Bioorganic & Medicinal Chemistry 19 (2011) 1649–1657

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of imidazolo[2,1-*b*]benzothiazole derivatives, as potential p53 inhibitors

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ARTICLE INFO

Article history:
Received 30 November 2010
Revised 14 January 2011
Accepted 19 January 2011
Available online 25 January 2011

Keywords:
p53 Inhibitors
Analogues of pifithrin-β
Benzothiazole derivatives
Tetrahydrobenzothiazole derivatives
Biochemical and biological evaluation
Combined treatment with taxanes

ABSTRACT

Since activation of p53 in response to cytotoxic stress may have proapoptotic or protective effects depending on the nature of the injury, inhibitors of p53 may have therapeutic interest as modulators of chemotherapy toxicity or efficacy. In an attempt to identify novel p53 inhibitors, a quality collection of compounds structurally related to pifithrin-β were designed and synthesized as potential inhibitors of p53. The biochemical and biological evaluations supported that compounds of the tetrahydrobenzothiazole series were inhibitors of the p53 transcriptional activity and were effective in enhancing paclitaxel-induced apoptosis. In contrast, in spite of the increased cytotoxic potency, selected compounds of the benzothiazole series were not able to modulate the transcriptional activity of p53, as indicated by lack of change of p21 expression. The therapeutic interest of the compounds of the former series in combination with taxanes was confirmed in a human tumor xenograft model.

1. Introduction

The tumor suppressor protein p53 is a transcription factor, which plays a critical role in cellular response to a variety of stresses including DNA damage.[1,2] The activation of p53 may induce growth arrest or apoptosis depending on the extent or nature of stress signals.[1–3] Based on these functions, p53 can modulate tumor cell sensitivity to chemotherapy or radiation. Since p53 has been implicated as a determinant of apoptosis induced by DNA damage, the loss of p53 function, as a consequence of mutation, could result in a relative drug resistance of tumors carrying p53 mutations.[4,5] However, the role of p53 in response to cytotoxic treatment is dependent on the activation of distinct functions and molecular context. For example, p53-mediated transcriptional activation of p21 after genotoxic stress may result in cell cycle arrest allowing DNA repair.[6,7] In this case, p53 plays a role of survival factor by allowing cells to reside in a growth arrested state, thereby reducing the risk of accumulation of genotoxic lesions during cell cycle progression. A protective role of the transcriptional activity of p53 has been documented in response to antimicrotubule agents.[8] Indeed, cyclic pifithrin (**PFT-β**, Fig. 1)[9] sensitizes wild-type p53 tumor cells to paclitaxel (PTX) and vinca alkaloids. Under these conditions, targeting p53 function may have therapeutic relevance to improve the effects of mitotic spindle damage. In addition, when apoptosis is impaired as a consequence of alterations in the apoptotic process, wild-type p53 may have a prosurvival function in response to chemotherapy through prolonged growth arrest allowing DNA repair. Therefore, although p53 inhibitors have been developed as potential modulators of chemotherapy-induced toxicity,[10–12] these agents may have potential applications to sensitize tumor cells under particular treatment conditions. In the present study, we report the synthesis of novel PFT-β analogues (Fig. 1) and the biological evaluation of the obtained compounds.

2. Results and discussion

2.1. Chemistry

We considered as possible modifications (a) the replacing of the tetrahydrobenzothiazole ring with a benzothiazole portion, (b) the functionalization of the methyl group, and (c) the replacement of the sulfur atom with alkylated nitrogen (Fig. 1). For the synthesis of analogues **3a–f** (Scheme 1) the commercially available 2-aminobenzothiazole **1a** was used, while the starting 2-aminotetrahydrobenzothiazole **1b** was obtained following the procedure described by King and Hlavacek[13] in which cyclohexanone was reacted with thiourea in the presence of iodine. Condensation of benzothiazoles **1a** and **1b**

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doi:10.1016/j.bmc.2011.01.039

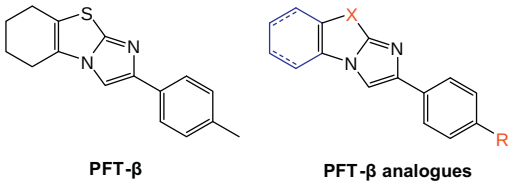


Figure 1. Structure of pifithrin-β (**PFT-β**) and the general structure of designed related analogues.

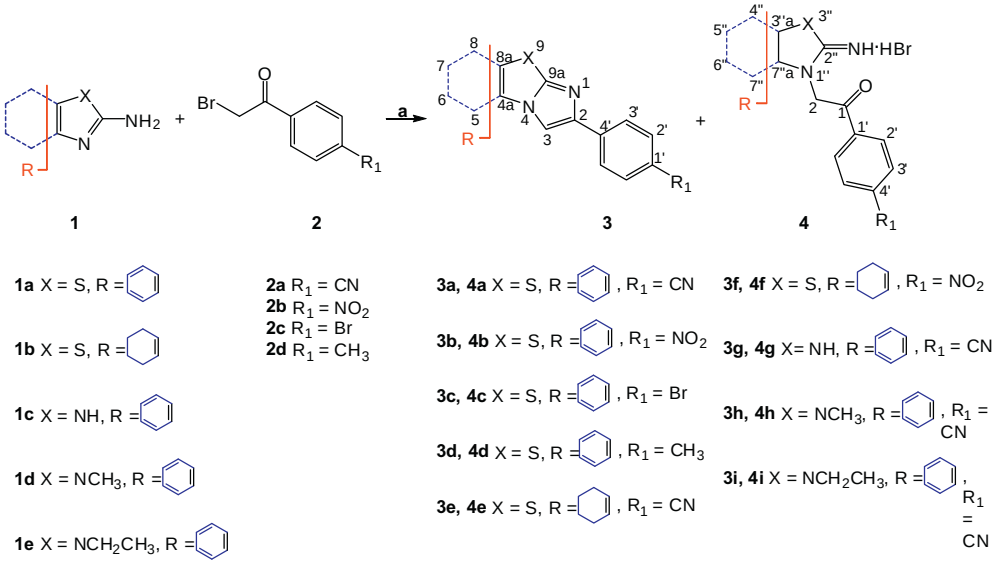
with a variety of α-bromoacetophenones **2a–d** provided the corresponding closed ring derivatives **3a–f** in moderate yields.

