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Review article

2-substituted benzothiazoles as antiproliferative agents: Novel insights on structure-activity relationships

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ABSTRACT

Given the wide spectrum of biological activities, benzothiazoles represent privileged scaffolds in medicinal chemistry, useful in drug discovery programs to modulate biological activities of lead compounds. A large body of knowledge about benzothiazoles has been reported in scientific literature, describing their antimicrobial, anticonvulsant, neuroprotective, anti-inflammatory, and antiproliferative effects. This review summarizes the results obtained in the structure-activity relationship studies on antiproliferative benzothiazoles, focusing on 2-substituted derivatives and on mechanism of action responsible for the antitumor effects of this class of compounds.

Contents

Abbreviations: Abeta, Amyloid beta peptide; ABAD, amyloid beta peptide binding alcohol dehydrogenase; GLOI, human glyoxalase I; Bcr-Abl, breakpoint cluster region-Abelson tyrosine kinase; CML, chronic myeloid leukemia; CSF-1R, macrophage colony stimulating factor receptor; VEGFR, vascular endothelial growth factor receptor; AhR, aryl hydrocarbon receptor; CYP1A1, cytochrome P450 isoform 1A1; COX, cyclooxygenase; RhoGDI, Rho GDP-dissociation inhibitor; JNK-1, Jun N-terminal kinase 1; PPARα, Peroxisome Proliferator-Activated Receptor α; CA IX, carbonic anhydrase IX; Hsp70, heat shock protein 70; PBMC, peripheral blood mononuclear cell.

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1. Introduction

Heterocycles represent valuable chemical structures found in several bioactive natural compounds. They are privileged scaffolds, precious in drug discovery programs, while they provide to the medicinal chemist the possibility to modulate physicochemical properties of lead compounds. Benzothiazoles belong to the class of bicyclic heteroaromatics, and they attracted the attention of scientists because of their valuable wide spectrum of biological activities as antimicrobial [1,2], anticonvulsant [3,4], neuroprotective [5,6], anti-inflammatory, antitumor and many others [7–9].

A number of drugs containing the benzothiazole core (Fig. 1) are commercially used to treat different pathologies. Modulating the glutamatergic system, riluzole (Rilutek®) exerts a neuroprotective action useful in the treatment of amyotrophic lateral sclerosis, whereas the sulfonamide derivative ethoxzolamide inhibits carbonic anhydrase and is therapeutically used in glaucoma and as antiepileptic drug. The phenylurea derivative frentizole has antiviral and immunosuppressant properties [10], but it has been also identified as inhibitor of the Abeta-ABAD interaction (amyloid beta peptide and amyloid beta peptide binding alcohol dehydrogenase) [11]. Zopolrestat is an aldose reductase inhibitor developed by Pfizer for the treatment of diabetic complications [12], and recently it was shown to inhibit human glyoxalase I (GLOI), an emergent target in anticancer research [13]. The 2-phenyl benzothiazole salt thioflavin T is a useful diagnostic tool of the amyloid structure [14].

Given the easy synthetic pathways and the great potential as bioactive compounds [15–17], the research in novel drugs based on benzothiazole nucleus has been receiving a growing interest [18–20]. In particular, the antitumor activity of benzothiazoles attracted a lot of interest, with a number of studies dedicated to antiproliferative benzothiazoles [21–23]. Indeed, the molecular mechanisms responsible for this activity have not been fully clarified, and different biological pathways have been indicated as possible targets of this class of molecules.

This review analyzes the 2-substituted benzothiazoles, a group of compounds showing interesting antiproliferative activity. Based on substituents at position 2, the molecules were classified into amide or ureido derivatives, hydrazones and semicarbazones, phenyl derivatives, mercapto derivatives and a mixed group of benzothiazole hybrids (Fig. 2).

2. Antiproliferative 2-substituted benzothiazoles

2.1. 2-Amido, ureido, and carbamate benzothiazoles

A series of 2-amido and 2-ureido benzothiazoles was developed in the search for novel Raf-1 inhibitors [24]. Raf kinases are involved in several organic functions, both under physiological and pathological conditions. Their involvement in cancer is well documented [25], and inhibitors directed to these kinases are currently under investigation. Sorafenib (BAY43-9006, Nexavar®), an inhibitor of Raf-1 and other kinases, has been approved by FDA in 2005 for renal cell carcinoma [26], then for hepatocellular carcinoma and metastatic thyroid cancer. Song and coworkers synthesized two series of 2-substituted benzothiazoles, bearing an amide (**1**) or a ureido (**2**) moiety as linker between the bicyclic core and the aromatic ring (Fig. 3). They explored the structure-antiproliferative relationships by varying substituents on benzothiazole (at 4, 5, 6, and 7 position) and on the phenyl ring; the cytotoxicity was measured in liver (SK-Hep-1), breast (MDA-MB-231) and gastric (NUGC-3) cancer cell lines. A marked *in vitro* antiproliferative effect was found for amide derivatives disubstituted on benzothiazole ring (at 5 and 7 position), and with electron-withdrawing substituents on the distal aromatic ring (Z = cyano, trifluoromethyl, nitro) (compounds **1a-c**, GI_{50} 0.29–1.74 μM). Among monosubstituted derivatives, the 4-methoxy (**1d**) and 6-nitro (**1e**) derivatives showed cytotoxic effect on the three human cancer models (GI_{50} 0.86–3.46 μM). Results for ureido derivatives **2** were not encouraging: they showed a moderate antiproliferative effect on the same cancer models, with the best compounds bearing a halogen on benzothiazole (at 6 position) and an electron withdrawing group in *meta* to the aromatic ring (**2a-b**). When tested against Raf-1, amide and urea compounds induced moderate to poor inhibition properties, suggesting that Raf-1 inhibition could not be the only mechanism involved in the antiproliferative activity of these molecules.

An interesting library of 2-ureido benzothiazoles were reported

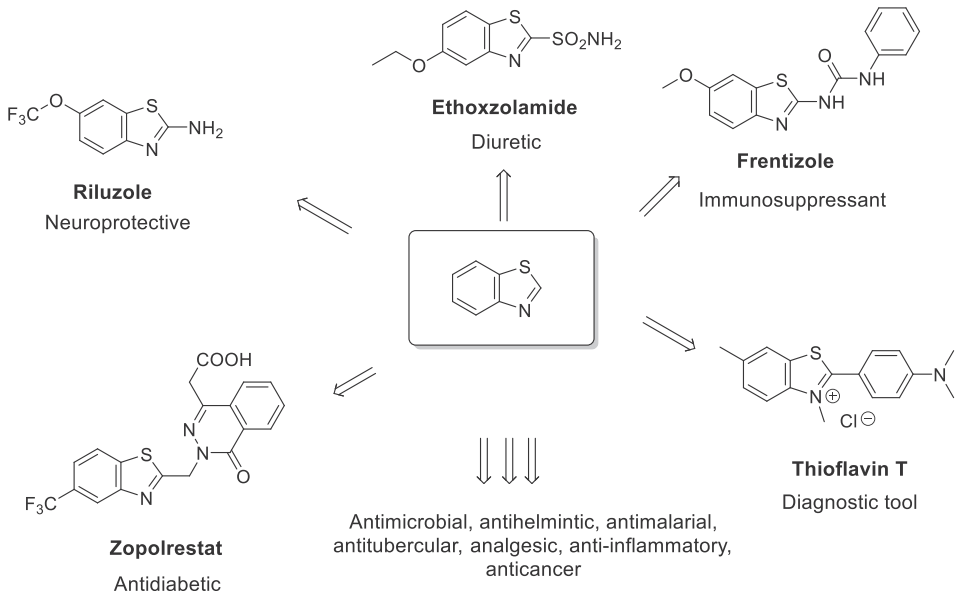


Fig. 1. The wide biological profiles of benzothiazoles. Chemical structures of benzothiazole-based drugs riluzole, frentizole, zopolrestat, ethoxzolamide and the diagnostic tool thioflavin T.

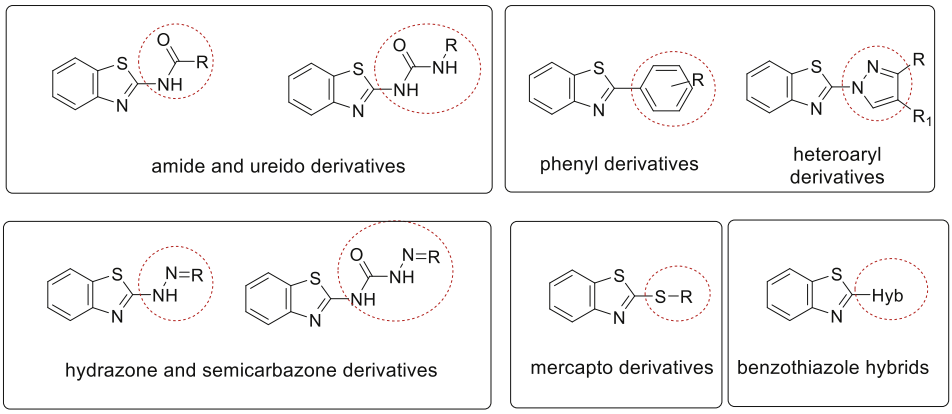


Fig. 2. 2-Substituted benzothiazoles bearing amide, urea, hydrazone, semicarbazone, phenyl, heteroaryl, and mercapto substituents.

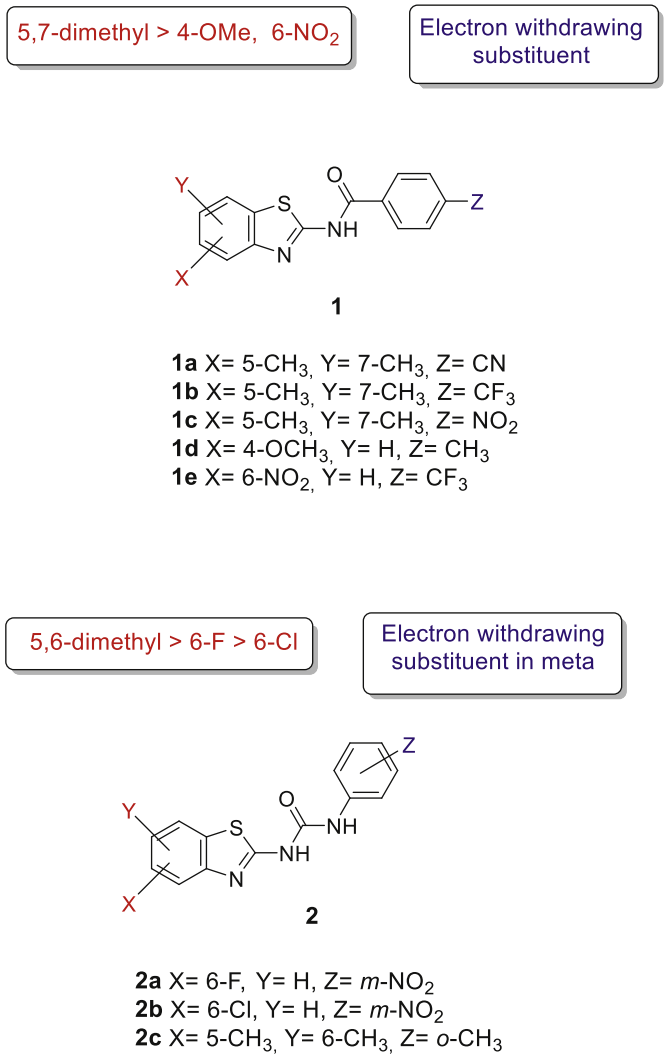


Fig. 3. 2-Amido (**1**) and 2-ureido (**2**) benzothiazoles tested in human liver, breast, and gastric cancer models.

as breakpoint cluster region-Abelson tyrosine kinase (Bcr-Abl) inhibitors effective against wild-type and T315I mutant kinases [27]. The fusion protein Bcr-Abl kinase represents a validated target for the development of therapeutic agents effective in chronic myeloid leukemia (CML) [28]. The first-generation inhibitor imatinib has been the standard therapy for CML due to its remarkable activity and mild toxicity. However, the development of mutations in Bcr-Abl kinase domain is the main reason for imatinib resistance in CML; many efforts were done by researchers to develop novel inhibitors, able to overcome the imatinib resistance, targeting the T315I mutation [29,30].

In this context, chemical modifications performed on benzimidazole compound nocodazole produced a family of 2-ureido benzothiazoles (**3** and **4** Fig. 4), displaying good inhibition profiles against wild-type Bcr-Abl and T315I mutant kinases. A first set of compounds were synthesized, bearing an ethylurea at C2 of benzothiazole, and aryl or heteroaryl rings at C6. The combination of 2-methoxybenzene, the benzothiazole nucleus and the ethylurea portion gave the most potent compounds **3a** and **3b** (IC_{50} 0.06 and 0.03 nM against Abl wild-type, 0.11 and 0.064 nM against Abl T315I, respectively). Compound **3a** was further studied to determine its selectivity against a wide panel of kinases, displaying a high degree of selectivity. In order to improve the modest cellular activity of this compound (Ba/F3 cells, IC_{50} 3.57 μM against wild-type and 2.14 μM against T315I), an optimization strategy was performed by introducing water-soluble groups, as morpholine, piperazine and hydroxy moieties. SAR studies indicated that derivatives incorporating a 2-methoxyphenyl group at the C6 position were superior to the corresponding 2-ethoxy and 2-ethyl phenyl derivatives in terms of enzyme and cell growth inhibition. Derivatives **4a** and **4b** retained an excellent enzymatic potency (IC_{50} 0.064 and 0.015 nM against Abl T315I, respectively), together with an increased potency in Ba/F3 cell line (IC_{50} 0.089 and 0.046 μM against wild-type, 0.14 and 0.078 μM against T315I line).

