absence of activity). Fluorinated ureas **8a**,**c** and **9a–c** demonstrated much higher activity against all cell lines tested in comparison with nonfluorinated aryl–alkyl urea **X** and bis-trifluoromethylated

Table 2
Overview of the results of the in vitro anticancer activity screening for compounds **8a**,**c** and **9a–c**.[a]

Cd	N	Number of cell lines giving positive log GI_{50}[b], log TGI[c], log LC_{50}[d]						MG_MID[e] and Δ[f] for:			Most sensitive cell lines
		log GI_{50}[b]		log TGI[c]		log LC_{50}[d]		log GI_{50}	log TGI	log LC_{50}	
		N1	Range[g]	N2	Range[g]	N3	Range[g]				
8a	57	49	−4.65 to −4.02	3	−4.20 to −4.03	0	>−4.00	−4.28/0.37	−4.01/0.19	−4.00/0.00	Breast cancer HS 578 T
8c	57	49	−7.10 to −4.07	5	−4.52 to −4.09	0	>−4.00	−4.32/2.78	−4.02/0.50	−4.00/0.00	Prostate cancer PC-3
9a	59	59	−5.49 to −4.42	39	−4.94 to −4 .03	9	−4.34 to −4.02	−4.62/0.87	−4.16/0.78	−4.02/0.32	Renal cancer UO-31
9b	59	54	−5.66 to −4.01	9	−5.11 to −4.04	2	−4.47 to −4.10	−4.45/1.21	−4.07/1.04	−4.01/0.46	Leukemia SR,CNS cancer SNB-75, Renal cancer UO-31
9c	57	57	−5.84 to −4.53	56	−4.76 to −4.15	53	−4.37 to −4.02	−4.77/1.08	−4.46/0.31	−4.19/0.19	Renal cancer UO-31, CNS cancer SNB-75

N – number of tested human tumor cell lines; N1, N2, N3 – number of cell lines giving positive log GI_{50}, log TGI, log LC_{50}.
[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells. Compound **8b** was inactive ($\log_{10} GI_{50} > 4.00$).
[b] The log of the molar concentration that inhibits 50% net cell growth.
[c] The log of the molar concentration leading to total growth inhibition.
[d] The log of the molar concentration leading to 50% net cell death.
[e] MG_MID = mean graph midpoint = arithmetical mean value for all tested cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.
[f] The reported data represent the logarithmic difference between the parameter value referred to the most sensible cell line and the same mean parameter. Delta is considered low if <1, moderate >1 and <3, high if >3.
[g] The value >−4.00 were excluded.

Table 3
The most sensitive cell lines in vitro anticancer activity for compounds **8a**,**c** and **9a**–**c**.

Cd	Cancer type	Most sensitive cell line	log GI_{50}	log TGI	log LC_{50}
8a	Breast Cancer	HS 578T	−4.65	−4.20	>−4.00
8c	Prostate cancer	PC-3	−7.10	−4.52	>−4.00
9a	Renal Cancer	UO-31	−5.49	−4.94	−4.34
9b	Leukemia	SR	−5.44	−4.90	>−4.00
	CNS Cancer	SNB-75	−5.12	−4.44	>−4.00
	Renal Cancer	UO-31	−5.66	−5.11	−4.47
9c	CNS Cancer	SNB-75	−5.84	−4.71	−4.28
	Renal Cancer	UO-31	−5.33	−4.76	−4.37

benzothiazolyl non-urea **XI**. This result can indicate an importance of all structural subunits that provide together bioactivity of compounds **8a**,**c** and **9a–c** such as bis(trifluoromethyl)alkyl EWG, urea core, and (benzo)thiazolyl heterocycle. Overall activity of fluorouracil **VI** (MG_MID −6.10) is higher than of any urea **8a**,**c** and **9a–c**. Nevertheless, all five compounds **8a**,**c** and **9a–c** demonstrate higher activity than standard fluorouracil **V** does against EKVX and HOP-92 (Non-Small Cell Lung Cancer) as well as SNB-75 (CNS Cancer) and SK-MEL-2 (Melanoma). Furthermore, benzothiazolyl ureas **9a–c** are more active than cyclic urea **V** against NCI-H226 (Non-Small Cell Lung Cancer). Last, but not least, anticancer activity of thiazolyl urea **8c** against cancer cell line PC-3 (Prostate Cancer) far exceeds that of anticancer drug **V**. From Tables 2–4 it follows that compound **8c** demonstrated the highest overall selective activity against prostate cancer cell line PC-3 (log GI_{50} −7.10). Additionally, it is worth noting the high antiproliferative activity (Tables 3 and 4) of compound **9b** against SR leukemia (log GI_{50} −5.44, log TGI −4.90) and SNB-75 CNS (log GI_{50} −5.12, log TGI −4.44); as well as urea **9c** against UO-31 renal (log GI_{50} −5.33, log TGI −4.76, log LC_{50} −4.37).