The use of 2-aminobenzoimidazole **1c** and 1-methyl-2-aminobenzoimidazole **1d** permitted the obtainment of the compounds that present the nitrogen atom replacing the sulfur atom. However, the condensation of imidazoles **1c** and **1d** with 2-bromo-4′-CN-acetophenone **2a** provided the open forms **4g** (yield 60%) and **4h** (yield 75%), respectively, as the main products. Only in the case of imidazole **1d** a very low yield (5%) of the closed product **3h** was observed. Having this evidence, we considered to introduce an aliphatic chain on the *N*-1 of the 2-aminobenzoimidazole. The 1-ethyl-2-aminobenzoimidazole **1e** was easily prepared from **1c** using ethyliodide in the presence of KOH. Although the condensation of **1e** with 2-bromo-4′-CN-acetophenone **2a** provided the desired derivative **3i** in higher yield (20%), still the open form **4i** remained the main product (60%) of the reaction (Scheme 1).

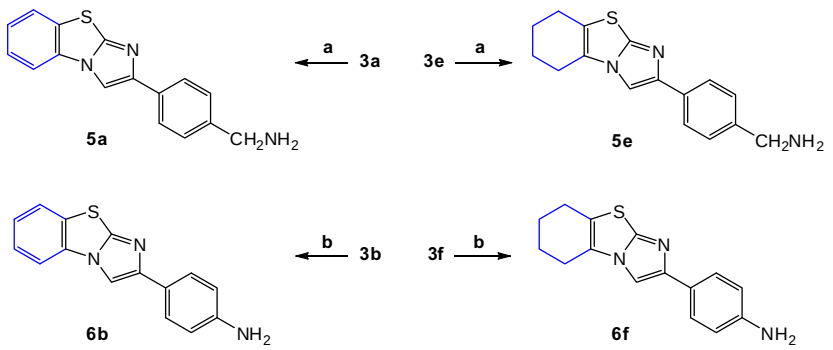
Continuously, the reduction of nitriles **3a** and **3e** with DIBALH in toluene provided the desired amines **5a** and **5e** in excellent yields (90%). Nitro derivatives **3b** and **3f** were also transformed to the corresponding amines **6b** and **6f** using H_2SO_4 in the presence of Fe in excellent yields (89%) (Scheme 2). The transformation of amines **5** and **6** in the corresponding hydrochloride salts was realized by a treatment with a solution of satd HCl in MeOH .

In order to investigate further the effects of substitution at the *para* position of the right phenyl ring, several derivatives were prepared. Acylation of amines **5a** and **5e** with acetylchloride in DMF provided the corresponding amide analogues **7a** and **7e**, respectively, in very high yields (85%). Analogue **8** was obtained via an aza-Michael addition of *N*-isopropylacrylamide in H_2O, while analogue **9** was prepared in a two step synthesis, using the Boc-protected β-alanine and subsequent removal of the terbutoxycarbonyl group (Scheme 3).

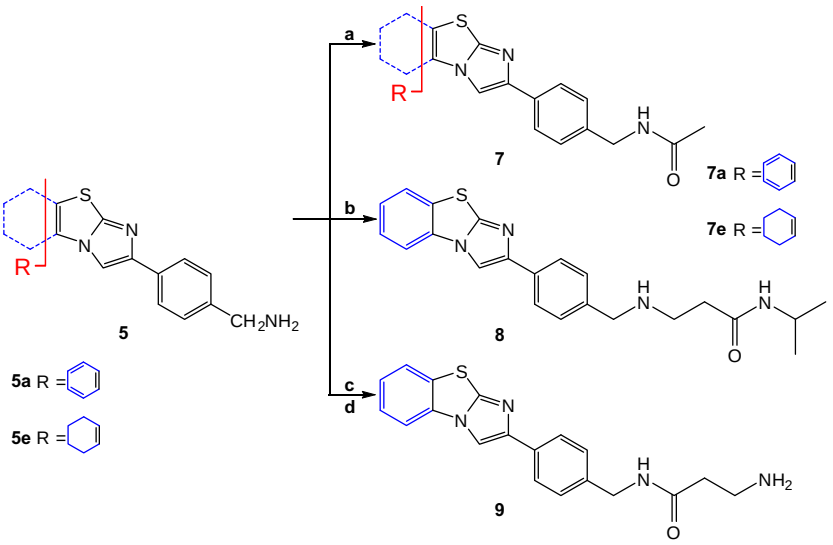
The obtainment of alcohol **12** and aldehyde **13** was planned in two different approaches: (a) transformation of nitrile to the aldehyde and subsequent reduction and (b) reduction to alcohol and oxidation to the desired aldehyde. Transformation of nitrile **3a** to aldehyde **13** using DIBALH in different solvents and temperatures resulted unsuccessfully. Thus, nitrile **3a** was first transformed into acid **10** in basic conditions in almost quantitative yield (96%). The subsequent reduction of acid **10** by $LiAlH_4$ in THF afforded the desired alcohol **12** in very low yield (10%). Therefore, acid **10** was converted by trimethylsilyldiazomethane into the corresponding



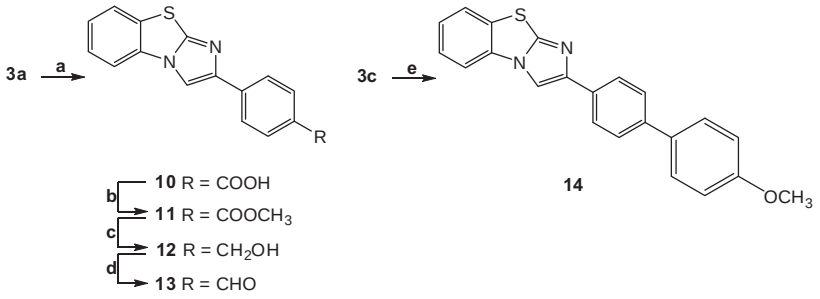
Scheme 1. Reagents and condition: (a) EtOH, reflux, 90 min.



Scheme 2. Reagents and conditions: (a) DIBALH, toluene, reflux, 2 h; (b) Fe, H_2SO_4/H_2O, 90 °C, 1 h.



Scheme 3. Reagents and conditions: (a) acetylchloride, Et_3N, DMF, 24 h; (b) *N*-isopropylacrylamide, H_2O, reflux, 16 h; (c) *N*-Boc-β-alanine, HATU, DIPEA, 20 h; (d) TMSCl, MeOH, 1 h.



Scheme 4. Reagents and conditions: (a) NaOH, EtOH, H_2O, reflux, 4 h; (b) trimethylsilyldiazomethane, MeOH, 30 min; (c) $LiAlH_4$, THF, 0 °C, 30 min; (d) Dess–Martin periodinane, DCM, 20 min; (e) 4-methoxyphenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, THF, EtOH, H_2O, reflux, 12 h.

ester **11** that by reaction with $LiAlH_4$ provided alcohol **12** in good yield. Oxidation of alcohol **12** using Dess–Martin periodinane in DCM furnished aldehyde **13** in quantitative yield (Scheme 4). The availability of the bromide derivative **3c** permitted the application of Suzuki coupling reaction with 4-methoxyphenyl boronic acid in EtOH to obtain compound **14**.