BLZ945 (**5**, Fig. 5), a 6-aryloxy-2-aminobenzothiazole derivative, has been discovered as potent inhibitor of macrophage colony stimulating factor receptor (CSF-1R, IC_{50} 1 nM) [31–33]. The inhibition of CSF-1R has the potential to treat diseases associated with normal and dysregulated functions of macrophages. BLZ945 was tested in a mouse glioblastoma model, inducing a block of tumor progression and a dramatic increase of survival [34]. This strong antitumor effect was explained with a "re-education" of macrophages, transformed in "good cells", able to promote tumor regression [35]. BLZ945 is currently under evaluation in clinical trials for its use as a single agent or in combination with PDR001 (spartalizumab) in adult patients with advanced solid tumors.

Novel 2-ureidobenzothiazoles were designed by merging the *N*-methylpicolinamide scaffold of BLZ945 with the 2-ureidobenzothiazole (**6** and **7**, Fig. 5). Ureido and thioureido derivatives **6** were tested for their antiproliferative activity against

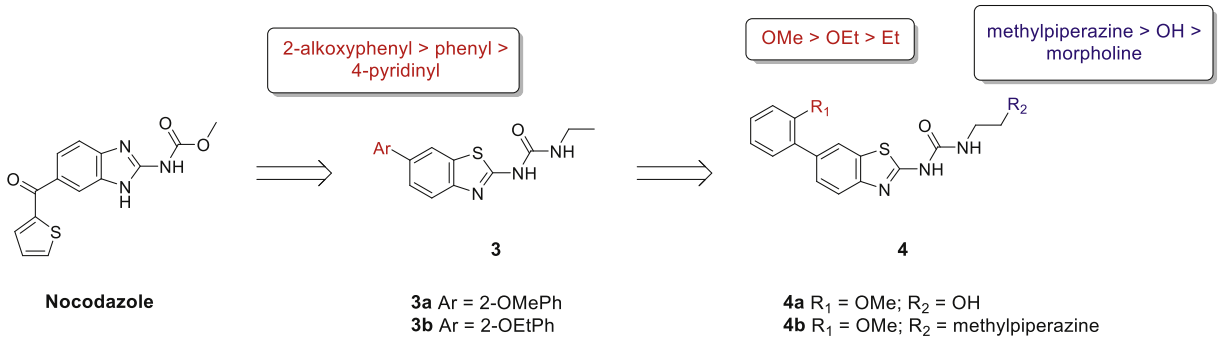


Fig. 4. From nocodazole to 2-ureidobenzothiazoles as potent inhibitors of wild-type Bcr-Abl and T315I mutant kinases.

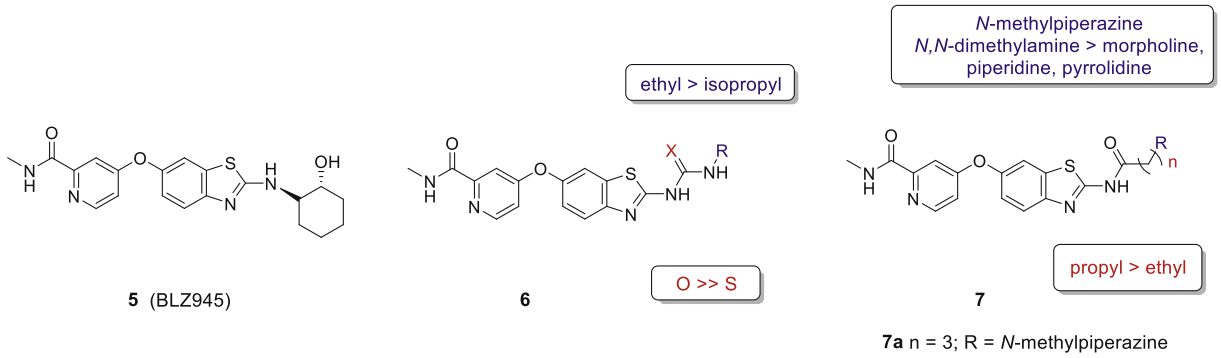


Fig. 5. From the clinical candidate BLZ945 to its ureido and amido derivatives.

wild-type and mutant T315I Bcr-Abl kinases [36]. The effects of urea substitution at C2 position of benzothiazole were studied, being the ethyl derivatives more active than corresponding isopropyl compounds. The replacement of urea with a thiourea was deleterious, with a 3–6 fold drop in activity. A further series of derivatives (**7**) were synthesized in the attempt to improve both enzymatic activity and water solubility. For this purpose, polar fragments (hydroxy, *N*,*N*-dimethylamino, pyrrolidine, piperidine, morpholine and *N*-methylpiperazine) were inserted in the distal portion of the molecules, connected by an aliphatic linker to the ureido moiety. Results obtained showed that propylurea derivatives with cyclic amines were more potent than corresponding ethylurea analogues; compound **7a** was the best in this series, displaying nanomolar inhibition activity both against wild-type and mutant T315I Bcr-Abl kinases (IC_{50} 18.2 and 39.9 nM, respectively). Generally, the propyl and butyl spacers were favorable to the activity, while the *N*-methylpiperazine and the *N*,*N*-dimethylamine resulted to be the best water solubilizing groups. The antiproliferative activity of a selection of synthesized compounds was further assessed through a wide screening against 60 human cancer cell lines, including lung, colon, ovary, kidney, prostate, breast, blood and CNS cancers. Tested compounds displayed significant growth inhibition values towards the Bcr-Abl dependent leukemia line K-562 at 10 μM concentration (GI range 76.7–92.9%).

Starting from the structure of sorafenib, an interesting study was performed by replacing the central aromatic ring with a benzothiazole, substituted in position 2 with an amide or a urea (Fig. 6) [37]. A preliminary evaluation of the antiproliferative effect was performed on two human cancer cell lines, namely colorectal carcinoma HCT-116 and breast cancer SK-BR3. Some compounds, belonging to the series of derivatives **8** and **9**, showed growth inhibition comparable to that of sorafenib, and were selected for a broad investigation on a panel of 60 cancer cell lines. Results revealed a modest anticancer activity for amide compounds **8**, with the only exception of compound **8a** (mean % GI 87.02), whose best activity was explained by the nature of lipophilic substituents on the aromatic ring. Overall, urea derivatives **9** were more potent than the corresponding amides: this result was explained by a more favorable geometry, probably responsible for a best fitting in the active site. In addition, the terminal NH may be involved in hydrogen bonds and stabilize the interaction with the biological target. The 3,5-bis-trifluoromethylphenyl urea **9b** displayed a wide spectrum of activity against several cancer cell lines (GI_{50} 0.301–1.67 μM), with superior potency and efficacy compared to sorafenib (GI_{50} = 0.301 μM in lung cancer HOP-92, 0.444 μM in colon cancer KM12, 0.399 μM in breast cancer MDA-MB-468).

The *in vitro* screening of **9b** over 10 oncogenic kinases showed a selective inhibition profile towards B-Raf^{V600E} and C-Raf; these findings revealed the benzothiazole urea derivatives **9b** and **9c** as novel promising candidates for the development of potent anticancer agents.

Further structural modifications afforded novel benzothiazole urea derivatives, endowed with a broad spectrum of antiproliferative activity mediated by a potent inhibition of kinases [38]. Compound **10** (KST016366) emerged as the best-in-class molecule, obtained by insertion of an ethylpiperazinomethyl moiety on the aromatic ring; it induced a strong antiproliferative effect against a wide panel of cancer cells, with excellent results in leukemia K-562 (GI_{50} = 51.4 nM) and colon carcinoma KM12 (GI_{50} = 19 nM). When tested against kinases, **10** showed a multikinase inhibitor profile, displaying nanomolar IC_{50} against Tie2 (IC_{50} = 0.82 nM), TrkA (IC_{50} = 3.81 nM), and ABL-1 (IC_{50} = 52.7 nM). Molecular docking studies pointed out the important role of the ethylpiperazinomethyl moiety in increasing the binding affinity toward multiple oncogenic kinases.

Both the excellent antiproliferative activity and the favorable

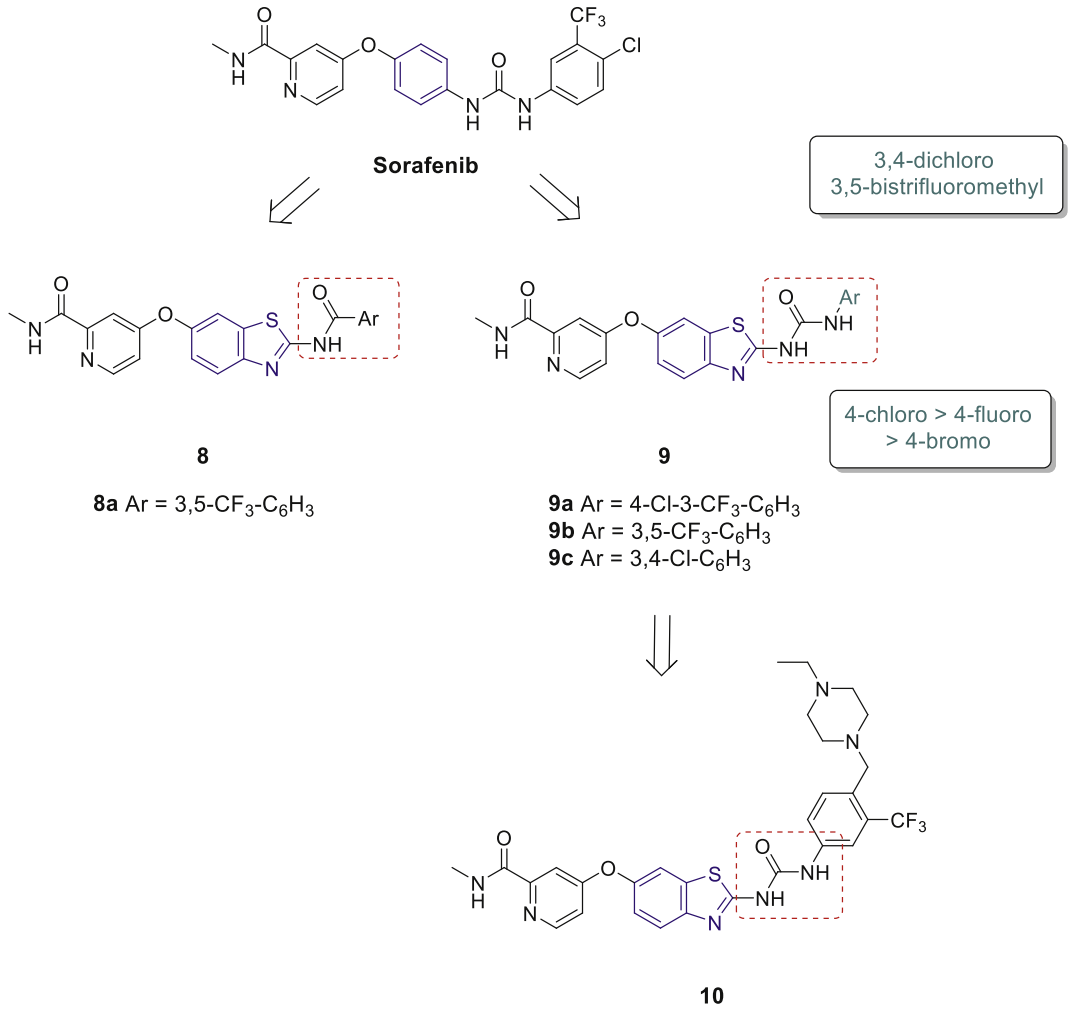


Fig. 6. 2-Amido (**8**) and 2-ureido (**9** and**10**) benzothiazole derivatives of sorafenib.

pharmacokinetic profile make compound **10** a promising candidate for further preclinical and clinical investigations.

A series of benzothiazole amides and carbamates was developed and tested against a wide panel of cancer cells (Fig. 7) [39]. Derivatives **11** showed good antiproliferative properties, mainly against breast cancer (MCF-7), leukemia (K562), melanoma (A375), and testicular embryonal carcinoma (NT2/D1). Carbamate derivatives **11a-b** showed a marked antiproliferative effect in NT2/D1 cells (**11a** IC_{50} = 0.2 μM, **11b** IC_{50} = 0.1 μM), whereas the amide derivative **11c** induced a moderate effect in breast (IC_{50} = 30.5 μM), leukemia (IC_{50} = 53.2 μM) and melanoma (IC_{50} = 77.5 μM) models. These compounds were also investigated for their mechanism of action in MCF-7 and NT2/D1 cells. In MCF-7, selected benzothiazoles were able to promote apoptosis by blocking cell cycle in G2/M phase. In NT2/D1 cells, strong effects on migration and invasiveness have been detected, but the exact mechanism of action remains to be clarified.