We have performed COMPARE [50] analyses for all the active compounds in order to investigate the similarity of their cytotoxicity pattern (mean graph fingerprints) with those of known anticancer standard agents, NCI active synthetic and natural compounds, which are present in public available databases [36]. Such analysis is based on comparing the patterns of differential growth inhibition for cultured cell lines and can potentially gain insight into the mechanism of the cytotoxic action. If the data pattern correlates well with that of compounds belonging to a standard agent database (a pairwise or Pearson correlation coefficient (PCC) >0.6), the compound of interest may have the same mechanism of action [51]. On the other hand, if the activity pattern does not correlate with any standard agent, it is possible that the compound has a novel mechanism of action. Standard COMPARE analyses were performed at the GI50 level for compounds **8a**, **c** and **9a–c**, where each of them was used as a seed compound in six NCI databases: BEC REFERRAL SET GI50, DIVERSITY SET GI50, MARKETED DRUGS GI50, MECHANISTIC SET GI50, STANDARD AGENTS GI50 and SYNTHETIC COMPOUNDS GI50 [36] (the best correlations are summarized in Supplementary information). Compounds **8a** and **8c** did not reveal correlations >0.6 with any previously tested compounds from the above databases. This result can be explained by a possible new mechanism of their bioactivity (a possible mechanism for inhibition of PC-3 human tumor cell line by fluorourea **8c** will be presented in the end of this chapter).

High correlations were demonstrated for urea **9a** by alkaloid Ecteinascidin 729 (ET729; NSC S638718; PCC = 0.757) and depsipeptide Dehydrodidemnin B (DDB; NSC S638719; PCC = 0.757) [52]. It is notable that these two natural derivatives showed also highest correlations almost 0.6 in COMPARE with seeded methyl derivative **9b**: ET729, PCC = 0.554; DDB, PCC = 0.554. Such similarity in COMPARE results could indicate the resemblance in mechanisms of action for benzothiazoles **9a** and **9b**. The reported mechanism of action for ET729 is alkylation of the minor groove of DNA [53]. This means that ET729 works as an alkylating agent. In the case of ureas **9a** and **9b**, they do not have alkylating moieties. Nevertheless, *O*-alkyl isoureas are strong alkylating agents [54–58]. Such intermediate *O*-alkyl isoureas could be formed *in situ* by *O*-alkylation of isoureid tautomers of **9a** and **9b**. To validate this suggestion it is necessary to synthesize and test at least two *O*-methylated isoureas from ureas **9a** and **9b**. The synthesis of such *O*-alkyl isoureas is under development. Another COMPARE analog of benzothiazoles **9a**,**b**, DDB, is reported to dramatically decrease ornithine decarboxylase activity and thereby to inhibit the production of polyamines, compounds required for cell division [59]. The latter putative mechanism can explain high cytostatic efficiency of ureas **9a** and **9b**. We therefore might assume that derivatives **9a** and **9b** inhibit ornithine decarboxylase, but this only provides limited information regarding the mechanism of action and demands future pharmacokinetic studies. In regard to ethyl derivative **9c**, there are three compounds from six above-mentioned NCI databases that correlate well enough (PCC > 0.6) with it, namely: 2-benzo[1,4]dioxin-2-yl-4*H*-[1,8]naphthyridine-1-carboxylic acid tert-butyl ester (BDNCB, NCS S717212, PCC = 0.642); alkaloid Trigilletine [60–62] (NCS S181486, PCC = 0.639) and flavanoid Calendulaglycoside D-6′-*O*-methyl ester [63] (CGDM, NCS S731922, PCC = 0.602). In literature we have found only a possible mechanism of action for CGDM [63]. It is inhibiting or reducing a transport function of P-glycoprotein (Pgp). There is no printed information about mechanisms of action of BDNCB and Trigilletine possibly because that they both were tested recently. Nevertheless, PubChem provides an access to online databases with the results of recent tests of these two compounds [37] (see Supplementary Information). We have retrieved data that Trigilletine revealed inhibition activity against 5 protein targets: (1) huntingtin; (2) NCV HS3 helicase; (3) IMP-1 metallo-beta-lactamase; (4) MEK Kinase PB1 Domains, specifically MEK5 binding to MEK Kinase 2 Wildtype; (5) MEK Kinase PB1 Domains, specifically MEK5 binding to MEK Kinase 2 mutant. As for BDNCB, it revealed activity against 32 protein targets (all of them are posted in Supplementary Information). Among them are huntingtin, beta-lactamase (both as for Trigilletine), tumor protein p53, Caspase-1, Caspase-7, 15-human lipogenase 2, HADH2, ATP-binding protein YjeE, HSD17B4, menin isoform 1, arachidonate 12-lipoxygenase, mitogen-activated protein kinase 1, et al. Such a broad spectrum of activity for these three COMPARE analogs of compound **9c** makes difficult thus far to specify its anticancer targets. However, it is worth doing so, because urea **9c** revealed the highest overall anticancer activity among compounds **8a–c** and **9a–c** as it follows from Tables 2–4.