2.2. Biological activity

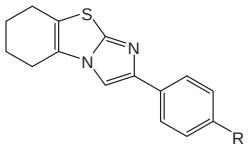
The biological activity of all compounds described in the present study was performed with an antiproliferative assay in the human ovarian carcinoma cell line, IGROV-1, following 72 h-exposure. This cell system was chosen, because it is able to grow in athymic nude mice. The results obtained in this cell system show that the compounds with the tetrahydrobenzothiazole scaffold of PFT-β (Table 1) retained a biological activity comparable to that of the parent compound (i.e., IC_{50} values in the range of 5–30 μM).

In contrast, the compounds of the benzothiazole series (Table 2) exhibited a substantial increase of the antiproliferative potency (i.e., IC_{50} values in the nanomolar range).

The values of *c* log *P*[14] for the compounds **3–14** are generally lower than the one related to PFT-β, which indicates that new compounds present a reduced lipophilicity.

Selected compounds of the two series were tested for their ability to modulate the expression of p53 and p21, a well-known p53 target, after paclitaxel (PTX) treatment.[8] Figure 2 shows that the compounds **6f** and **5e·HCl** were able to reduce the up-regulation of p21 induced by paclitaxel. In contrast **5a·HCl**, benzothiazole analogue of **5e·HCl,** was not able to modulate the expression of p21, thus suggesting that the biological activity of the benzothiazoles was independent from the inhibition of p53. Thus the mechanism of action of compounds of the latter series remains to be determined.

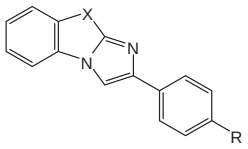
Table 1



Compound	R	IC_{50}* (μM)	*c* log *P***
PFT-β	CH_3	23 ± 4	4.397
3e	CN	8 ± 3	3.703
5e	CH_2NH_2	12 ± 2	3.133
5e·HCl	CH_2NH_2·HCl	31 ± 3	0.588
6f	NH_2	4 ± 2	3.024
6f·HCl	NH_2·HCl	19 ± 4	0.691
7e	$CH_2NHCOCH_3$	28 ± 2	2.868

* IC_{50} = concentration of compound that reduces cell proliferation by 50%.
** *c* log *P* = calculated octanol–water partition coefficient.

Table 2



Compound	X	R	IC_{50}* (μM)	*c* log *P***
3a	S	CN	0.03 ± 0.01	3.967
3b	S	NO_2	2 ± 1	4.171
3c	S	Br	0.3 ± 0.1	5.021
3d	S	CH_3	0.3 ± 0.2	4.661
3h	NCH_3	CN	7 ± 3	3.290
3i	NCH_2CH_3	CN	4.5 ± 0.5	3.666
5a	S	CH_2NH_2	0.03 ± 0.01	3.396
5a·HCl	S	CH_2NH_2·HCl	0.3 ± 0.1	0.852
6b	S	NH_2	1 ± 0.8	3.288
6b·HCl	S	NH_2·HCl	5 ± 2	0.955
7a	S	$CH_2NHCOCH_3$	12 ± 4	3.132
8	S	$CH_2NH(CH_2)_2CONHCH(CH_3)_2$	5 ± 2	3.821
9	S	$CH_2NHCO(CH_2)_2NH_2$	3 ± 1	2.167
12	S	CH_2OH	7 ± 4	3.550
13	S	CHO	6 ± 3	4.002
14	S	Ph (*p*OCH_3)	<1	6.064

** *c* log *P* = calculated octanol–water partition coefficient.
* IC_{50} = concentration of compound that reduces cell proliferation by 50%.

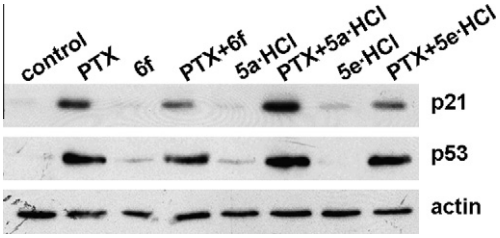


Figure 2. Expression of p53 and p21waf1 in IGROV-1 cells treated for 24 h with paclitaxel (dose corresponding to IC_{50}: PTX 0.12 μM) or selected compounds alone (dose corresponding to IC_{50}: **6f** 5 μM; **5a·HCl** 0.3 μM; **5e·HCl** 10 μM) or their combination. In the combined treatment, selected compounds were added 2 h before treatment with PTX. Whole-cell extracts were prepared and analyzed by Western Blot analysis. Actin is shown as a control of protein loading.

Table 3
Antitumor activity of compounds **5a·HCl** and **5e·HCl** in the treatment of the human ovarian carcinoma IGROV-1 model

Drugs	Dose (mg/kg)	TVI%[a]	BWL%[b]	Tox[c]
5a·HCl	3	15	3	0/4
	9	46*	5	0/4
5e·HCl	9	21	6	0/4
	18	27	4	0/4

Animals were treated iv every four days for a total of 4 administrations (q4dx4).
* *P* <0.05 by Student's test versus control mice.
[a] Tumor volume inhibition in treated versus control mice.
[b] Body weight loss induced by drug treatment.
[c] Tox, that is, animals exhibiting lethal toxicity in each group.

The antitumor activity of **5e·HCl** and **5a·HCl** was compared in the ovarian carcinoma xenograft, IGROV-1. The results indicate that under comparable conditions only **5a·HCl** exhibited a significant tumor growth-inhibition (Table 3).

Since at the tested doses the compound was well tolerated, an improvement of the efficacy is expected by increasing the dose level. On the basis of previous studies indicating the ability of pifithrin-β to sensitize wild-type p53 tumor cells to paclitaxel,[8] we have explored the ability of **6f**, the most potent analogue of the tetrahydrobenzothiazole series, to modulate the tumor cell sensitivity to paclitaxel. Using drug concentrations causing only antiproliferative effects (IC_{50}), as indicated by the low level of apoptosis, the combination of paclitaxel and **6f** resulted in a substantial enhancement of apoptosis induction (Fig. 3), thus suggesting that the cellular sensitization by the p53 inhibitor reflected an increased susceptibility to apoptosis resulting in a cytotoxic, rather than cytostatic, outcome.

On the basis of this observation, the efficacy of this combination was evaluated in vivo using the human osteosarcoma U2OS xenograft, a model characterized by wild-type p53 (Fig. 4). Using well-tolerated doses of each agent, the combination resulted in an increase of efficacy as compared to paclitaxel in terms of tumor growth-inhibition and complete response.