Cindric and coworkers reported on the antiproliferative activity of a series of molecules (**12**), showing the 2-amidobenzothiazole and a benzothiophene as pharmacophores. Compound **12a** showed a remarkable cytotoxic effect in cervical carcinoma HeLa (IC_{50} = 1.16 μM), colorectal adenocarcinoma SW620 (IC_{50} = 7.05 μM), hepatocellular carcinoma (IC_{50} = 14.17 μM), and ductal pancreatic adenocarcinoma CFPAC-1 (IC_{50} = 5.04 μM) [40]. The hydrochloride salt **12b** induced a potent antiproliferative effect in breast cancer (MCF-7, IC_{50} = 40 nM) and a micromolar activity in colon carcinoma HTC116 (IC_{50} = 4 μM) [41]. The mode of action of these derivatives was investigated by DNA binding assay, but results did not correlate with the antiproliferative effects observed in cellular assays.

A series of benzothiazole-based imidazole derivatives (**13**, Fig. 7) were easily prepared by a two-step synthesis and their cytotoxicity against rat glioma (C6) and human liver (HepG2) tumor cell lines was determined [42]. Compound **13g** proved to be the most cytotoxic (IC_{50} = 15.67 μg/mol), followed by derivatives **13e** (IC_{50} = 16.33 μg/mol), **13f** (IC_{50} = 24.33 μg/mol) and **13g** (IC_{50} = 19.33 μg/mol) against C6 tumor cell line. In contrast, compound **13h** showed no apparent cytotoxicity (IC_{50} > 500 μg/mol). Compounds **13b**, **13d**, **13e** and **13f** showed high cytotoxicity against HepG2 tumor cells (IC_{50} = 26.33, 29.33, 31.67 and 28.67 μg/mol, respectively), unlike their analogues **13h**, **13i** and **13j**, whose IC_{50} values could not be calculated due to their low cytotoxicity, even at high concentrations (>500 μg/mol).

A series of benzothiazole-pyrazole hybrids has been recently proposed as anticancer agents [43]. An amide linker has been placed between two heterocycles, providing a series of 2-amidobenzothiazoles substituted on the benzothiazole ring (at C6) and in *para* to the aromatic ring (**14**, Fig. 7). Cytotoxicity of these compounds was evaluated in colon (HT29), prostate (PC3), lung (A549) and glioblastoma (U87MG) cancer cell lines; results showed

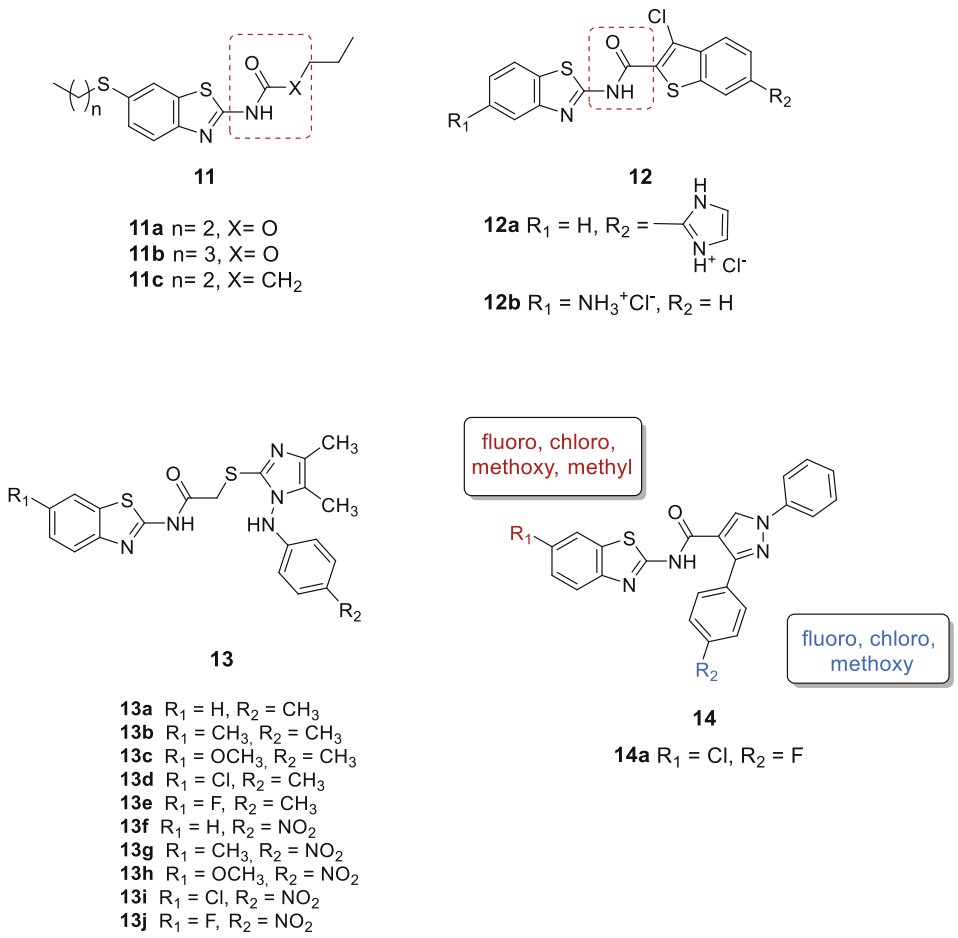


Fig. 7. 2-Carbamate (**11a-b**) and 2-amido (**11c**, **12**–**14**) benzothiazoles.

a good ability to inhibit cell viability, with the best compounds displaying IC_{50} in the low micromolar range. **14a** was selected as the most promising compound among these hybrids, and its cytotoxic profile was further analyzed in PC3 cell line ($IC_{50} = 3.17$ μM), where it significantly inhibited colony growth and cell migration. The cell cycle analysis revealed a dose-dependent arrest in G0/G1 phase, whereas its antiangiogenic profile was assessed in an *in vivo* transgenic zebrafish model. Finally, these hybrids showed the ability to inhibit the vascular endothelial growth factor receptor (VEGFR-2) (**14a** $IC_{50} = 97$ nM), a successful clinical target in anticancer therapy.

Sugano's group carried out a SAR study starting from the structure of the benzothiazole-pyrazole hybrid **15**, that led to design a new family of antitumor agents (Fig. 8) [44]. The starting benzothiazole-pyrazole hybrid **15** is a potent and selective cytotoxic inhibitor of cancer cell line WI-38 VA-13 subline 2RA (VA-13) ($EC_{50} = 26$ ng/mL), with no cytotoxic effect against the normal parental cell line, WI-38 ($EC_{50} > 4000$ ng/mL); however, it showed no satisfactory *in vivo* activity, which had been attributed to its metabolic instability provided by the nitro group. The structural optimization of the left-hand region of **15** showed that the nitropyrazole ring could be replaced by a properly substituted phenyl group. Among all tested compounds, the *ortho*-dihalosubstituted ones displayed stronger biological activity, being the dichlorophenyl amide **16a** more potent than the hit **15**. Some urethane, urea, thiourea and sulfonamide isosteres were also tested, but they were less potent than the amide analogues. Unfortunately, like the hit **15**, compound **16a** did not show any significant therapeutic effect *in vivo*. The solution to this problem was achieved with the subsequent modulation of the right-hand region of **15**. The cyclopropyl derivative **16b** was selected as the best one, showing both biological potency and excellent plasma concentration, with a strong *in vivo* inhibitory effect on tumor growth.

A series of benzothiazoles linked to a pyrazolo[1,5-*a*]pyrimidine scaffold (**17**, Fig. 8) were synthesized and evaluated for their anticancer activity [45]. Compounds **17** were tested on a panel of 60 human cancer cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. While compounds **17a** and **17b** showed significant anticancer activity, they were screened against a panel of five human cancer cell lines, A549 (lung), DU-145 (prostate), MCF-7 (breast), ACHN (renal) and Hela (cervical). They displayed IC_{50} values ranging from 2.01 to 7.07 μM and 1.94–3.46 μM, respectively. Moreover, cell cycle arrest in G2/M and reduction in Cdk1 expression level were observed upon treatment with these compounds, and they also induced caspase-3 dependent apoptosis.

SAR studies were performed on this family of compounds, showing the negligible effect on activity played by substituents on benzothiazole ring (R_4 and R_5), whereas the substitution of phenyl linked to the pyrazolo[1,5-*a*]pyrimidine subunit (R_1, R_2, R_3) plays a role on the cytotoxic activity. The presence of 3,4,5-trimethoxy, 4-fluoro and 4-methoxy substitutions increased the cytotoxicity of these compounds.

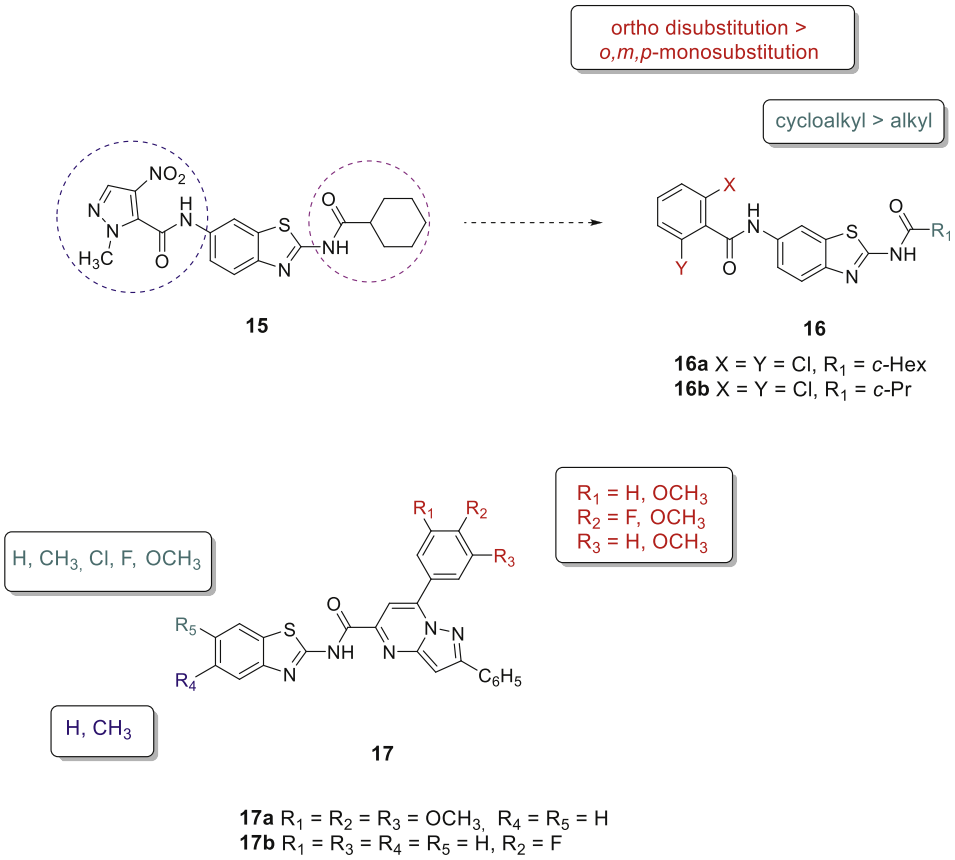


Fig. 8. From lead compound **15** to 2-amido benzothiazoles **16** and **17**.

2.2. Hydrazone and semicarbazone benzothiazoles

A series of (*E*)-2-benzothiazole hydrazones (**18**, Fig. 9) was synthesized and tested against human leukemia (HL-60), breast (MDAMB-435) and colorectal (HCT-8) cancer cell lines [46]. A strong cytotoxic effect was obtained by compounds **18a-c**, all displaying a dihydroxy substitution in the aromatic ring. **18a-b** inhibited leukemia cells at submicromolar concentration (IC_{50} = 0.35 and 0.59 μM, respectively); **18c** induced a good cytotoxicity in leukemia and colorectal models, at low micromolar concentration (IC_{50} = 1.89 and 3.43 μM, respectively). The observed cytotoxicity seems to be not related to membrane damage.

A group of 2-anilinopyridyl benzothiazoles linked through a hydrazone (**19**, Fig. 9) were identified and screened for their antiproliferative activities [47]. These molecules were obtained by combination of bioactive pharmacophores, benzothiazole and 2-anilinopyridyne, both endowed with cytotoxic properties. Structure-activity relationships were analyzed by inserting electron donating and electron withdrawing substituents on both pharmacophores. As summarized in Fig. 9, the best cytotoxic effects were displayed by derivatives substituted on benzothiazole (C6) with a methyl group (**19a-b**) or electron donating groups; as regard anilinopyridine, the *p*-chloro substitution was beneficial, and substituents like trifluoromethyl and fluorine were well tolerated. Interestingly, both aromatic rings were found essential for the cytotoxicity: their removal led to complete loss of activity. The most active compounds **19a-b** displayed antiproliferative activity at low micromolar concentration in breast cancer (MCF-7, MDA-MB-231), lung adenocarcinoma (A549), melanoma (B16F10). Given the interesting results obtained for **19a-b** in MCF-7 cells (IC_{50} = 1.03 and 1.69 μM, respectively), further studies were performed to clarify the mechanism of action. Overall, these compounds were able to induce cell cycle arrest in S and G2/M phase, and the apoptotic pathway was confirmed by other cell-based assays.