The relationship of activity from length of alkyl R (H–Me–Et) in the fluoroalkyl group was not quite well defined (Tables 2–4). The highest activity has been shown by urea **9c** (R = Et). The longer alkyl chain seems to be more active in this case. The absence of the direct correlations of activity and alkyl length can be explained from a structural point of view. Compounds **8a** and **9a** contain 2H-perfluoroisopropyl moiety with a relatively acidic hydrogen atom that makes possible dehydrofluorination followed by an amidating of $=CF_2$ with $-NH_2-$ group of a protein or nucleic acid. At the same time all fluorine atoms in ureas **8b**, **8c**, **9b**, **9c** are inert and bioactivity of these compounds could be due their stereo properties and hydrophobicity/philicity of different parts of the molecules. Alternatively these fluoroalkyl ureas **8a–c** and **9a–c** can form their isourea tautomers with active OH-group that could be in situ modified (e.g., alkylation, acylation or phosphorylation) [38–40] and interfere with biological processes. However this suggestion is not proven yet. The bulkier ethyl derivatives **8c** and **9c** show

Table 4
In vitro anticancer activity of compounds **8a,c** and **9a–c** against 60 human cancer cell lines in comparison with data of NCI's compounds.[a,b,c]

Panel/Cell line	Compound/growth inhibitory activity (log GI_{50})						
	8a	**8c**	**9a**	**9b**	**9c**	**X**[a]	**V**[b]
Leukemia							
CCRF-CEM	–	−4.71	−4.48	−4.56	−4.63	−4.00	−5.01
HL-60(TB)	−4.14	–	−4.58	−4.01	–	−4.00	−5.64
K-562	−4.44	−4.46	−4.60	−4.77	−4.63	−4.00	−5.45
MOLT-4	−4.39	−4.51	−4.53	−4.74	−4.53	−4.00	−6.45
RPMI-8226	–	–	−4.47	−4.41	–	−4.00	−7.35
SR	−4.62	−4.52	−4.59	−5.44	−4.77	−4.00	−7.62
Non-Small Cell Lung Cancer							
A549/ATCC	−4.22	−4.35	−4.57	−4.79	−4.80	−4.00	−6.72
EKVX	−4.38	−4.45	−4.68	−4.13	−4.77	−4.00	−4.21
HOP-62	>−4.00	>−4.0	−4.42	−4.50	−4.75	−4.00	−6.40
HOP-92	−4.42	−4.22	−4.47	−4.12	−4.72	−4.00	−4.11
NCI-H226	−4.24	−4.15	−4.59	−4.41	−4.80	−4.00	−4.26
NCI-H322 M	−4.06	>−4.0	−4.74	−4.57	−4.59	−4.00	−6.75
NCI-H460	−4.46	−4.41	−4.65	−4.61	−4.67	−4.00	−7.25
NCI-H522	−4.63	−4.42	−4.85	−4.61	−4.77	−4.00	−5.14
NCI-H23	–	–	–	–	–	−4.00	−6.48
Colon Cancer							
COLO 205	−4.28	−4.13	−4.75	−4.42	−4.79	−4.00	−6.80
HCC-2998	−4.50	−4.13	−4.70	−4.41	−4.72	−4.00	−7.28
HCT-116	−4.42	−4.33	−4.78	−4.38	−4.74	−4.00	−6.64
HCT-15	−4.18	−4.27	−4.59	>−4.00	−4.75	−4.00	−6.96
HT29	−4.04	−4.38	−4.71	−4.71	−4.71	−4.00	−6.75
KM12	−4.40	−4.33	−4.53	−4.33	−4.69	−4.00	−6.67
SW-620	>−4.00	−4.08	−4.47	−4.34	−4.68	−4.00	−6.03
CNS Cancer							
SF-268	−4.53	−4.22	−4.53	−4.53	−4.77	−4.00	−5.80
SF-295	−4.36	−4.28	−4.63	−4.22	−4.77	−4.00	−6.64
SF-539	>−4.00	>−4.00	−4.57	−4.61	−4.74	−4.00	−7.20
SNB-19	−4.31	>−4.00	−4.45	−4.61	−4.68	−4.00	−5.42
SNB-75	−4.26	−4.71	−4.77	−5.12	−5.84	−4.13	−4.10
U251	−4.47	−4.39	−4.61	−4.47	−4.76	−4.00	−6.04
Melanoma							