3. Discussion

In this report we described a novel series of compounds structurally related to pifithrin-β as potential inhibitors of p53. These compounds were prepared in an attempt to identify novel analogues more suitable for in vivo use than the parent compound. We show evidence that selected compounds of the tetrahydrobenzothiazole series (i.e., **6f** and **5e** containing an amino group in the side chain), characterized by improved physico-chemical properties in terms of solubility and stability, also exhibited an increased potency as antiproliferative agents. These changes are expected to influence the pharmacokinetic behaviour. In particular, the changed molecules may have a reduced uptake of an intracellular accumulation. Indeed the changed forms of **5e** (Table 1) and **5a** (Table 2) exhibited a reduced antiproliferative activity. This finding is consistent with a modulation of the cellular pharmacokinetic behaviour. The water-solubility of **5e·HCl** and **5a·HCl** allowed iv administration. At the tested doses, both compounds, were well tolerated (Table 3). The significant antitumor activity of **5a·HCl** was consistent with its increased antiproliferative activity as compared to **5e·HCl**.

The tested compounds **5e** and **6f** were found to be able to reduce the up-regulation of p21 induced by paclitaxel. This effect provides evidence that these agents are inhibitors of the transcriptional activity of p53, which is activated in response to the mitotic spindle damage induced by paclitaxel. In contrast, at equitoxic concentrations, a related compound of the benzothiazole series (i.e., **5a·HCl**) was not able to modify the expression of p21, thus indicating lack of inhibition of p53 in spite of its substantial antiproliferative potency. Consistent with this interpretation is lack of potentiation of paclitaxel toxicity by compounds of the benzothiazole series (not shown).

In contrast, a representative compound of the tetrahydrobenzothiazole series **6f** was very effective in sensitizing ovarian carcinoma cells to paclitaxel-induced apoptosis (Fig. 3). The ability of **6f** to enhance the cytotoxic effects of paclitaxel may have pharmacological relevance as documented by a substantial increase of antitumor activity of the combination of paclitaxel/**6f** in the treatment of a human tumor xenograft (Fig. 4). The physical properties allow the iv administration of **6f**, which was well tolerated at the dose and schedule used in this experiment. Future studies aimed at optimizing the treatment schedule will better define the pharmacological profile of the novel p53 inhibitors and the therapeutic interest of the combination. Finally, selected compounds of the benzothiazole series (e.g., **5a·HCl**) exhibited a significant antitumor activity at well tolerated doses. A better understanding of the mechanism of action should provide the basis for optimization of the pharmacological profile.

In conclusion, the obtained results are a further demonstration of the importance of the chemical manipulation of small molecules

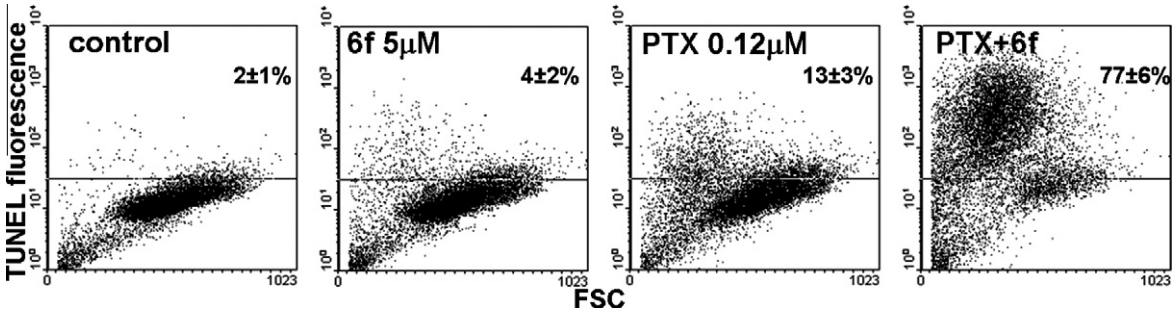


Figure 3. Induction of apoptosis in IGROV-1 cells exposed to an antiproliferative concentration of paclitaxel (PTX), **6f**, or their combination. Drug treatment was performed with IC_{50} concentrations for both agents, as indicated in legend to Figure 2. Cells were pretreated with **6f** for 2 h and then exposed to the antimicrotubule agent. Apoptosis was determined by TUNEL assay and FACScan analysis after 72 h of treatment. The percentages of TUNEL-positive cells are indicated in each panel. Representative of at least three independent experiments is shown.

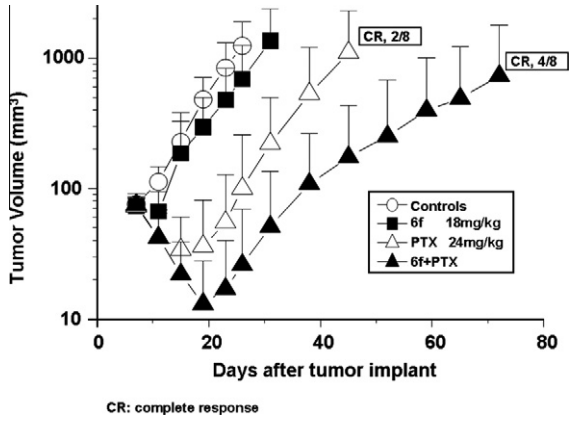


Figure 4. Antitumor activity of paclitaxel, compound **6f** and their combination against the osteosarcoma model U2OS. Animals were treated iv at the indicated doses, every four days for 4 administrations. (○) Control (solvent-treated animals); (■) **6f** (Δ) or PTX-treated animals; (▲) combined treatment. Drug treatment started when tumors were just measurable (around 80 mm^3). CR: complete response (the indicated ratio is referred to the ratio of the number of the complete tumor regression/number of the treated tumors). No relevant manifestations of toxicity were recorded.

known as inhibitors to improve the performance or to modulate the biological activity and throw light on the rule of p53.

4. Experimental section

4.1. General experimental conditions

Thin-layer chromatography (TLC) was performed on Merck precoated aluminium sheets of Silica Gel 60 F_{254} and visualization of products being accomplished by UV absorbance at 254 nm. Flash column chromatography was performed on Merck Silica gel (240–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 400 MHz on Brucker spectrometers in the indicated solvents. Chemical shifts (δ) for proton and carbon resonances are reported in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard. EI mass spectra were recorded at an ionizing voltage of 6 keV on a VG 70-70 EQ. All reactions were carried out in dry solvents.