An interesting family of cytotoxic compounds was obtained combining a benzothiazole and an indole, linked by a semicarbazone linker (**20**, Fig. 9) [48]. Synthesized compounds were tested in human colon (HT29), lung (H460 and A549) and breast (MDA-MB-231) cancer cell lines, showing good to excellent inhibition of cell viability. From a structure-activity relationship study emerged the key substitutions in C6 of benzothiazole and in the *N*-benzylindole. The introduction of amine moieties in R_1 highlighted the critical role of the steric hindrance in this position, with the following activity order: dimethylamine > diethylamine > 4-methylpiperidine. The benzylic substituent on the indole ring had a great influence on the antiproliferative activity: a chlorine atom or a weak electron donating group (CH_3) produced compounds with strong antiproliferative activity, whereas the dichloro substitution (2,4 or 3,4) decreased the activity. In addition to electronic effects, steric effects were also studied: the bulky *t*-butyl group in *para* resulted in a strong decrease of activity. In this series of semicarbazone benzothiazole-indoles, **20a** emerged as a novel *lead compound*, endowed with an excellent antiproliferative profile against the tested cancer cell lines, with IC_{50} = 0.024 (HT29), 0.29 (H460), 0.84 (A549), and 0.88 μM (MDA-MB-231). Further studies about the mechanism of action indicated that **20a** could act by activating procaspase-3, (70.9% at concentration 10 μM) and determining cell cycle arrest. However, its enzymatic potency does not correlate with the strong and broad-spectrum antitumor activity, suggesting other mechanisms could be responsible for the

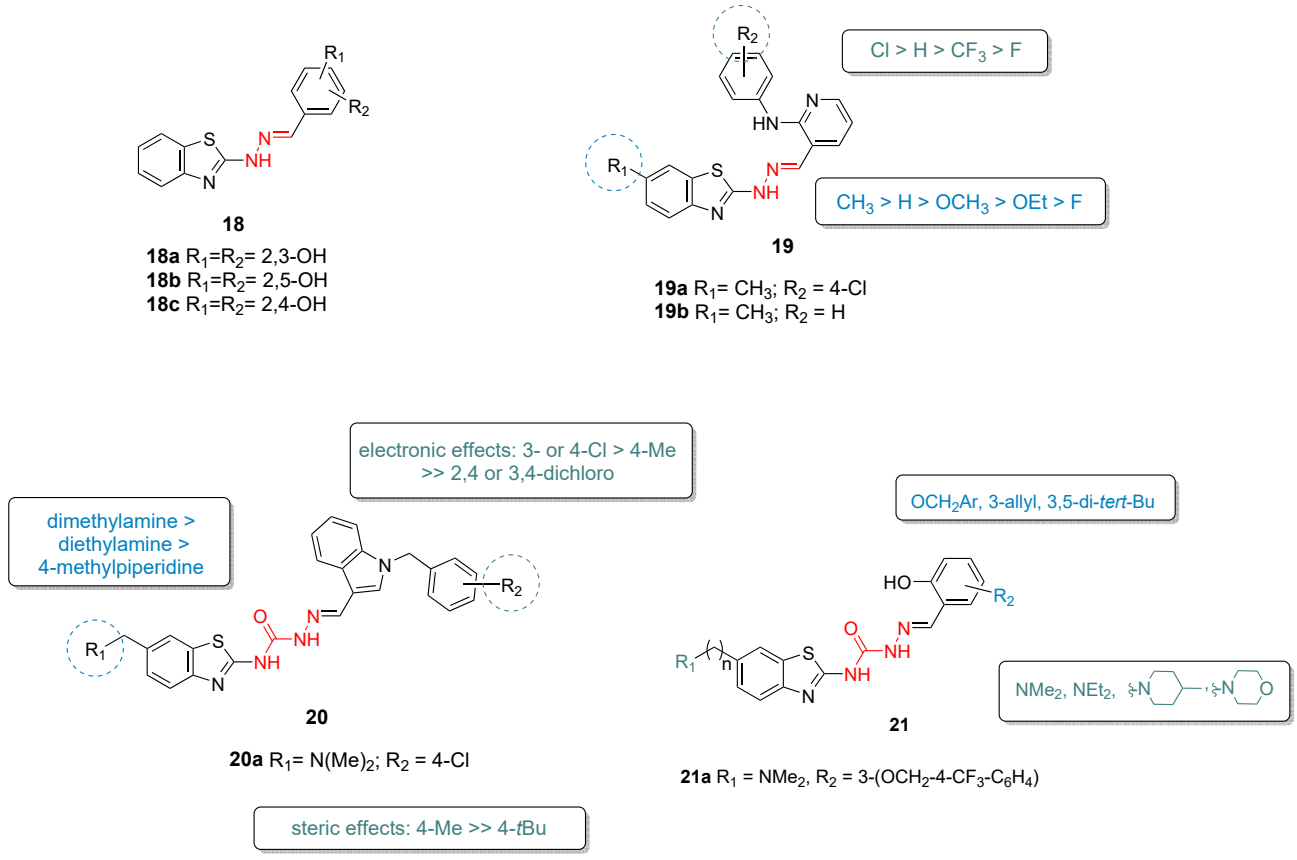


Fig. 9. Benzothiazoles bearing hydrazone (**18**, **19**) or semicarbazone (**20**, **21**) functional groups.

observed cytotoxicity.

A series of benzothiazole derivatives bearing an *ortho*-hydroxy-*N*-acylhydrazone moiety (**21**, Fig. 9) were synthesized and evaluated for their ability to activate procaspase-3 kinase [49]. The antiproliferative effects against five cancer cell lines (NCI-H226, SK-N-SH, HT29, MKN-45, and MDA-MB-231) were also assessed. Most of synthesized compounds showed moderate to excellent activity against procaspase-3 kinase and cytotoxic activity against tested cancer lines. The most promising compound **21a** (procaspase-3 $EC_{50} = 0.31$ μM) displayed IC_{50} values ranging from 0.24 to 0.92 μM against all tested cell lines, being 4.24–12.2 times more active than PAC-1 (procaspase-3 $EC_{50} = 0.41$ μM), a known apoptosis-inducing compound.

SAR studies indicated that the phenyl group on the 2-hydroxyphenyl ring was critical for the pharmacological activity *in vitro*. In addition, the introduction of a benzyloxy group with a mono-electron-withdrawing group at the 4-position was also more favorable to antitumor activity. The reduction of the electron density in the phenyl ring of the benzyloxy group caused a strong drop in activation of procaspase-3 kinase *in vitro*.

2.3. 2-Phenyl benzothiazoles

A study from Shi and coworkers identified a novel series of antiproliferative 2-(4-aminophenyl) benzothiazoles (**22**, Fig. 10) [50]. These compounds were designed starting from the chemical structures of quercetin and genistein, by inserting the benzothiazole ring and modifying the hydroxyl substituents [51]. Parent compound **22a** (CJM126) displayed a strong cytotoxicity at nanomolar concentration when tested against a panel of human breast cancer cell lines (MCF-7 $IC_{50} = 0.0003$ μM, MDA 468 $IC_{50} = 0.0016$ μM). Interestingly, the effect was selective for breast cancer, being **22a** inactive against other cancer cell lines as colon, ovarian, prostatic, and bladder. Extensive SAR studies were performed on these derivatives, showing the importance of the benzothiazole ring to exert the antiproliferative effect (benzothiazole > benzoxazole ≫ benzimidazole); the introduction of hydroxyl or alkoxy substituents on benzothiazole appeared detrimental for activity, whereas the substitution of the aromatic ring produced good results, especially with methyl or halogens at 3' position. These latter substitutions extended the cytotoxic activity to ovarian, lung, and renal cancer cell lines. The *in vivo* evaluation in breast carcinoma models in mice displayed the most potent effect for compound **22b** (DF203). The mechanism of action explaining the strong cytotoxicity of these aminophenyl benzothiazoles was not identified.

Fluorinated derivatives of **22b** were synthesized in the attempt to prevent undesired hydroxylation on benzothiazole; this medicinal chemistry screening led to the discovery of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole **23** (5F 203), displaying excellent *in vitro* antitumor effects [52]. To overcome unfavorable pharmacokinetic properties, prodrug forms of **23** were developed through the insertion of aminoacidic moieties. Water soluble lysyl amide prodrug **24** (Phortress) showed improved pharmacokinetic profile compared to parent compound, by releasing the active form in *vitro* and *in vivo* experiments [53,54]. Investigations on mode of action of **24** revealed the ability to bind the cytosolic aryl hydrocarbon receptor (AhR); the benzothiazole derivative is selectively sequestered by sensitive tumor cells and translocated into the nucleus, where it stimulates the cytochrome P450 isoform 1A1

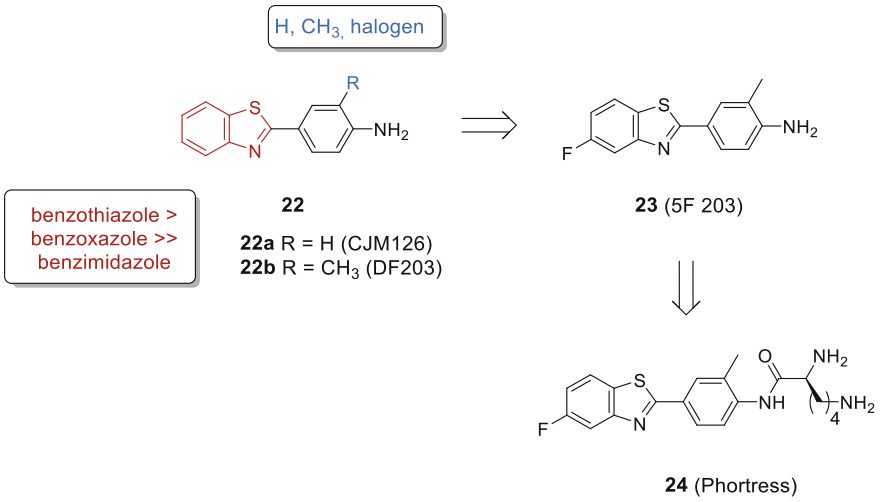


Fig. 10. 2-Phenylbenzothiazoles: from the *lead compound* CJM126 (**22a**) to the clinical candidate Phortress (**24**).

(CYP1A1) transcription. The formation of reactive electrophilic metabolites accounts for the cellular toxicity of this compound. Thanks to its favorable profile, **24** was indicated as a suitable candidate for Phase I clinical trials.

A wide library of 2-phenyl benzothiazoles bearing oxygenated substituents on the phenyl moiety was synthesized by Mortimer and coworkers [55]. The insertion of a fluorine atom at C_5 of benzothiazole and the dimethoxy substitution on aromatic ring afforded compound **25** (PMX610), displaying strong antiproliferative activity when tested in human breast (MCF-7 $GI_{50} < 0.0001$ μM, MDA 468 $GI_{50} < 0.0001$ μM) and colon (KM12 $GI_{50} = 0.29$ μM, HCC 2998 $GI_{50} = 0.00025$ μM) cancer cell lines (Fig. 11). This study allowed to disclose some structure-activity relationships: regarding benzothiazole, unsubstituted analogues retained submicromolar activity only against MDA 468 cell line, whereas the replacement of fluorine with chlorine or bromine dramatically decreased the inhibitory potency. 4- and 6-fluoro regioisomers retained nanomolar activity only against breast cancer cell lines. The antiproliferative activity decreased by replacing one or both methoxy groups with hydroxy, ethoxy and other oxygenated groups; the 3′,5′-dimethoxy analog showed micromolar potency, whereas the 3′,4′,5′-trisubstituted derivative retained nanomolar potency against two breast cancer lines. In studies about the mechanism of action, **25** displayed high affinity for AhR ($IC_{50} = 25$ nM) and CYP1A1, but these properties do not fully account for its strong antitumor activity.

The scaffold of 2-phenylbenzothiazole was further explored, in the attempt to improve the antitumor potency and expand the activity to a wider panel of cancer cell lines. A study from Kumbhare et al. proposed novel 2-phenylbenzothiazoles incorporating isoxazole (**26**) or triazole (**27**, Fig. 11) [56]. These molecules, tested against lung, colon, and breast cancer cell lines, displayed promising results, with cytotoxic effects at micromolar concentration. SAR studies revealed the best cytotoxic activity for triazole derivatives **27** compared to isoxazoles **26**, with 3-trifluoromethylphenyl as better substituent (**27a**). Investigations on their mechanism of action revealed for **27a** caspase-mediated apoptotic pathway.