LOX IMVI	−4.23	−4.14	−4.64	−4.44	−4.74	−4.00	−6.61
MALME-3 M	−4.53	−4.23	−4.69	−4.82	−4.81	−4.00	−7.29
M14	>−4.00	−4.42	−4.72	−4.43	−4.73	−4.00	−6.01
SK-MEL-2	−4.34	−4.70	−4.64	−4.52	−4.80	−4.00	−4.25
SK-MEL-28	>−4.00	−4.14	−4.45	−4.14	−4.75	−4.00	−5.99
SK-MEL-5	−4.41	−4.47	−4.81	−4.31	−4.75	−4.00	−6.33
UACC-257	−4.45	−4.49	−4.69	−4.29	−4.70	−4.00	−5.45
UACC-62	−4.20	−4.39	−4.61	>−4.00	−4.81	−4.00	−6.28
Ovarian Cancer							
IGROV1	−4.02	>−4.00	−4.42	−4.38	−4.77	−4.00	−5.91
OVCAR-3	−4.37	−4.21	−4.74	−4.68	−4.78	−4.00	−7.80
OVCAR-4	−4.40	−4.08	−4.54	−4.66	−4.73	−4.00	−5.35
OVCAR-5	−4.04	>−4.00	−4.56	−4.09	−4.71	−4.00	−4.96
OVCAR-8	−4.40	−4.27	−4.47	−4.28	−4.74	−4.00	−5.76
SK-OV-3	>−4.00	>−4.00	−4.51	−4.28	−4.75	−4.00	−4.66
Renal Cancer							
786-0	>−4.00	>−4.00	−4.54	−4.69	−4.70	−4.00	−6.14
A498	−4.02	−4.33	−4.70	>−4.00	−4.87	−4.00	−6.40
ACHN	−4.26	−4.21	−4.55	−4.54	−4.74	−4.00	−6.53
CAKI-1	−4.26	−4.08	−4.54	−4.41	−4.72	−4.00	−7.14
RXF 393	−4.11	−4.08	−4.55	−4.19	−4.76	−4.00	−5.58
SN12C	−4.36	−4.26	−4.85	−4.59	−4.79	−4.00	−6.30
TK-10	−4.29	−4.50	−4.50	−4.31	−4.66	−4.00	−5.95
UO-31	−4.45	−4.02	−5.49	−5.66	−5.33	−4.00	−5.85
Prostate Cancer							
PC-3	−4.42	−7.10	−4.60	−4.46	−4.74	−4.00	−5.63
DU-145	−4.20	−4.16	−4.52	−4.04	−4.73	−4.00	−6.44
Breast Cancer							
MCF7	−4.39	−4.37	−4.55	−4.42	−4.80	−4.00	−7.10
NCI/ADR-RES	−4.48	−4.53	−4.56	>−4.00	−4.71	−4.00	−6.50
MDA-MB-231/ATCC	−4.23	−4.07	−4.65	−4.46	−4.79	−4.00	−5.18
HS 578T	−4.65	−4.28	−4.57	−4.73	−4.67	−4.00	−5.01
MDA-MB-435	−4.10	−4.21	−4.50	−4.38	−4.70	−4.00	−7.14
MDA-N	>−4.00	−4.22	−4.63	−4.01	−4.73	−4.00	–
BT-549	−4.33	−4.39	−4.55	>−4.00	−4.74	−4.00	−4.97
T-47D	−4.34	−4.41	−4.77	−4.42	−4.81	−4.00	−5.09
MG_MID[d]	−4.28	−4.32	−4.62	−4.45	−4.77	−4.006	−6.10
Delta[e]	0.37	2.78	0.87	1.21	1.08		

[a] NCI's data for *N*-methyl-*N*′-phenyl urea **X**, NCS 10989.
[b] NCI'data for 5-fluorouracil **V**, NCS 19893 (NCI standard compound).
[c] Third reference compound **2**-(3,3,3-trifluoro-2-trifluoromethyl-propenyl)-benzothiazole (**XI**, NSC 168629) is not active and not shown in Table 4.
[d] MG_MID = mean graph midpoint = arithmetical mean value for all tested cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.
[e] Delta – Difference in log GI_{50} value of the most sensitive cell lines and the MG_MID value. Delta is considered low if <1, moderate >1 and <3, high if >3.

a higher activity. On the other hand, the benzothiazolyl ureas are more active in comparison with the thiazolyl ones. It should be noted that thiazolyl H-homolog **8a** proved to be nonselective with broad spectrum anticancer activity against the nine tumor subpanels, thiazolyl Me-homolog **8b** was inactive even in the pre-test that could be explained by its small size and chemical inertness. Nevertheless thiazolyl Et-homolog **8c** revealed the best selective activity against prostate cancer cell line PC-3 (log GI_{50} −7.10).