4.2. Synthesis

4.2.1. Preparation of compounds 3a–3i

4.2.1.1. 4-(Imidazo[2,1-*b*]benzothiazol-2′-yl) benzonitrile (3a). To a solution of 2-aminobenzothiazole **1a** (0.93 g, 6.0 mmol) in ethanol (92 mL), 2-Br-4′-CN-acetophenone **2a** (1.5 g, 6.6 mmol) was added and the solution was refluxed for 90 min. After the completion of the reaction, the solution was cooled to 0 °C. The resulting precipitate was filtered and washed with ethanol to provide **3a** as foam (yield 35%). ^{1}H NMR ($CDCl_3$ 400 MHz): δ = 8.09 (s, 1H, H-3), 7.98 (d, 2H, J = 8.2 Hz, H-3′), 7.75 (d, 1H, J = 8.0 Hz, H-5), 7.70 (d, 2H, J = 8.2 Hz, H-2′), 7.66 (d, 1H, J = 8.0 Hz, H-8), 7.51 (t, 1H, J = 8.0 Hz, H-6), 7.41 (t, 1H, J = 8.0 Hz, H-7); ^{13}C NMR ($CDCl_3$, 400 MHz): δ = 149.7 (C-9a), 146.4 (C-2), 138.9 (C-4′), 133.2 (C-2′), 132.6 (C-4a), 131.1 (C-8a), 127.1 (C-6), 126.2 (C-3′), 126.1 (C-7), 125.2 (C-8), 119.6 (CN), 113.5 (C-5), 111.3 (C-1′), 109.1 (C-3). Anal. Calcd for $C_{16}H_9N_3S$: C, 69.80; H, 3.29; N, 15.26. Found: C, 69.83; H, 3.24; N, 15.22; EI/MS m/z = 275.

4.2.1.2. 2-(4′-Nitrophenyl)imidazo[2,1-*b*]benzothiazole (3b). According to the general procedure analogue **3b** was obtained from 2-aminobenzothiazole **1a** and 2-Br-4′-NO_2-acetophenone **2b** as yellow solid (yield 26%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 9.12 (1H, s, H-3), 8.32 (2H, d, J = 9.2 Hz, H-2′), 8.13 (2H, d, J = 9.2 Hz, H-3′), 8.08 (1H, d, J = 8.1 Hz, H-5), 8.02 (1H, d, J = 8.1 Hz, H-8), 7.63 (1H, t, J = 8.1 Hz, H-6), 7.49 (1H, t, J = 8.1 Hz, H-7). Anal. Calcd for $C_{15}H_9N_3O_2S$: C, 61.01; H, 3.07; N, 14.23. Found: C, 61.06; H, 3.11; N, 14.25; EI/MS m/z = 295.

4.2.1.3. 2-(4′-Bromophenyl)imidazo[2,1-*b*]benzothiazole (3c). According to the general procedure analogue **3c** was obtained from 2-aminobenzothiazole **1a** and 2,4′-dibromoacetophenone **2c** as yellow solid (yield 23%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.86 (s, 1H, H-3), 8.06 (d, 1H, J = 8.1 Hz, H-5), 7.99 (d, 1H, J = 8.1 Hz, H-8), 7.83 (d, 2H, J = 8.4 Hz, H-2′), 7.64 (d, 2H, J = 8.4 Hz, H-3′), 7.58 (t, 1H, J = 7.8 Hz, H-6), 7.45 (t, 1H, J = 7.8 Hz, H-7); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 148.7 (C-9a), 146.1 (C-2), 134.2 (C-4′), 132.8 (C-2′), 130.4 (C-4a), 129.6 (C-6), 127.9 (C-3′), 127.5 (C-8a), 126.5 (C-7), 126.2 (C-8), 121.2 (C-1′), 114.5 (C-5), 110.8 (C-3). Anal. Calcd for $C_{15}H_9BrN_2S$: C, 54.72; H, 2.76; N, 8.51. Found: C, 54.74; H, 2.71; N, 8.54 EI/MS m/z = 329 (M).

4.2.1.4. 2-(4′-Methylphenyl)imidazo[2,1-*b*]benzothiazole (3d). According to the general procedure analogue **3d** was obtained from 2-aminobenzothiazole **1a** and 2-Br-4′-methylacetophenone **2d** as yellow solid (yield 23%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.90 (s, 1H, H-3), 8.14 (d, 1H, J = 8.1 Hz, H-5), 8.09 (d, 1H, J = 8.1 Hz, H-8), 7.75 (d, 2H, J = 8.1 Hz, H-3′), 7.64 (t, 1H, J = 7.8 Hz, H-6), 7.52 (t, 1H, J = 7.8 Hz, H-7), 7.30 (d, 2H, J = 8.1 Hz, H-2′), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 148.7 (C-9a), 144.4 (C-2), 138.4 (C-1′), 131.5 (C-4′), 131.6 (C-4a), 130.6 (8a), 130.5 (C-2′), 130.2 (C-6), 127.7 (C-3′), 126.6 (C-7), 126.7 (C-8), 126.3 (C-5), 114.3 (C-3), 21.90

(CH_3). Anal. Calcd for $C_{16}H_{12}N_2S$: C, 72.70; H, 4.58; N, 10.60. Found: C, 72.73; H, 4.51; N, 10.64; EI/MS *m/z* = 264.

4.2.1.5. 4-(5′,6′,7′,8′-Tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzonitrile (3e). According to the general procedure analogue **3e** was obtained from 2-amino-4,5,6,7-tetrahydrobenzothiazole **1b** and 2-Br-4′-CN-acetophenone **2a** as white solid (yield 30%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.42 (s, 1H, H-3), 8.00 (d, 2H, *J* = 8.6 Hz, H-3′), 7.83 (d, 2H, *J* = 8.6 Hz, H-2′), 2.72–2.68 (m, 4H, H-5 and H-8), 1.90–1.86 (m, 4H, H-6 and H-7); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 147.8 (C-9a), 143.4 (C-2), 139.1 (C-4′), 133.1 (C-2′), 126.9 (C-4a), 125.4 (C-3′), 122.4 (C-8a), 119.6 (CN), 110.4 (C-1′), 109.3 (C-3), 24.27 (C-5), 23.05 (C-7), 22.54 (C-8), 21.52 (C-6). Anal. Calcd for $C_{16}H_{13}N_3S$: C, 68.79; H, 4.69; N, 15.04. Found: C, 68.75; H, 4.72; N, 15.05; EI/MS *m/z* = 279.

4.2.1.6. 2-(4′-Nitrophenyl)-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole (3f). According to the general procedure analogue **3f** was obtained from 2-amino-4,5,6,7-tetrahydrobenzothiazole **1b** and 2-Br-4′-NO_2-acetophenone **2b** as white solid (yield 40%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.53 (s, 1H, H-3), 8.27 (d, 2H, *J* = 8.8 Hz, H-2′), 8.09 (d, 2H, *J* = 8.8 Hz, H-3′), 2.72–2.70 (m, 4H, H-5 and H-8), 1.91–1.89 (m, 4H, H-6 and H-7). Anal. Calcd for $C_{15}H_{13}N_3O_2S$: C, 60.18; H, 4.38; N, 14.04. Found: C, 60.14; H, 4.33; N, 14.08.