A small series of 2-phenylbenzothiazole derivatives (**28**, Fig. 12) was synthesized, with different nitrogenated cycles (piperidine, morpholine, and methylpiperazine), as distal substituents [57]. Novel derivatives, as well as the benzoxazole analogues, showed potent antitumor activity against human breast cancer cell lines MCF7 and MDA231, with IC_{50} values of nanomolar order (8–34 nM for MDA231 and 10–29 nM for MCF7). Among tested compounds, the most potent cytotoxic effect was obtained with the 4-metylpiperazine-1-yl-acetamide derivative **28c** (X = NMe), with IC_{50} values of 10 nM for MCF7 and 8 nM for MDA231.

A highly diversified library of N-substituted 2-(3-aminophenyl)-benzothiazole derivatives (**29**, Fig. 12) was prepared and their *in vitro* antiproliferative activity against various human cancer cell lines (A549, HeLa, HepG2, MCF-7, MV4-11 and DB) were studied [58]. Among tested compounds, *N*-[3-(benzo[*d*]thiazol-2-yl) phenyl]nicotinamide (**29a**) displayed significantly improved antiproliferative activity toward A549 and MV4-11 cells, with IC_{50} values of 5.42 and 7.51 μM, respectively, and an interesting range of activities against the rest of the cell lines tested (IC_{50} range 15–24 μM). Flow cytometry analysis indicated that the nicotinamide derivative **29a** blocked the G1 phase of A549 cell cycle and was an effective apoptosis-inducing agent.

A series of 2-phenyl substituted benzothiazole amide derivatives (**30**, Fig. 12) with antibacterial activity was selected for the evaluation of cytotoxicity and apoptotic properties against SiHa HPV16-positive and C-33A HPV-negative human cervical cancer cell lines [59]. Compounds **30a**, **30c**, **30g** and **30h** showed potent cytotoxic activity against both cancer lines and selectivity compared with the normal cell line HEK-293. Compound **30g** was the most active against SiHa cells ($IC_{50} = 6.13$ μM); starting from cytotoxicity results, preliminary SAR studies suggested that compounds with electron-donating groups on the phenyl ring were generally more potent than compounds with electron-withdrawing substituents. The exploration of mode of action revealed for these compounds the accumulation of cells in the G1 and S phase of the cell cycle in SiHa and C33-A cells, respectively.

Some nitro-amidino and amino-amidino 2-phenylbenzothiazoles (**31**, Fig. 13) were synthesized and tested as antiproliferative agents against a panel of tumor cell lines (breast, colon, lung carcinoma and leukemia) [60]. They displayed tumor cell-growth inhibitory activity and cytotoxicity, with IC_{50} in the micromolar range (IC_{50} 1–3 μM for nitro-derivatives, 0.2–4 μM for amino-derivatives). The best antiproliferative activity was observed for compound bearing the amino and the imidazolinyl group as substituents, with IC_{50} values in submicromolar range of concentration, selectivity towards cancer cells and very low toxicity against normal fibroblasts.

A second series of linear and cyclic amidino 2-

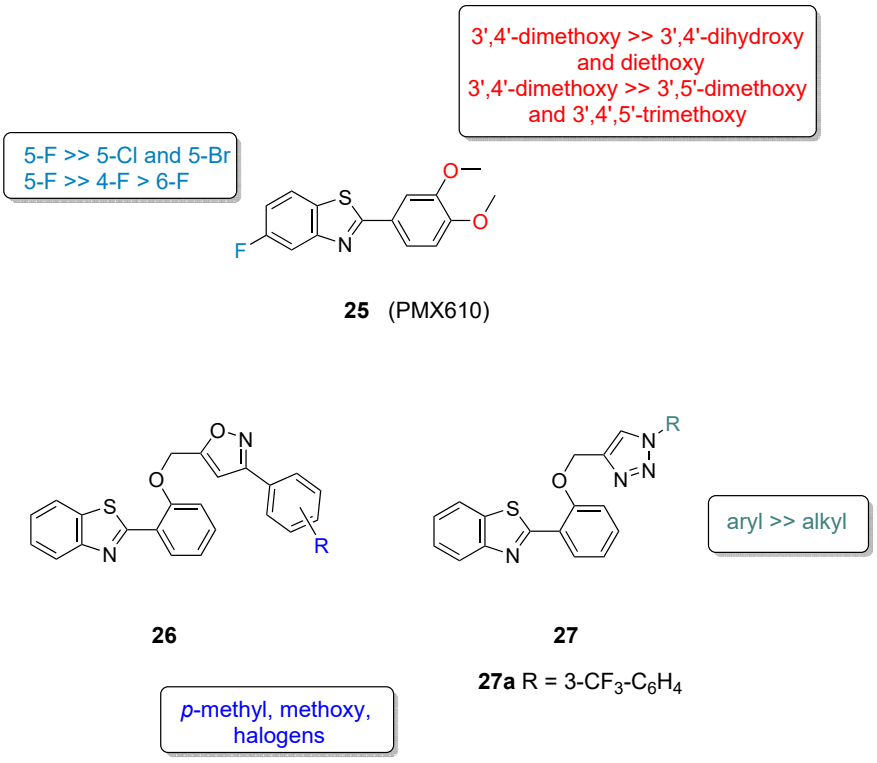


Fig. 11. SAR of PMX610 (**25**) and 2-phenylbenzothiazoles incorporating isoxazole (**26**) or triazole (**27**).

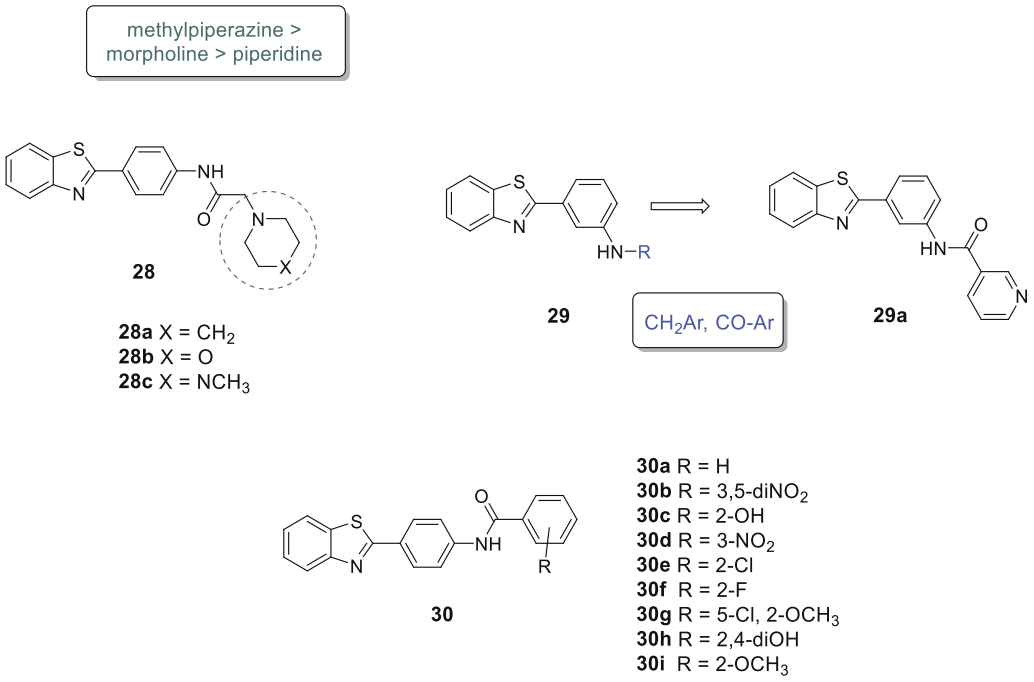


Fig. 12. 2-Phenylbenzothiazoles incorporating an amido group.

phenylbenzothiazole derivatives (**32**, Fig. 13) has been explored for their antiproliferative activity *in vitro* on different cancer cell lines, in order to determine the influence of the amidino substituents in the interaction with the corresponding targets responsible for the antitumor activity [61]. Amidines seem to contribute significantly to the affinity for biological targets through hydrogen bonding and electrostatic interactions.

Amidino substituted benzothiazoles **32** were tested against HCT116 (colon carcinoma), H460 (lung carcinoma) and MCF-7 (breast carcinoma) cancer cell lines. Overall, moderate antiproliferative activities were observed, similar for both amidino and imidazole group, except for dihydroxy derivative ($R_1 = R_2 = OH$),

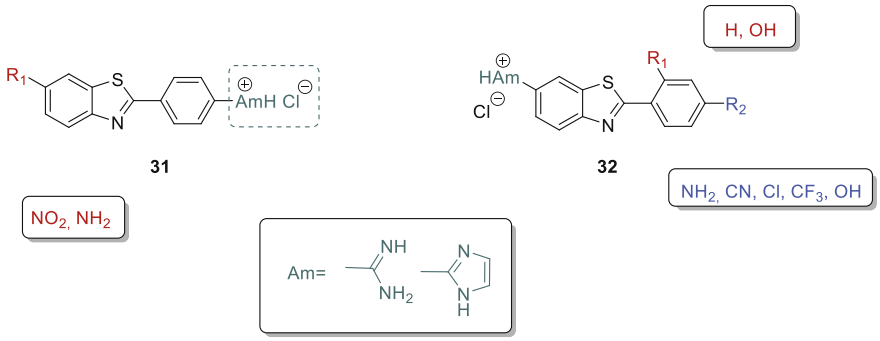


Fig. 13. Amidino substituted 2-phenylbenzothiazoles.

which did not show any activity. SAR studies revealed that benzothiazole derivatives displayed significantly better activity in comparison to their benzimidazole analogues.

A family of 2-phenylbenzothiazoles incorporating an imidazole ring (**33**, Fig. 14) was designed and synthesized in order to obtain various phenyl ring and heteroaromatic systems with a *cis* conformation, similar to that of the natural combretastatin, a potent cytotoxic agent with high tubulin binding ability [62]. SAR studies confirmed the importance of *cis* stereochemistry. All the invention compounds were evaluated as anticancer agents against sixty human cancer cells derived from nine cancer types (leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate, renal cancer and breast cancer cell lines), showing GI_{50} values of μM order. Most promising derivatives were also analyzed for their mechanism of action: compound **33a** was found the most effective in causing G2/M cell cycle arrest and inducing disrupted microtubulin organization. The treatment of MCF-7 cells with **33a** increased p21 and caspase-9 levels, markers of apoptosis.

A series of *cis*-restricted triazole/tetrazole mimics of combretastatin, incorporating a benzothiazole scaffold (Fig. 15) were synthesized and evaluated for their cytotoxic potential against selected human cancer cell lines [63]. In recent years, combretastatin emerged as an effective lead compound in the development of novel tubulin polymerization inhibitors, because of its potency and ease of synthesis [64,65]. Triazole (**34** and **35**) and tetrazole (**36**) derivatives of combretastatin were tested against prostate (DU-145), cervix (HeLa), lung adenocarcinoma (A549), liver (HepG2) and breast (MCF-7) cancer cell lines. Overall, many of these compounds displayed a broad spectrum of growth inhibition against all tested cancer cell lines; the most potent compounds **35a** and **35b**, incorporating a 1,2,4-triazole, showed a range of inhibition in the low micromolar range (IC_{50} 0.16–1.13 μM for **35a**, 0.15–1.41 μM for **35b**). SAR studies suggested that derivatives bearing the 1,2,4-triazole (**35**) showed stronger antiproliferative activities than corresponding bioisosteres containing 1,2,3-triazole (**34**) and 1,2,3,4-tetrazole (**36**). The substitution of benzothiazole ring influenced the activity, with fluoro, methoxy, dimethoxy and trimethoxy substituents (R_2) displaying better activity. Also, the presence of a methyl at C_3 of central aromatic ring exhibited a significant increase in antiproliferative activity.

The growth inhibitory effect of **35a** and **35b** was related to the arrest in G2/M phase during cell cycle progression, in a dose-dependent manner. They were also screened for their tubulin polymerization inhibition, displaying a significant effect, with IC_{50} values of 1.67 and 1.00 μM, respectively.

A number of 2-(4-aminophenyl)benzothiazole analogues (Fig. 16) were synthesized and tested for their antiproliferative activity against lung (A549), cervical (HeLa) and breast (MDA-MB-231) cancer cell lines [66]. These molecules were obtained by adding to the benzothiazole nucleus other classic pharmacophores derived from bioactive molecules, as piperazine (**37**), triazole (**38**), and pyrazolinone (**39**).