While it is too early to claim a definite mechanism for PC-3 inhibition with compound **8c**, today we could only suggest some reasons of such specific activity. As urea **8c** did not reveal any activity correlation COMPAREd with already described compounds we dare to suggest a novel mechanism of its action against PC-3 prostate cancer cell line. The suggestion will be done based not on the compound structure but the cell properties. The PC-3 is a peculiar cell line, which is extremely sensitive to pH. PC-3 tumor pH is acidic [64]. Its increase can cause inhibition of the cells proliferation and even their death. That is why any biochemical distortion that decreases intracellular acidity and/or increases basicity inside PC-3 cells can be a reason for antiproliferative activity of urea **8c** against this cell line. One of the mechanisms of such acid–base interconversion might be the reverse Warburg effect, which is opposite to the "direct" Warburg effect. The "direct" Warburg effect [65,66] is a metabolic feature of many cancers that causes them even in the presence of sufficient oxygen to preferentially metabolize pyruvate, which is a product of glycolysis [67–69] or/and glutaminolysis [70,71] (glutamine is a part of the test medium, see Experimental part), to lactic acid with NADH, catalyzed by lactate dehydrogenase (LDH), thus providing acidolysis of tumor cells. The "direct" Warburg effect may confer a survival advantage upon tumor cells, and a major question is whether cancer cells are selectively killed if this effect is reversed. There are two possible approaches to reversing the Warburg effect and thereby reactivating the mitochondria: first by activating pyruvate dehyrogenase (PDH), which facilitates pyruvate entry into tricarboxylic acid (TCA) cycle, and second, by inhibiting LDH [72]. The just mentioned inhibition suppresses the conversion to lactate and thereby shifts pyruvate metabolism towards bicarbonate (a base) and acetyl coenzyme A (Acetyl-CoA). Acetyl-CoA, in turn, enters the Krebs cycle and eventually produces bicarbonate too. A dramatic increase of bicarbonate/lactate ratio under LDH-A inhibition has recently been demonstrated by hyperpolarized ^{13}C NMR method [73]. Additionally, Robey et al. have recently reported the PC-3 tumor pH increase by bicarbonate and consequent inhibition of spontaneous metastasis [74]. This is one hypothetical explanation of

a possible mechanism of thiazolyl urea **8c** action against PC-3 human prostate cancer cells. A future work will demonstrate whether this suggestion is valid.

It is necessary to note that anticancer trifluoromethyl-containing antitumor compounds **I**–**IV**, mentioned in the Introduction, have never been tested *in vitro* against whole NCI panel of standard 60 cancer cell lines. However available literature data indicate quite a moderate activity of these compounds against selected cancer cell lines, when tested individually [75]. The activity of drugs **I**–**IV** dramatically enhanced in combination with other drugs due to synergistic effect [76–78]. This information can open door for future investigations not only in structure modification, but also in pharmacological studies of *N*-bis(trifluoromethyl)alkyl-*N*′-thiazolyl and -benzothiazolyl ureas.

3. Conclusion

Herein we demonstrated the *in vitro* anticancer activity of some new *N*-fluoroalkyl-*N*′-thiazolyl and -benzothiazolyl ureas. Although the critical biochemical targets of these molecules have not been identified yet, they show an interesting antiproliferative profile against different human tumor-derived cell lines, especially thiazolyl urea **8c** against PC-3 (prostate cancer), benzothiazolyl ureas **9a**–**c** against UO-31 (renal cancer), benzothiazolyl ureas **9b**,**c** against SNB-75 (CNS cancer), and benzothiazolyl urea **9b** against SR (leukemia). With this result *N*-fluoroalkyl-*N*′-(benzo)thiazolyl ureas represent a new perspective class of physiologically active compounds.

4. Experimental

4.1. General methods

The ^{1}H and ^{19}F NMR spectra were recorded on Bruker DXP at 200 and 188 MHz, respectively, in $CDCl_3$, DMSO-d_6 and acetone-d_6 using tetramethylsilane (TMS) as an internal standard and CF_3COOH as an external standard. Chemical shifts are reported in ppm units with the use of δ scale. Mass-spectra were recorded on Finnigan 4021. The elemental analyses (C, H, F, N) were performed in the laboratory of analytic chemistry of IPAC RAS. Melting points were measured in open capillary tubes and are uncorrected. Perfluoroisobutene **1** was obtained by pyrolysis of octafluorocyclobutane [30]. Alkenyl ethers **3** were obtained according to procedures [30,42]. Haloanhydrides **2** and **4** were synthesized after methods [42] and [30,42], respectively. Starting isocyanates **5a**–**c** were prepared by Curtius reaction [28,43]. 2-Aminothiazole **6** and 2-aminobenzothiazole **7** (Aldrich) were sublimed in vacuum (10^{-2} Torr) before the reaction. Compounds **8c** and **9c** were synthesized according to our procedure [28].