4.2.1.7. 4-(9′-Methyl-9*H*-imidazo[1,2-*a*]benzimidazol-2′-yl) benzonitrile (3h). According to the general procedure analogue **3h** was obtained from 1-methyl-2-aminobenzimidazole **1d** and 2-Br-4′-CN-acetophenone **2a** as white solid (yield 5%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.44 (s, 1H, H-3), 8.03 (d, 2H, *J* = 8.4 Hz, H-3′), 7.83 (d, 2H, *J* = 8.4 Hz, H-2′), 7.78 (d, 1H, *J* = 7.8 Hz, H-5), 7.56 (d, 1H, *J* = 7.8 Hz, H-8), 7.37 (t, 1H, *J* = 7.8 Hz, H-7), 7.24 (t, 1H, *J* = 7.8 Hz, H-6), 3.79 (s, 3H, C*H*$_3$N); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 150.6 (C-9a), 141.9 (C-2), 140.1 (C-4′), 136.6 (C-8a), 133.1 (C-2′), 125.4 (C-3′), 124.2 (C-7), 124.1 (C-4a), 120.7 (C-6), 119.7 (CN), 111.9 (C-5), 110.8 (C-8), 108.8 (C-1′), 106.1 (C-3), 29.54 (CH_3N). Anal. Calcd for $C_{17}H_{12}N_4$: C, 74.98; H, 4.44; N, 20.58. Found: C, 74.94; H, 4.47; N, 20.53; EI/MS *m/z* = 272.

4.2.1.8. 4-(9′-Ethyl-9*H*-imidazo[1,2-*a*]benzimidazol-2′-yl) benzonitrile (3i). According to the general procedure analogue **3i** was obtained from 1-ethyl-2-aminobenzimidazole **1e** and 2-Br-4′-CN-acetophenone **2a** as yellow solid (yield 20%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.80 (s, 1H, H-3), 8.05 (d, 2H, *J* = 8.4 Hz, H-3′), 7.99 (d, 2H, *J* = 8.4 Hz, H-2′), 7.98 (d, 1H, *J* = 8.2 Hz, H-5), 7.84 (d, 1H, *J* = 8.2 Hz, H-8), 7.53 (t, 1H, *J* = 7.8 Hz, H-7), 7.43 (t, 1H, *J* = 7.8 Hz, H-6), 4.46 (q, 2H, *J* = 7.2 Hz, CH_3CH_2N), 1.47 (t, 3H, *J* = 7.2 Hz, CH_3CH_2N); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 148.1 (C-9a), 136.0 (C-2 and C-4′), 135.7 (C-8a), 134.1 (C-2′), 126.5 (C-3′), 126.1 (C-7), 124.8 (C-4a), 123.0 (C-6), 119.9 (CN), 113.5 (C-5), 112.6 (C-8), 111.1 (C-1′), 108.0 (C-3), 38.90 (CH_3CH_2N), 14.59 (CH_3CH_2N). Anal. Calcd for $C_{18}H_{14}N_4$: C, 75.50; H, 4.93; N, 19.57. Found: C, 75.47; H, 4.98; N, 19.62; EI/MS *m/z* = 286.

4.2.2. Preparation of benzylamines 5a, 5e

4.2.2.1. 4-(Imidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine (5a). To a cooled at 0 °C mixture of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzonitrile **3a** (0.25 g, 0.90 mmol) in dry toluene (5 mL) was added 1 M diisobutylaluminum hydride solution in hexane (4.5 mL, 4.5 mmol) and the solution was refluxed for 2 h. Then, the solution was poured into ice-water (50 mL), an aqueous NaOH solution (1 N, 15 mL) was added and the organic layer was extracted with Et_2O (3 × 35 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, and evaporated in vacuum to dryness to provide **5a** as yellow solid (yield 90%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.75 (s, 1H, H-3), 8.03 (d, 1H, *J* = 8.0 Hz, H-5), 7.98 (d, 1H, *J* = 8.0 Hz, H-8), 7.83 (d, 2H, *J* = 8.1 Hz, H-3′), 7.57 (t, 1H, *J* = 8.0 Hz, H-6), 7.43 (t, 1H, *J* = 8.0 Hz, H-7), 7.41 (d, 2H, *J* = 8.1 Hz, H-2′), 3.78 (s, 2H, C*H*$_2$NH$_2$); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 147.9 (C-9a), 147.6 (C-2), 143.1 (C-1′), 133.4 (C-4′), 133.0 (C-4a), 130.3 (C-8a), 128.7 (C-2′), 127.8 (C-6), 126.2 (C-8), 126.1 (C-7), 126.0 (C-3′), 114.4 (C-5), 109.9 (C-3), 46.18 (CH_2NH_2). Anal. Calcd for $C_{16}H_{13}N_3S$: C, 68.79; H, 4.69; N, 15.04. Found: C, 68.73; H, 4.65; N, 15.09; EI/MS *m/z* = 279.

4.2.2.2. 4-(5′,6′,7′,8′-Tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine (5e). According to the general procedure benzylamine **5e** was obtained from 4-(5′,6′,7′,8′-tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzonitrile **3e** as yellow solid (yield 90%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.14 (s, 1H, H-3), 7.76 (d, 2H, *J* = 8.2 Hz, H-3′), 7.72 (d, 2H, *J* = 8.2 Hz, H-2′), 3.71 (s, 2H, C*H*$_2$NH$_2$), 2.68–2.66 (m, 4H, H-5 and H-8), 1.88–1.86 (m, 4H, H-6 and H-7); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 146.9 (C-9a), 145.7 (C-2), 142.9 (C-1′), 133.0 (C-4′), 127.7 (C-2′), 126.7 (C-4a), 124.8 (C-3′), 120.9 (C-8a), 107.6 (C-3), 45.76 (CH_2NH_2), 24.20 (C-5), 23.12 (C-7), 22.58 (C-8), 21.60 (C-6). Anal. Calcd for $C_{16}H_{17}N_3S$: C, 67.81; H, 6.05; N, 14.83. Found: C, 67.78; H, 6.10; N, 14.80; EI/MS *m/z* = 283.

4.2.3. Preparation of benzenamines 6b, 6f

4.2.3.1. 4-(Imidazo[2,1-*b*]benzothiazol-2′-yl) benzenamine (6b). To a solution of 2-(4′-nitrophenyl)imidazo[2,1-*b*]benzothiazole **3b** (2.0 g, 6.7 mmol) in ethanol (150 mL) were added iron powder (7.5 g, 1.34 mol), H_2O (30 mL) and H_2SO_4 12 N (750 mL) and the mixture was stirred at 90 °C for 1 h. The hot solution was filtered through Celite and washed with hot ethanol. The solvent was removed in vacuum and the crude precipitate was treated with $NaHCO_3$ (400 mL). The organic layer was extracted with CH_2Cl_2 (3 × 150 mL), dried with Na_2SO_4 and evaporated in vacuum to dryness to give **6b** as white solid (yield 89%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.52 (1H, s, H-3), 8.05 (1H, d, *J* = 8.7 Hz, H-5), 7.95 (1H, d, *J* = 8.7 Hz, H-8), 7.61–7.51 (3H, m, H-3′ and H-6), 7.40 (1H, t, *J* = 8.7 Hz, H-7), 6.65 (2H, d, *J* = 9.3 Hz, H-2′), 5.25 (2H, s, N*H*$_2$). Anal. Calcd for $C_{15}H_{11}N_3S$: C, 67.90; H, 4.18; N, 15.84. Found: C, 67.87; H, 4.22; N, 15.87.