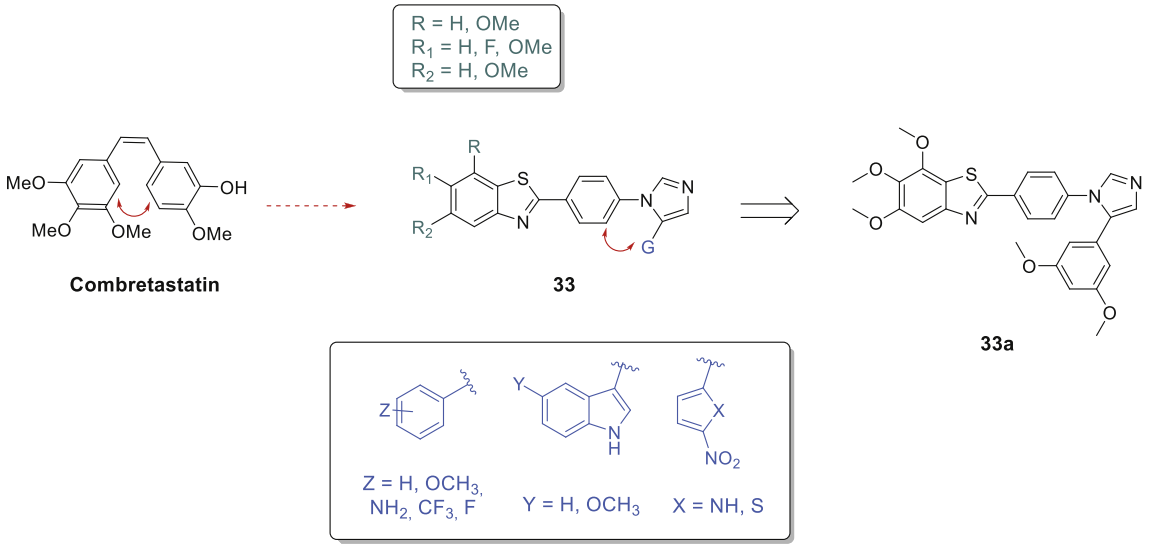


Fig. 14. From combretastatin to 2-phenylbenzothiazoles bearing imidazole.

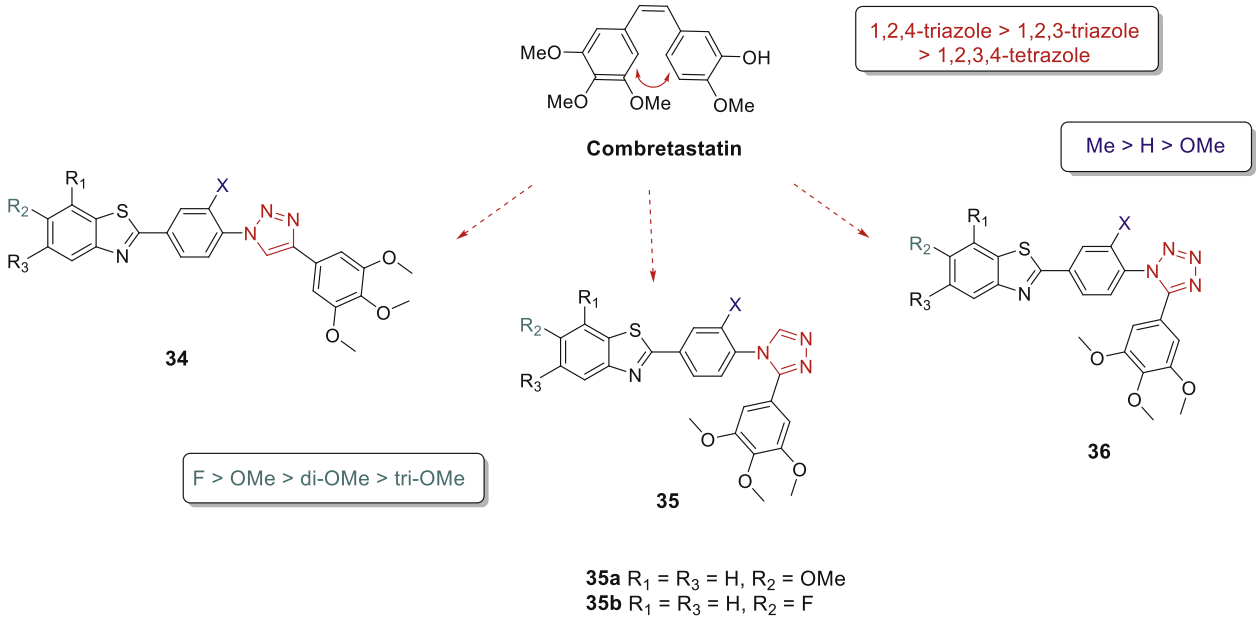


Fig. 15. *Cis*-restricted benzothiazole mimics of combretastatin incorporating triazole (**34** and **35**) or tetrazole (**36**).

Derivatives **37a-c** and **38a-b** displayed effective growth inhibition against three tested cancer cell lines at low micromolar concentrations (GI_{50} = 0.2–1.7 μM). SAR studies revealed that electron donating groups at *para* position of phenylpiperazine (**37c**) were beneficial for activity, whereas phenyltriazole derivatives **38** showed improved activity with *meta* methoxy (**38a**) or chloro (**38b**) substitution. **38a** was further studied to give insights about the mechanism of action of this class of compounds: it induced a G2/M arrest in cell cycle in treated HeLa cells, and displayed a significant tubulin polymerization inhibition when tested at 5 μM.

A series of benzothiazole derivatives incorporating a differently substituted pyrazolinone ring (**39**, Fig. 16) were prepared and evaluated for their antiproliferative activity against breast carcinoma (MCF-7) and non-small cell lung cancer (A549) cell lines [67]. Substituting the pyrazolinone nucleus at N_2 with an acetyl (**39b**) or phenyl (**39c**), the antiproliferative activities increased (IC_{50} = 12.30–15.92 μM), compared with the unsubstituted derivative **39a** (IC_{50} > 25 μM).

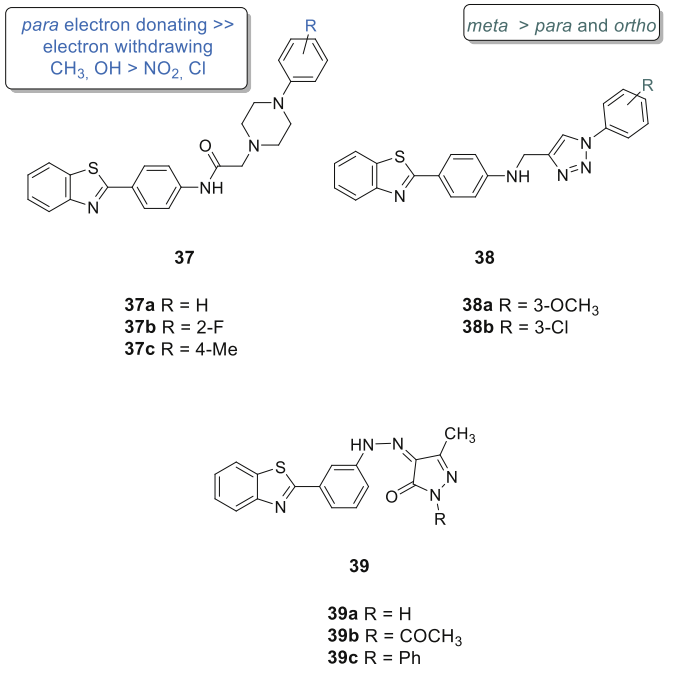


Fig. 16. Piperazine (**37**), triazole (**38**), and pyrazolinone (**39**) derivatives of 2-phenylbenzothiazole.

In order to determine their mechanism of action as anticancer agents, some cyclooxygenase (COX) inhibition assay studies for COX-1 and COX-2 were performed at 10 μM and 0.5 μM, respectively. A weak effect towards COX-1 isoform (IC_{50} = 9.56–10.41 μM) and a high potency against COX-2 enzyme (IC_{50} = 0.10–0.36 μM) were observed, being the *N*-phenyl derivative **39c** the most potent COX-2 inhibitor (IC_{50} = 0.10 μM). A docking study of **39c** was performed to determine its interactions within the binding site of COX-2, showing the same binding mode to that of co-crystallized S-58 ligand, and pointing its therapeutic interest as a potential anti-inflammatory agent.

Starting from the structure of 2-phenylbenzothiazole, a research group developed novel derivatives substituted at C_5 and C_6 of benzothiazole, that were screened against a panel of 60 human cancer cell lines [68]. This extensive work led to the synthesis of compounds (**40**, Fig. 17), in which three different regions of benzothiazole were modified (substitution at C_2, C_5 and C_6). Methoxy, methyl, fluorine, cyano and thiomethyl groups (R_1) were placed in *para* to the aromatic ring in C_2. About the substitution in R_2, secondary amines, sulfonamides, amides and ureas were developed, whereas in R_3 piperazine, piperidine, morpholine, amines and ethers were introduced.

The derivative **40a** induced good antiproliferative effect against six selected cancer cell lines (leukemia, lung adenocarcinoma, colon adenocarcinoma, glioblastoma, ovarian carcinoma, breast cancer), showing an interesting range of activity (GI_{50} 0.68–1.09 μM), and an average GI_{50} (calculated over 60 cancer cell lines) of 1.41 μM. The replacement of ureido moiety with an amine produced **40b**, that displayed an improved antiproliferative activity, with an average GI_{50} of 0.87 μM. A similar effect was obtained by changing the piperazine in C6 with a diethylaminopiperidine (**40c**, GI_{50} = 0.87 μM). A remarkable increase in antiproliferative activity

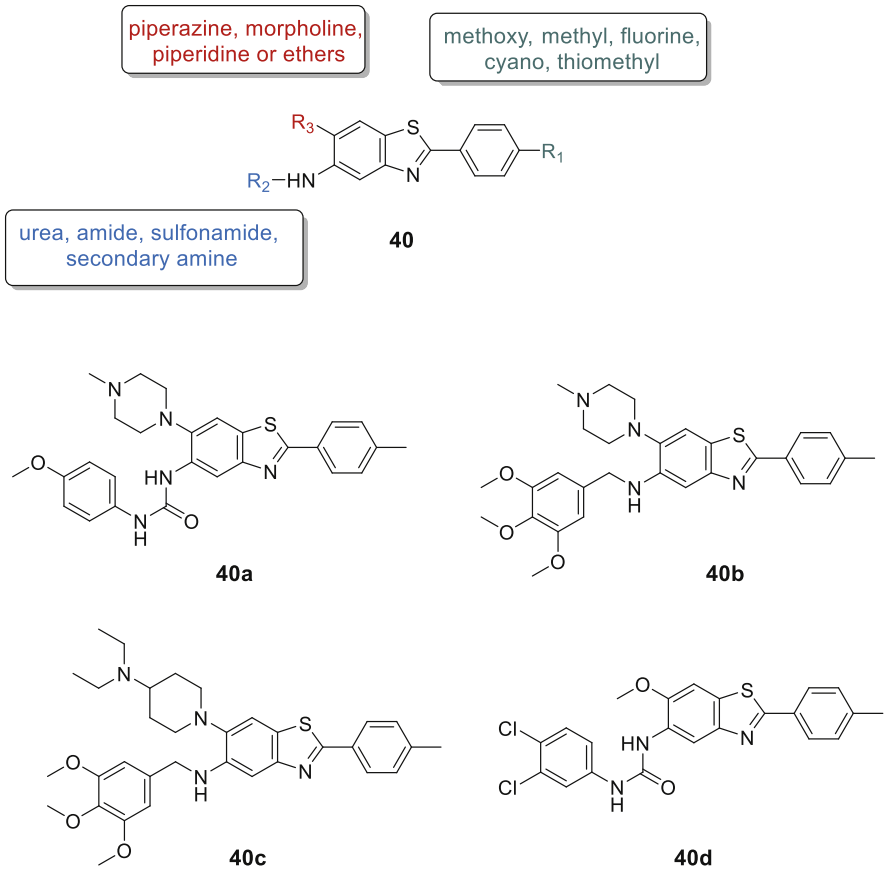


Fig. 17. C_5 and C_6 substituted 2-phenylbenzothiazoles (**40**).

was achieved with **40d**, bearing a dichlorophenylurea (R_2) and a methoxy group (R_3), with an average GI_{50} of 0.38 μM. Overall, these chemical modifications outlined the need for aromatic rings in C_2 and in the C_5 substituent; aromatic rings could be variously substituted, in order to balance the physicochemical properties. The *N*-methylpiperazine in C_6 could be replaced by a methoxy or other bioisosteres.

2.4. 2-Mercaptobenzothiazole derivatives

Whereas many studies focused on the development of 2-amido, 2-ureido, and 2-phenylbenzothiazole derivatives, a limited number of 2-mercaptobenzothiazoles were synthesized and tested for their antiproliferative activity. Despite this, some of these derivatives showed interesting properties for their ability to induce cytotoxicity in several cancer cell lines. A family of benzothiazole 2-thiol derivatives was designed and tested for their antiproliferative profile (**41**, Fig. 18) [69,70]. SAR studies on molecules **41** explored different substitutions over the amide substituent on C6 of benzothiazole and on the terminal benzylic portion. From SAR studies emerged the good contribution to the antiproliferative activity for chloromethyl in R_1, whereas substitutions in R_2 were found less significant related to the observed cytotoxicity. A preliminary screening was performed over HepG2 and MCF-7 cell lines, with the best compounds showing IC_{50} in low micromolar concentration (0.6 and 1.1 μM for **41a**, 1.1 and 4.2 μM for **41b**, respectively). These compounds showed a broad spectrum of antiproliferative activity when tested in several cancer cell lines, with excellent results in colon (HCT116), epidermoid cancer (A431) and melanoma (A375) cell lines. Further studies about the mechanism of action revealed that compound **41a** inhibits the proliferation of HepG2 cells by inducing apoptosis in a concentration-dependent way.