4.1.1. 3,3,3-Trifluoro-2-trifluoromethyl-propionyl chloride *2*

Perfluoroolefin **1** (2 mol) was carefully dissolved at r.t by slow bubbling into a 10 L polyethylene reactor containing a mixture of 5 L of acetone, 1 L of water and 500 g of ground glass powder. The reaction mixture was left in a ventilation hood for 72 h and agitated every 12 h for 5–10 min. The upper transparent solution was decanted and extracted with an equal volume of diethyl ether 2 times. The extract was evaporated under reduced pressure (20 Torr). The residue was loaded into a 2 L 1-neck glass round-bottom flask, mixed with an equal volume of 95% H_2SO_4 and moved under high vacuum (1–2 Torr) into 1 L glass flask cooled with liquid nitrogen. Thus obtained crude hexafluoroisobutiric acid $(CF_3)_2CHCO_2H$ was mixed with another equal volume of 95% H_2SO_4 and distilled under atmospheric pressure. Yield 70%, colorless crystals, b.p. 126–128 °C, m.p. 50–52 °C. ^{1}H and ^{19}F NMR spectra are in accordance with the previously reported data [79,80].

Phosphorus pentachloride (1.6 mol) was slowly added to a 1.5 L 2-neck round-bottom flask equipped with an efficient reflux condenser and containing $(CF_3)_2CHCO_2H$ (1.5 mol). After a vigorous reaction stopped the reaction mixture was boiled for 2 h. The fraction boiling below 110 °C was distilled off from the reaction mixture. Product **2** was isolated by rectification. Yield 66% relative to starting compound **1** (94% relative to $(CF_3)_2CHCO_2H$), colorless liquid, b.p. 54–55 °C. ^{1}H and ^{19}F NMR spectra correspond to the previously reported data [81].

4.1.2. 1,1,1,3,3,3-Hexafluoro-2-isocyanato-propane *5a*

Chloroadhydride **2** (0.7 mol), sodium azide (1.4 mol), trimethylsilyl azide (7.5 mmol) and benzyltriethylammonium chloride (4.4 mmol) were loaded into a 1 L 2-neck round bottom flask containing dry diglyme (200 mL) and dry dibutyl ether (200 mL) and equipped with an efficient reflux condenser and magnetic stirrer. The temperature was gradually allowed to reach 100 °C. After the reaction was cooled down the fraction boiling below 140 °C was distilled off from the reaction mixture. Isocyanate **5a** was isolated by rectification. Yield 75%, colorless liquid, b.p. 48–50 °C. ^{1}H and ^{19}F NMR spectra correspond to previously reported data [82].

4.2. General procedure for the synthesis of compounds 3

A 2 L stainless steel bomb was loaded with 3 mol of dry R^1OH (R^1=CH_3 or CH_3CH_2), the bottom was cooled with liquid nitrogen, then 2 mol of precooled (−40 °C) perfluoroolefin **1** was added. The bomb was sealed and kept at r.t., shaken from time to time. After 48 h the bomb was opened, vented for ∼1 h. Then the content was poured into iced water, washed once with water (1 L), once with saturated $NaHCO_3$ water solution (1 L), then with water again (1 L). The heavy organic layer was separated and dried over $CaCl_2$. This liquid ether was slowly added dropwise into a 3 L round-bottom 3-neck flask equipped with a condenser, a mechanical stirrer and a dropping funnel and contained 1.5 L of dry diethyl ether and 4 mol of solid KOH. The reaction mixture was stirred for ∼5 h. The speed of adding and agitation was regulated manually to avoid vigorous reflux. If necessary an ice water bath was used to slow down the reaction. Product **3** was isolated by rectification.

4.2.1. 1,3,3,3-Tetrafluoro-1-methoxy-2-trifluoromethyl-propene (*3*, R^1=CH_3)

Yield 69% (relative to alkene **1**), colorless liquid, b.p. 99–102 °C. ^{1}H and ^{19}F NMR spectra correspond to the previously reported data [83,84].

4.3. 1-Ethoxy-1,3,3,3-tetrafluoro-2-trifluoromethyl-propene (*3*, R^1=CH_3CH_2)

Yield 62% (relative to alkene **1**), colorless liquid, b.p. 114–117 °C. ^{1}H NMR ($CDCl_3$): δ 1.42 (t, 3H, J = 7 Hz); 4.38 (q, 2H, J = 7 Hz). ^{19}F NMR: δ 11.35 (qq, 1F, J_1 = 25 Hz, J_2 = 11 Hz); 18.75 (dq, 3F, J_d = 11 Hz, J_q = 8); 19.55 (dq, 3F, J_d = 25 Hz, J_q = 8 Hz). EI-MS (m/z): 226 (M^+). Anal. Calcd. for $C_7H_5F_6N_3OS$: C, 31.87%; H, 2.23%; F, 58.82%; Found: C, 31.51%; H, 2.30%; F, 58.67%.

4.4. General procedure for the synthesis of fluoroanhydrides 4

A 400 mL stainless steel bomb containing 1.5 mol of alkoxyheptafluoroolefin **3** (R^1=CH_3 or CH_3CH_2) and 0.15 mol of triethylamine was heated at 130 °C 24 h. Product **4** was isolated by rectification.