4.2.3.2. 4-(5′,6′,7′,8′-Tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzenamine (6f). According to the general procedure benzenamine **6f** was obtained from 2-(4′-nitrophenyl)-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole **3f** as white solid (yield 89%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 7.87 (s, 1H, H-3), 7.50 (d, 2H, *J* = 8.3 Hz, H-3′), 6.58 (d, 2H, *J* = 8.3 Hz, H-2′), 5.12 (s, 2H, N*H*$_2$), 2.68–2.66 (m, 4H, H-5 and H-8), 1.89–1.87 (m, 4H, H-6 and H-7). Anal. Calcd for $C_{15}H_{15}N_3S$: C, 66.88; H, 5.61; N, 15.60. Found: C, 66.84; H, 5.65; N, 15.55.

4.2.4. Preparation of hydrochloride salts 5a·HCl, 5e·HCl, 6b·HCl, 6f·HCl

4.2.4.1. 4-(Imidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine hydrochloride salt (5a·HCl). A solution of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine **5a** (0.25 g, 0.89 mmol) and satd HCl in methanol (50 mL) was stirred for 1 h and then evaporated till dryness. The resulting precipitate was filtered and washed with DEE to provide **5a**·HCl as white solid (yield 95%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.03 (s, 1H, H-3), 8.13 (d, 1H, *J* = 8.0 Hz, H-5), 8.01 (d, 1H, *J* = 8.0 Hz, H-8), 7.94 (d, 2H, *J* = 8.1 Hz, H-3′), 7.66–7.61 (m, 3H, H-2′ and H-6), 7.51 (t, 1H, *J* = 8.0 Hz, H-7), 4.05 (q, 2H, *J* = 5.6 Hz, C*H*$_2$NH$_2$).

4.2.4.2. 4-(5′,6′,7′,8′-Tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine hydrochloride salt (5e·HCl). According to the procedure described above **5e**·HCl was obtained from **5e** as a white

solid (yield 95%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.59 (s, 1H, H-3), 7.95 (d, 2H, J = 8.3 Hz, H-3′), 7.62 (d, 2H, J = 8.3 Hz, H-2′), 4.06 (q, 2H, J = 5.8 Hz, CH_2NH$_2$), 2.77–2.76 (m, 4H, H-5 and H-8), 1.92–1.87 (m, 4H, H-6 and H-7).

4.2.4.3. 4-(Imidazo[2,1-*b*]benzothiazol-2′-yl) benzenamine hydrochloride salt (6b·HCl). According to the general procedure benzenamine hydrochloride salt **6b**·HCl was obtained from 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzenamine **6b** as white solid (yield 95%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.89 (1H, s, H-3), 8.08 (1H, d, J = 8.0 Hz, H-5), 8.04 (1H, d, J = 8.0 Hz, H-8), 7.96 (2H, d, J = 8.4 Hz, H-3′), 7.61 (1H, t, J = 7.8 Hz, H-6), 7.47 (1H, t, J = 7.8 Hz, H-7), 7.44 (2H, d, J = 8.4 Hz, H-2′).

4.2.4.4. 4-(5′,6′,7′,8′-Tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzenamine hydrochloride salt (6f·HCl). According to the general procedure benzenamine hydrochloride salt **6f**·HCl was obtained from 4-(5′,6′,7′,8′-tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzenamine **6f** as white solid (yield 95%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.61 (s, 1H, H-3), 7.94 (d, 2H, J = 8.2 Hz, H-3′), 7.38 (d, 2H, J = 8.2 Hz, H-2′), 2.78–2.76 (m, 4H, H-5 and H-8), 1.91–1.89 (m, 4H, H-6 and H-7).

4.2.5. Preparation of acetamides 7a, 7e

4.2.5.1. *N*-(4-(Imidazo[2,1-*b*]benzothiazol-2′-yl)benzyl) acetamide (7a). To a solution of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine **5a** (0.10 g, 0.36 mmol) in dry DMF (0.9 mL) dry Et_3N (0.33 mL, 2.4 mmol) was added and the solution was cooled to 0 °C. Then, acetyl chloride was added (0.16 mL, 2.2 mmol) and the solution was stirred overnight at rt. After the completion of the reaction EtOAc was added and the organic layer was washed with 5% NH_4Cl, water and brine, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (EtOAc) to provide **7a** as white solid (85% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.43 (s, 1H, H-3), 7.91 (d, 1H, J = 8.2 Hz, H-5), 7.88 (d, 1H, J = 8.2 Hz, H-8), 7.83 (d, 2H, J = 8.2 Hz, H-3′), 7.56 (t, 1H, J = 8.2 Hz, H-6), 7.44 (t, 1H, J = 8.2 Hz, H-7), 7.36 (d, 2H, J = 8.2 Hz, H-2′), 4.40 (s, 2H, CH_2NH), 2.03 (s, 3H, CH_3CO); ^{13}C NMR (CD_3OD-d_4, 400 MHz): δ = 172.0 (CO), 147.5 (C-9a), 147.1 (C-2), 138.1 (C-1′), 132.5 (C-4′), 132.4 (C-4a), 129.8 (C-8a), 127.7 (C-2′), 126.5 (C-6), 125.2 (C-7), 125.0 (C-3′), 124.3 (C-8), 113.0 (C-5), 108.0 (C-3), 42.61 (CH_2NH), 21.15 (CH_3CO). Anal. Calcd for $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07. Found: C, 67.23; H, 4.73; N, 13.12; EI/MS m/z = 321.