The cytotoxic profile of **41b** (named also SKLB-163), studied by Peng and coworkers, exhibited anticancer activity at low micromolar concentration [71]. It was tested in human melanoma (A375, IC_{50} = 1.82 μM), lung adenocarcinoma (SPC-A1, IC_{50} = 3.89 μM), colorectal (SW620, IC_{50} = 5.12 μM), cervical (Hela, IC_{50} = 5.13 μM), and prostate (PC-3, IC_{50} = 6.58 μM) cancers. In addition, antitumor activity of **41b** was explored in melanoma A375 and lung adenocarcinoma SPC-A1 *in vivo* models; about the mechanism of action, experiments revealed the involvement of RhoGDI and JNK-1 signaling pathways, leading to apoptosis and inhibition of proliferation. The introduction of a 5-bromopyridine in R_2 afforded compound **41c** (YLT205), whose cytotoxicity was assessed in a panel of cancer cell lines, including colorectal, lung, breast, liver, and melanoma [72]. The good antiproliferative effects (IC_{50}s ranging from 0.59 to 13.18 μM) were deeply investigated in colorectal cancer cell lines, in which **41c** induced apoptosis in a dose-dependent way. The mechanisms underlying the apoptotic action were identified in the activation of caspases 9 and 3, the down-regulation of Bcl-2 and the up-regulation of Bax. *In vivo* experiments in mouse xenograft models showed the inhibition of colorectal tumor growth without serious side effects.

Compound **41d** (named also YLT322) was extensively studied for its antitumor properties, showing growth cancer inhibition against a panel of 24 established cancer cell lines [73]. **41d** induced a significant cell viability inhibition in tested cancer cell lines, with IC_{50} ranging from 0.39 to 7.70 μM. In HepG2 cells, **41d** induced apoptosis in concentration- and time-dependent way; the observed apoptosis was associated with activation of caspase-3 and 9, but not

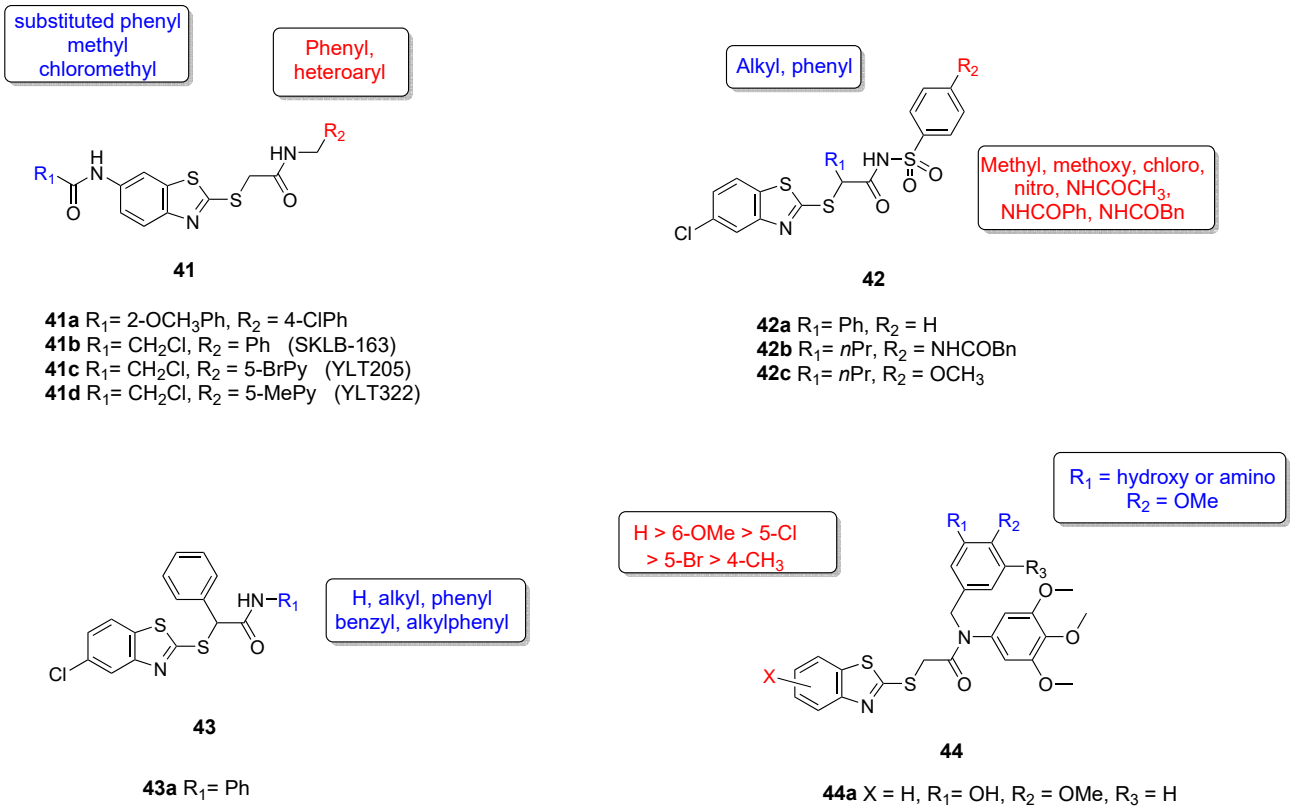


Fig. 18. 2-Mercaptobenzothiazole derivatives bearing amide (**41**, **43**, **44**) or sulfonimide (**42**) functional groups.

of caspase-8. This compound was also studied in liver xenograft models in mice, where it suppressed the tumor growth without showing significant toxicity. The promising antitumor activity of **41d** requires in depth studies to clarify its mechanism of action and its molecular target.

Novel derivatives of 2-mercaptobenzothiazole were developed, bearing sulfonimide (**42**) or amide (**43**) functional groups (Fig. 18). These molecules were designed as antagonists of nuclear receptor PPARα (Peroxisome Proliferator-Activated Receptor α), whose involvement in cell differentiation and tumor proliferation has been recently studied [74,75]. A number of derivatives were synthesized and their antiproliferative activity was assessed in different cancer cell lines. Interesting results were obtained for compound **42a**, displaying strong effects in glioblastoma primary cells [76]. In this cancer model, **42a** induced death, increased the radiosensitivity and affected the colony formation and the migration of cells. **42a-b** were also tested in pancreatic (Capan-2 and AsPC-1) and colorectal (HT-29 and SW480) cancer cell lines, showing cytotoxic effect at high concentrations [77]. Interestingly, compound **42c** showed a remarkable inhibition of carbonic anhydrase IX (CA IX) (IC_{50} = 0.49 μM), a validated antitumor target [78]. Amide derivatives **43** were tested for their antiproliferative activity in pancreatic (Capan-2 and AsPC-1), colorectal (HT-29 and SW480) and paraganglioma cell lines (PTJ64i and PTJ86i). Marked effects on cell viability were observed for the phenyl derivative **43a**, especially in two paraganglioma models (IC_{50} = 8.49 μM and 16.7 μM, respectively); an additional inhibition effect on colony formation was detected for this compound on both paraganglioma models [79].

A very recent work by Song and coworkers identified novel 2-mercaptobenzothiazole derivatives displaying good antiproliferative activity in gastric cancer [80]. The authors designed compounds as potential colchicine binding site tubulin inhibitors and as activators of Hippo pathway. Hippo is a highly conserved signaling pathway, acting as a central node in the regulation of cell division in many cancers [81–83]. Synthesized compounds (**44**, Fig. 18) were screened for their antiproliferative activity against gastric (MGC-803), colon (HCT-116) and prostate (PC-3) cancer cell lines: derivative **44a** was the most potent compound, with IC_{50} of 0.035, 0.182, and 2.11 μM against the three cancer cell lines, respectively. SAR studies conducted on these compounds showed that the methoxy group at R_2 gives an important contribution to the antiproliferative activity, and the introduction of an amino, hydroxy or azido group in R_1 could significantly improve the activity. The substitution of benzothiazole (X), instead, did not produce significant improvement of the antiproliferative activity. About the mechanism of action, further experiments demonstrated that **44a** inhibits tubulin polymerization (IC_{50} 1.9 μM) binding to colchicine binding site and it was able to activate the Hippo signaling pathway. In two gastric cell lines (MCG-803 and SGC-7901), **44a** arrested the cell cycle at G2/M phase and inhibited cell colony formatting.

2.5. Benzothiazole hybrids

2.5.1. Benzothiazole-rhodacyanine hybrids

Benzothiazole-rhodacyanine hybrids were developed as novel heat shock protein 70 (Hsp70) inhibitors. This family of molecular chaperones is highly expressed in tumors, where they play a role in proliferation and survival of cancer cells [84]. Benzothiazole derivatives were developed as promising Hsp70 inhibitors, able to induce antiproliferative effects in different cancer models. MKT-077 (**45**, Fig. 19) was evaluated in Phase 1 clinical trials for solid tumors, but it was discontinued for its modest efficacy and metabolic instability [85]. Medicinal chemistry programs led to the

discovery of Hsp70 allosteric inhibitors **46**, in which SAR analysis explored the substitution on benzothiazole (R_1), on the central rhodacyanine ring (R_2) and on the distal benzyl ring (R_3). Small electron donating substituents in R_1 improved the antiproliferative activity, whereas in the central rhodacyanine ring, the best activities were obtained with small hydrophobic substituents, as ethyl and allyl groups. In R_3 the *ortho* substitution with halogens enhanced the activity.

JG-231 (**46a**) and JG-294 (**46b**) emerged as the best compounds, both endowed with nanomolar potency in breast cancer cells MCF7 (IC_{50} = 0.12 and 0.10 μM, respectively) and MDA-MB-231 (IC_{50} = 0.25 and 0.18 μM, respectively) [86]. Further experiments revealed for **46a** the ability to suppress tumor growth in MDA-MB-231 xenograft mice.

Despite the interesting properties displayed by these compounds, their fluorescence and unfavorable pharmacokinetic led the researchers to develop novel neutral analogues. By replacing the charged group with a neutral pyridine, a group of derivatives was synthesized, with **47** (JG2-38) showing the best antiproliferative activity against breast (MCF-7) and prostate (22Rv1, PC3) cancer cell lines (IC_{50} = 0.10, 0.15 and 0.07 μM, respectively) [87]. SAR studies outlined the positive contribution to the activity of *ortho* fluorine substitution of aniline and the methyl on the benzothiazole ring.

2.5.2. Benzothiazole-pyrimidine hybrids

A recent study from literature describes the discovery of novel antiproliferative agents, obtained by combination of 2-aminobenzothiazole and 2,4-diaminopyrimidine, as privileged scaffolds in bioactive compounds [88]. Hybrids **48** (Fig. 20) were synthesized as novel CDK2 inhibitors and tested for their cytotoxic activity in selected human cancer cell lines, including breast (MDA-MB-231, MCF7), cervical (HeLa), colorectal (HCT116), and prostate (PC-3) cancer. Chemical modifications were performed on the benzothiazole ring at C_6 (R_1), the pyrimidine at C_5 (R_2) and the sulfonyl group (R_3). Regarding R_3 substitution, methylsulfonyl and sulfamoyl groups displayed the best results, and were kept fix for the optimization of R_1 and R_2. The introduction of halogens (fluorine and chlorine), methyl or methoxy groups at C_6 of benzothiazole resulted in a marked antiproliferative effect against MCF7 and PC-3, whereas derivatives with a hydrogen in R_2 exhibited excellent activities. The most promising compound **48a** showed a similar potency against HeLa, HCT116, PC-3 and MDA-MB-231 (IC_{50} = 0.45, 0.70, 0.92 and 1.80 μM, respectively). This compound was further studied to assess the mechanism of its antiproliferative action: it was tested *in vitro* against CDK2/cyclin A2, displaying an excellent inhibitory activity (94% inhibition at 1 μM, IC_{50} = 15.4 nM). The cell cycle analysis in HCT116 cells revealed that **48a** could induce cell cycle arrest and apoptosis in a concentration-dependent way.

2.5.3. Benzothiazole-pyrazole hybrids

The multiple pharmacological effects played by benzothiazole-based compounds and pyrazole derivatives led researchers to develop novel hybrids containing both these heterocycles, as potential antiproliferative compounds [89]. From this study emerged a novel group of molecules bearing the benzothiazole, the pyrazole and an aminoguanidine moiety (**49**, Fig. 20).