*4.4.1. 3,3,3-Trifluoro-2-methyl-2-trifluoromethyl-propionyl fluoride (**4**, R^1=CH_3)*

Yield 89%, colorless liquid, b.p. 47–48 °C. ^{1}H and ^{19}F NMR spectra correspond to the previously reported data [44,84].

*4.4.2. 2,2-Bis-trifluoromethyl-butyryl fluoride (**4**, R^1=CH_3CH_2)*

Yield 75%, colorless liquid, b.p. 75–78 °C. ^{1}H and ^{19}F NMR spectra correspond to the previously reported data [44].

4.5. General procedure for the synthesis of polyfluoroisocyanates 5b and 5c

A solution of trimethylsilyl azide (1 mol) in xylenes (100 mL) was slowly added dropwise to a boiling solution of acid fluoride **4** (1 mol) and benzyltriethylammonium chloride (0.004 mol) in xylenes (150 mL) in a round-bottom two-neck flask with a dropping funnel and an efficient reflux condenser. After the addition of trimethylsilyl azide, the reaction mixture was boiled for 4 h. The reaction was monitored by ^{19}F NMR spectroscopy. The fraction boiling below 140 °C was distilled off from the reaction mixture. The product was isolated by rectification.

*4.5.1. 1,1,1,3,3,3-Hexafluoro-2-isocyanato-2-methyl-propane (**5b**)*

Yield 89%, colorless liquid, b.p. 62–64 °C. ^{1}H and ^{19}F NMR spectra correspond to the previously reported data [28].

*4.5.2. 1,1,1-Trifluoro-2-isocyanato-2-trifluoromethyl-butane (**5c**)*

Yield 92%, colorless liquid, b.p. 82–84 °C. ^{1}H and ^{19}F NMR spectra correspond to the previously reported data [28].

4.6. General procedure for the synthesis of compounds 8a and 9a

A solution of 1 mmol of isocyanate **5a** and 1 mmol of thiazole in 10 ml of dry diethyl ether was stirred during 4 h at r.t and left overnight. The resulting precipitate was filtered and recrystallized from benzene.

*4.6.1. 1-Thiazol-2-yl-3-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-urea (**8a**)*

Yield 97%, colorless crystals, m.p. 300–302 °C (decomp). ^{1}H NMR (DMSO-d_6): δ 5.47 (m, 1H, $CH(CF_3)_2$), 6.92 (d, J = 3.5 Hz, 1H, SCH), 7.28 (d, J = 3.7 Hz, 1H, NCH), 7.90 (br s, 1H, NH), 10.32 (br s, 1H, NH). ^{19}F NMR: δ 5.81 (d, J = 7 Hz, 6F). EI-MS (m/z): 293 (M^+). Anal. Calcd. for $C_7H_5F_6N_3OS$: C, 28.68%; H, 1.72%; F, 38.88%; N, 14.33%; Found: C, 28.53%; H, 1.81%; F, 38.57%; N, 14.20%.

*4.6.2. 1-Benzothiazol-2-yl-3-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-urea (**9a**)*

Yield 96%, colorless crystals, m.p. 346–348 °C (from benzene). ^{1}H NMR (DMSO-d_6): δ 5.54 (m, 1H, $CH(CF_3)_2$), 7.21 (t, J = 7 Hz, 1H, $H_{m\text{-}arom}$), 7.35 (t, J = 7 Hz, 1H, $H_{m\text{-}arom}$), 7.63 (d, J = 7 Hz, 1H, $H_{o\text{-}arom}$), 7.95 (d, J = 7 Hz, 1H, $H_{o\text{-}arom}$), 8.17 (br s, 0.5H, OH) (urea–isourea equilibrium), 8.22 (br s, 0.5H, NH) (urea–isourea equilibrium); 10.60 (br s, 1H, NH); ^{19}F NMR: δ 5.95 (d, J = 7 Hz, 6F). EI-MS (m/z): 343 (M^+). Anal. Calcd. for $C_{11}H_7F_6N_3OS$: C, 38.49%; H, 2.06%; F, 33.21%; N, 12.24%; Found: C, 38.65%; H, 1.97%; F, 33.29%; N, 12.31%.

4.7. General procedure for the synthesis of compounds 8b,c and 9b,c

To a solution of 1 mmol of isocyanate **5b** or **5c** and 1 mmol of thiazole in 10 ml of dry diethyl ether 0.2 mL of saturated ether solution of catalyst trimethylamine was added. The mixture was stirred during 4 h at r.t and left overnight. The resulting precipitate was filtered and recrystallized from benzene.