SARs were derived by changing the substituent at C6 of benzothiazole, with aliphatic and aromatic substituents, with the most promising compound **49a** bearing a *p*-fluoro phenyl. It induced antiproliferative effects against four cancer cell lines (breast cancer MCF-7 and MDA-MB-231, hepatocarcinoma HepG2 and SMMC-7721) at low micromolar concentrations (IC_{50} = 2.23, 2.41, 3.75, 2.31 μM, respectively). The length of aliphatic substituents at C6 of

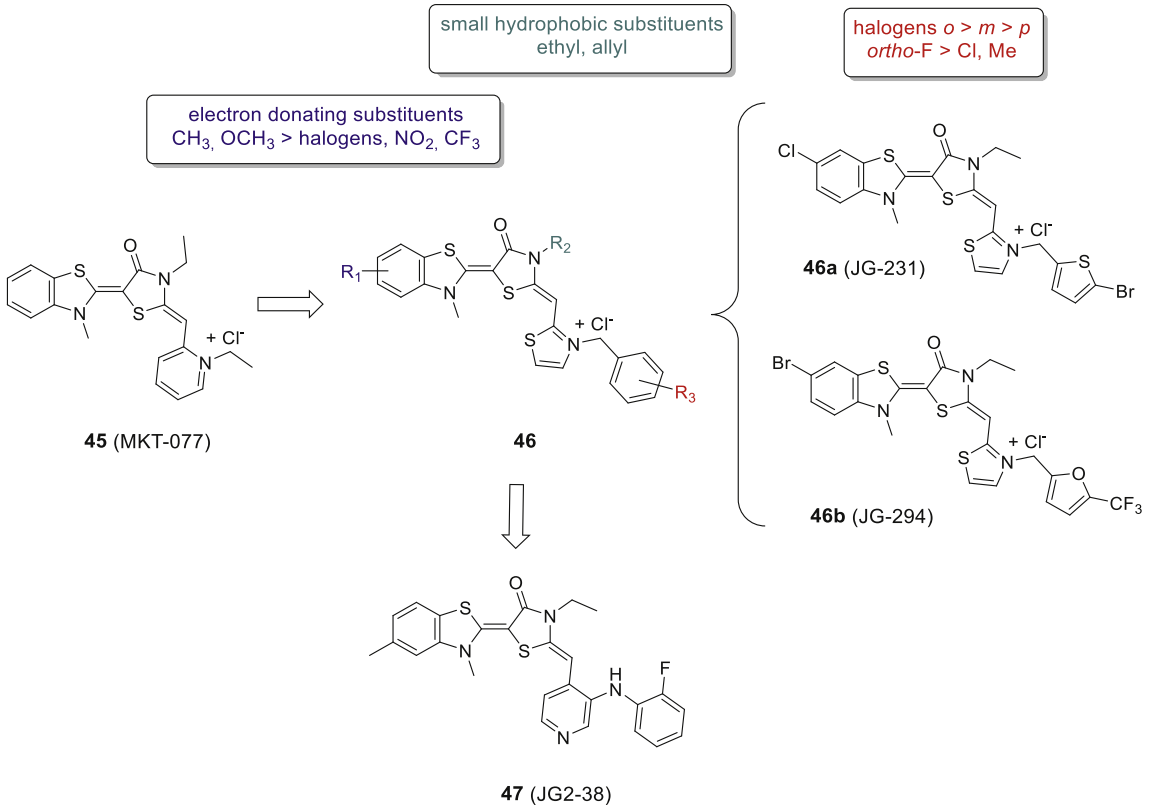


Fig. 19. Benzothiazole-rhodacyanine hybrids.

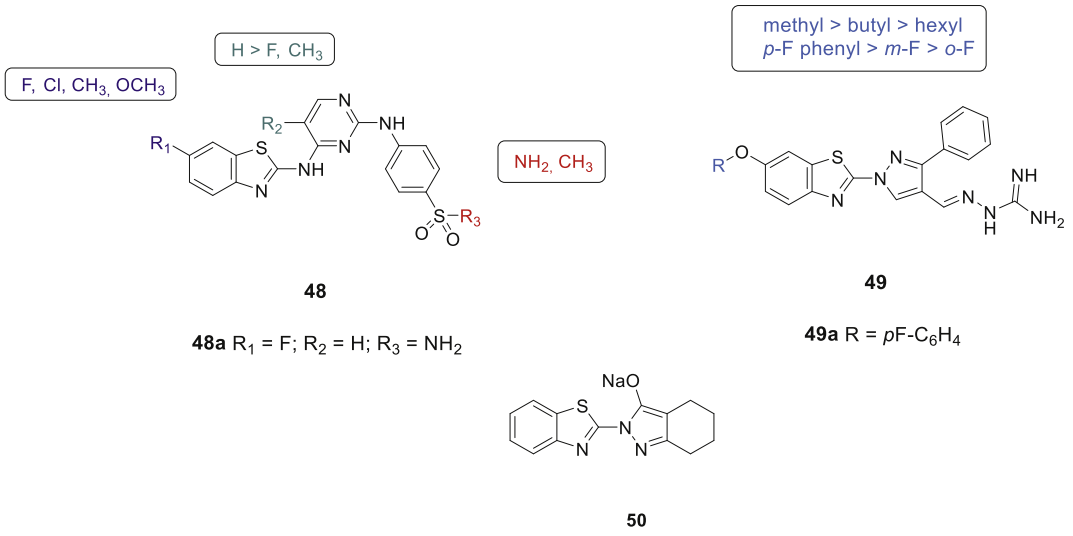


Fig. 20. Benzothiazole-pyrimidine (**48**) and benzothiazole-pyrazole (**49** and **50**) hybrids.

benzothiazole influenced the antiproliferative activity, with the best results given by methoxy group. In benzyloxy derivatives, the introduction of a fluorine produced good activities, with the order *p*-F > *m*-F > *o*-F. The activity of corresponding chlorine derivatives was weaker, and the introduction of two chlorine atoms further decreased the antiproliferative effects.

Further studies about the mechanism of action revealed that **49a** inhibits the proliferation of MDA-MB-231 cells, by inducing apoptosis in a concentration-dependent manner, with down-regulation of Bcl-2 and upregulation of Bax protein.

In order to increase the water solubility of benzothiazole derivatives, which is usually quite low and limits their clinical applications, Li's group developed a water soluble ionic benzothiazole-pyrazole hybrid (**50**, Fig. 20) [90]. This sodium salt showed antitumor activity on tumor cell lines including B lymphoma cells, T lymphoma cells and myeloma cells, with IC_{50} from 6.0 to 42.2 μM, depending on the cell line, with low toxicity in peripheral blood mononuclear cells (PBMC). The study of the mechanism of action showed that **50** induced apoptosis through both, mitochondrial and endoplasmatic pathways, causing cell cycle arrest at G0/G1 stage. The ability of this heteroaryl benzothiazole derivative to induce apoptosis was associated with overproduction of ROS.

2.5.4. Benzothiazole-piperazine hybrids

Benzothiazole-piperazine hybrids (Fig. 21) were synthesized and tested for their antiproliferative activity. *N*-arylsulfonylpiperazine derivatives with a carbohydroxamic group in the phenyl ring (**51**) were synthesized, proving that the position of the carbohydroxamic group in the phenyl ring was crucial for the activity; the *meta* derivative **51b** was not active, while the *para* substituted derivative **51a** exhibited activity as a HDAC1 inhibitor (IC_{50} = 4.8 μM), with selective antiproliferative activity against lung cancer cell lines (HCC4017 IC_{50} = 1.63 μM, HCC4018 IC_{50} = 4 μM) [91]. A wide screening across 60 tumor cell lines revealed for **51a** good antiproliferative effects in leukemia, melanoma, colon and prostatic cancers (GI_{50} 1.77–4.01 μM). A similar cytotoxic behavior was observed in the benzoxazole and *N*-methylbenzimidazole analogues.

A series of benzothiazole-piperazine hybrids bearing arylsulfonamides (**52**, Fig. 21) was synthesized and tested against a large group of human tumor-derived cell lines to evaluate their *in vitro* antiproliferative activity [92]. Compounds **52a**, **52b** and **52c** were the most potent analogues in this series, showing activity against both cell lines derived from haematological and solid tumors (CC_{50} range = 8–24 μM). Compound **52a** was especially active against the human prostate carcinoma (DU-145) cell line (CC_{50} = 8 μM) and

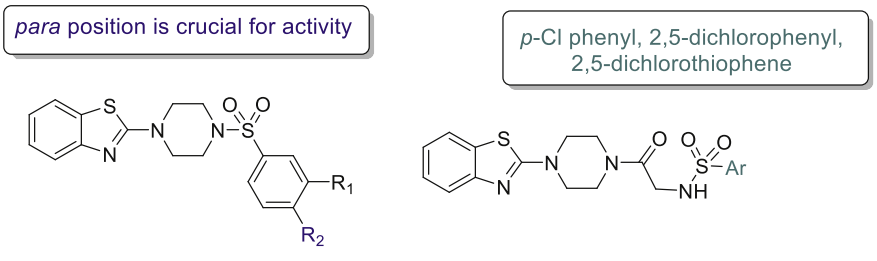
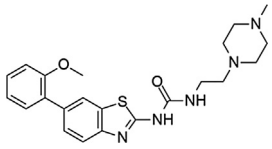
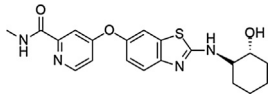
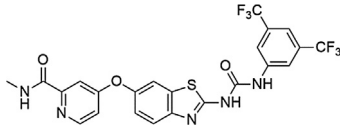
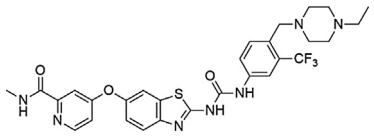
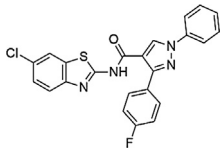
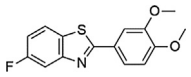
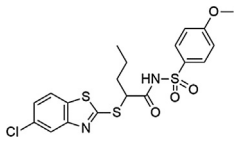
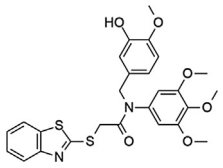
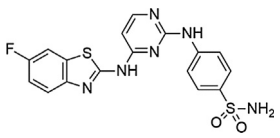


Fig. 21. Benzothiazole-piperazine hybrids.

Table 1
Representative antiproliferative 2-substituted benzothiazoles and their molecular targets.

Hit	Molecular target	Potency (IC_{50})	Reference
4b	Bcr-Abl T315I	0.015 nM	[27]
			
5 (BLZ945)	CSF-1R	1 nM	[31]
			
9b	B-Raf^{V600E} C-Raf	1.23 μM 0.566 μM	[37]
			
10 (KST016366)	Tie2 TrkA ABL-1	0.82 nM 3.81 nM 52.7 nM	[38]
			
14a	VEGFR-2	97 nM	[43]
			
25 (PMX610)	AhR	25 nM	[55]
			
42c	CA IX	0.49 μM	[78]
			
44a	Tubulin polymerization (colchicine binding site)	1.9 μM	[80]
			
48a	CDK2/cyclin A2	15.4 nM	[88]
			

52b against the human hepatocellular carcinoma (HepG2) and human prostate (DU-145) cell lines (CC_{50} = 8 μM and 9 μM, respectively). Only the dichlorophenyl derivative **52b** was found to be selective and not cytotoxic to human lung fibroblasts.

About SARs, the introduction of a chlorine, dichlorophenyl and dichlorothiophene generally enhanced the potency; the replacement of either (or both) of the chlorine atoms by hydrogen, methyl, nitro, trifluoromethyl, or methoxy substituents had a detrimental effect.

3. Conclusion and perspectives

Benzothiazoles represent a wide class of molecules which attracted much attention for their valuable biological properties. Several efforts in medicinal chemistry led to the discovery of a large library of benzothiazole derivatives with anticancer, anti-inflammatory, antibacterial, anticonvulsant, antidiabetic and other activities.

In this review, antiproliferative 2-substituted benzothiazoles were examined, pointing at the structure-activity relationships and at their mechanisms of action.

Among 2-ureido derivatives, novel Raf-1 and Bcr-Abl kinase inhibitors were identified, displaying strong cytotoxic effects in different cancer cell lines, sometimes with a better potency than sorafenib. The 2-aminobenzothiazole derivative BLZ945, a nanomolar CSF-1R inhibitor, represents an interesting drug candidate for the treatment of solid tumors; it is currently under evaluation in clinical trials as single agent or in combination with spartalizumab. Chemical modifications led to the discovery of novel 2-ureidobenzothiazoles, endowed with a broad-spectrum of antiproliferative activity. Among these compounds, the multikinase inhibitor **10** represents a promising candidate for further preclinical and clinical investigations.

Several antiproliferative 2-phenylbenzothiazoles were discovered in last decades, starting from CJM126, that served as *lead compound* for the identification of novel promising molecules. The different pathways of substitution of aromatic ring and the evaluation of pharmacokinetic properties led to the discovery of the clinical candidate Phortress. A large library of 2-phenylbenzothiazoles was obtained by inserting amide groups and heterocycles as isoxazole, triazole, imidazole, tetrazole, pyrazolinone.

2-Mercaptobenzothiazole derivatives were also identified, showing interesting cytotoxic effects in cancer cell models. In these molecules, amide and sulfonimide groups were inserted, obtaining compounds targeting different targets, such as PPARα, CA IX, and tubulin.

Despite the excellent cytotoxic effects induced in several cancer cell lines, for many of these compounds it is not clear the mechanism of action, and different biological targets have been explored at molecular level. Sometimes, the observed antiproliferative activity was not fully correlated to the potency of inhibition of a specific target, requiring more detailed studies to progress with the development of such compounds.

Molecular hybridization strategies allowed to obtain novel benzothiazole hybrids, connecting the benzothiazole with different pharmacophores present in antiproliferative compounds, as rhodacyanine, pyrimidine, pyrazole, piperazine. Table 1 summarizes the representative derivatives analyzed in this study and their molecular targets.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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