*4.7.1. 1-Thiazol-2-yl-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethyl-ethyl)-urea (**8b**)*

Yield 97%, colorless crystals, m.p. 302–304 °C (decomp). ^{1}H NMR (DMSO-d_6): δ 2.03 (s, 3H, CH_3), 6.87 (d, J = 3.7 Hz, 1H, SCH), 7.25 (d, J = 3.7 Hz, 1H, NCH), 7.48 (br s, 1H, NH), 10.37 (br s, 1H, NH); ^{19}F NMR: δ 1.07 (s, 6F). EI-MS (m/z): 307 (M^+), 308 ($M^+ + 1$). Anal. Calcd. for $C_8H_7F_6N_3OS$: C, 31.28%; H, 2.30%; F, 37.10%; N, 13.68%; Found: C, 31.12%; H, 2.25%; F, 37.18%; N, 13.56%.

*4.7.2. 1-(1,1-Bis-trifluoromethyl-propyl)-3-thiazol-2-yl-urea (**8c**)*

Yield 95%, colorless crystals, m.p. 158–159 °C (from benzene). ^{1}H NMR (acetone-d_6): 1.10 (t, J = 7 Hz, 3H), 2.70 (q, J = 7 Hz, 2H), 7.10 (d, J = 4 Hz, 1H), 7.40 (d, J = 4 Hz, 1H), 7.90 (br s, 1H), 8.90 (br s, 1H). ^{19}F NMR: δ 4.45 (s, 6F). EI-MS (m/z): 321 (M^+). Anal. Calcd. for $C_9H_9F_6N_3OS$: C, 33.65%; H, 2.82%; F, 35.48%; N, 13.08%; Found: C, 33.49%; H, 2.77%; F, 35.56%; N, 12.96%.

*4.7.3. 1-Benzothiazol-2-yl-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethyl-ethyl)-urea (**9b**)*

Yield 98%, colorless crystals, m.p. 348–350 °C (from benzene). ^{1}H NMR (DMSO-d_6): δ 2.06 (s, 3H, CH_3), 7.20 (t, J = 8 Hz, 1H, $H_{m\text{-}arom}$), 7.34 (t, J = 7 Hz, 1H, $H_{m\text{-}arom}$), 7.62 (d, J = 7 Hz, 1H, $H_{o\text{-}arom}$), 7.77 (d + br s, J = 7 Hz, 1H, $H_{o\text{-}arom}$ + NH), 7.85 (br s, 1H), 10.62 (br s, 1H, NH); ^{19}F NMR: δ 1.19 (s, 6F). EI-MS (m/z): 357 (M^+). Anal. Calcd. for $C_{12}H_9F_6N_3OS$: C, 40.34%; H, 2.54%; F, 31.91%; N, 11.76%; Found: C, 40.36%; H, 2.55%; F, 31.83%; N, 11.57%.

*4.7.4. 1-Benzothiazol-2-yl-3-(1,1-bis-trifluoromethyl-propyl)-urea (**9c**)*

Yield 94%, colorless crystals, m.p. 167–168 °C (decomp). ^{1}H NMR (DMSO-d_6): δ 1.12 (t, J = 7 Hz, 3H), 2.63 (q, J = 7 Hz, 2H), 7.15 (t, J = 7 Hz, 1H), 7.30 (t, J = 7 Hz, 1H), 7.65 (d, J = 7 Hz, 1H), 7.70 (d, J = 7 Hz, 1H), 7.80 (br s, 1H), 10.55 (br s, 1H). ^{19}F NMR: δ 4.65 (s, 6F). EI-MS (m/z): 371 (M^+). Anal. Calcd. for $C_{13}H_{11}F_6N_3OS$: C, 42.05%; H, 2.99%; F, 30.70%; N, 11.32%; Found: C, 41.86%; H, 2.80%; F, 30.83%; N, 11.18%.

5. Pharmacology

5.1. Primary anticancer assay

The compounds were tested by NCI in an in vitro three-cell line, one dose primary anticancer assay as a primary cancer screen. The three-cell line panel consists of the MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). Each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at single 10^{-4} M concentration and the culture incubated for 48 h. End-point determinations are made with alamar blue [49]. Results for each test agent are reported as the percent of growth of the treated cells when compared to untreated control cells. Compounds, which reduce the growth of any one of the cell lines to approximately 32% or less, are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

5.2. Determination of GI_{50}, TGI, and LC_{50} values

A total of 60 human tumor cell lines, derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate and breast) formed the basis of this test.

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well. [47,85]. Density of inoculum depends on the type of tumor cell and from its growth characteristics [46]. After cell

inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of drugs. The drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final concentration and stored frozen prior use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-folds or half log serial dilutions were made to provide a total five drug concentrations plus control. Aliquots of 100 μL of these different drugs dilutions (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M) were added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentration. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air and 100% relative humidity. Cells were fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. Then staining unbound dye was removed by washing (five times with 1% acetic acid) and plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. The cytotoxic effects were evaluated and the assay results and dose-response parameters were calculated as previously described [86].

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.08.007.

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