4.2.5.2. *N*-(4-(5′,6′,7′,8′-Tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl)benzyl) acetamide (7e). According to the general procedure acetamide **7e** was obtained from 4-(5′,6′,7′,8′-tetrahydroimidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine **5e** after purification by flash column chromatography (EtOAc) as yellow solid (85% yield). ^{1}H NMR (CD_3OD-d_4, 400 MHz): δ = 7.87 (s, 1H, H-3), 7.75 (d, 2H, J = 8.2 Hz, H-3′), 7.32 (d, 2H, J = 8.2 Hz, H-2′), 4.38 (s, 2H, CH_2NH), 2.72–2.70 (m, 4H, H-5 and H-8), 2.02 (s, 3H, CH_3CO), 1.96–1.94 (m, 4H, H-6 and H-7); ^{13}C NMR (CD_3OD-d_4, 400 MHz): δ = 172.3 (CO), 148.8 (C-9a), 146.4 (C-2), 138.3 (C-1′), 133.7 (C-4′), 128.2 (C-2′), 127.1 (C-4a), 125.5 (C-3′), 122.4 (C-8a), 107.1 (C-3), 43.30 (CH_2NH), 24.36 (C-5), 23.45 (C-6), 22.72 (C-8), 21.98 (C-7), 21.81 (CH_3CO). Anal. Calcd for $C_{18}H_{19}N_3OS$: C, 66.43; H, 5.88; N, 12.91. Found: C, 66.41; H, 5.84; N, 12.95; EI/MS m/z = 325.

4.2.6. Preparation of 3-(4′-(Imidazo[2,1-*b*]benzothiazol-2″-yl) benzylamino)-*N*-isopropylpropanamide (8)

A mixture of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzylamine **5a** (0.051 g, 0.18 mmol), and *N*-isopropylacrylamide (0.031 g, 0.27 mmol) in H_2O (1 mL) was refluxed for 16 h. After the completion of the reaction the organic layer was extracted repeatedly with EtOAc dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (EtOAc/MeOH/TEA 9:1:0.01) to provide **8** as yellow solid (yield 13%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.92 (s, 1H, H-3), 7.83 (d, 2H, J = 8.0 Hz, H-3′), 7.68 (d, 1H, J = 8.0 Hz, H-5), 7.60 (d, 1H, J = 8.0 Hz, H-8), 7.48–7.45 (m, 3H, H-2′ and H-6), 7.32 (t, 1H, J = 7.7 Hz, H-7), 4.06–3.90 (m, 3H, CH(CH$_3$)$_2$, CH_2NH), 3.06 (t, 2H, J = 6.0 Hz, CH_2CH$_2$CO), 2.61 (t, 2H, J = 6.0 Hz, CH$_2$CH_2CO), 1.15 (d, 6H, J = 6.0 Hz, CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 173.4 (CO), 150.9 (C-9a), 143.0 (C-2), 138.7 (C-1′), 136.1 (C-4′), 135.8 (C-4a), 129.6 (C-8a), 127.8 (C-2′), 126.8 (C-6), 125.3 (C-7), 124.4 (C-3′), 123.8 (C-8), 113.5 (C-5), 108.8 (C-3), 52.58 (CH_2NH), 47.86 (CH_2CH$_2$CO), 42.90 (CH(CH$_3$)$_2$), 35,74 (CH$_2$CH_2CO), 22.65 (CH(CH_3)$_2$). Anal. Calcd for $C_{22}H_{24}N_4OS$: C, 67.32; H, 6.16; N, 14.27. Found: C, 67.35; H, 6.11; N, 14.22; EI/MS m/z = 392.

4.2.7. Preparation of *N*-(4′-(imidazo[2,1-*b*]benzothiazol-2″-yl)benzyl)-3-aminopropanamide (9)

To a solution of *tert*-butyl (*N*-[3-oxo-3-(4′-(imidazo[2,1-*b*]benzothiazol-2″-yl) phenylmethylamino)propyl]) carbamate (0.050 mg, 0.11 mmol) in MeOH (3 mL) was added slowly TMSCl (0.077 mL, 0.50 mmol). After the completion of the reaction, $NaHCO_3$ was added and the organic layer was extracted twice with DCM. The combined organic layers were dried with Na_2SO_4, filtered, and evaporated in vacuum to dryness to provide **9** as yellow solid (yield 60%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.74 (s, 1H, H-3), 8.38 (m, 1H, NH), 8.04 (d, 1H, J = 8.0 Hz, H-5), 8.00 (d, 1H, J = 8.0 Hz, H-8), 7.84 (d, 2H, J = 8.2 Hz, H-3′), 7.58 (t, 1H, J = 7.3 Hz, H-6), 7.47 (t, 1H, J = 7.3 Hz, H-7), 7.34 (d, 2H, J = 8.2 Hz, H-2′), 4.32 (d, 2H, J = 5.6 Hz, CH_2NH), 2.83 (t, 2H, J = 6.6 Hz, COCH_2), 2.29 (t, 1H, J = 6.6 Hz, CH_2NH$_2$); ^{13}C NMR (CD_3OD-d_4, 400 MHz): δ = 172.0 (CO), 147.5 (C-9a), 146.7 (C-2), 139.2 (C-1′), 132.9 (C-4′), 132.3 (C-4a), 129.7 (C-8a), 128.1 (C-2′), 127.2 (C-6), 125.6 (C-7), 125.5 (C-3′), 125.1 (C-8), 113.8 (C-5), 109.4 (C-3), 42.30 (CH_2NH), 40.00 (CH_2NH_2), 38.90 (COCH_2). Anal. Calcd for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.15; H, 5.14; N, 15.95; EI/MS m/z = 350.

4.2.8. Preparation of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzoic acid (10)

A mixture of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzonitrile **3a** (0.50 g, 1.8 mmol) and NaOH (0.92 g, 23 mmol) in 1:1 EtOH–H_2O (20 mL) was heated under reflux for 4 h. The solvent was removed in vacuum and the residue was triturated with water (50 mL). The suspension was acidified with 10% aq HCl to pH 1 and stirred for 2 h at room temperature. The precipitate was filtered off, washed with water (100 mL) and DEE (100 mL), and dried over P_2O_5 atmosphere to afford **10** as white solid (yield 96%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.94 (s, 1H, H-3), 8.05 (d, 1H, J = 8.0 Hz, H-8), 8.02 (d, 2H, J = 8.6 Hz, H-2′), 8.01–7.98 (m, 3H, H-3′ and H-5), 7.59 (t, 1H, J = 7.8 Hz, H-6), 7.45 (t, 1H, J = 7.8 Hz, H-7); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 168.2 (COOH),148.6 (C-9a), 146.4 (C-2), 139.1 (C-4′), 132.8 (C-4a), 131.0 (C-2′), 130.4 (C-1′ and C-8a), 127.9 (C-6), 126.5 (C-7), 126.2 (C-8), 125.7 (C-3′), 114.6 (C-5), 111.7 (C-3). Anal. Calcd for $C_{16}H_{10}N_2O_2S$: C, 65.29; H, 3.42; N, 9.52. Found: C, 65.28; H, 3.40; N, 9.55; EI/MS m/z = 294.

4.2.9. Preparation of methyl 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzoate (11)

To a mixture of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzoic acid **10** (0.50 g, 1.7 mmol) in MeOH (16 mL), a 2 M solution of trimethylsilyldiazomethane in DEE (4 mL, 8.0 mmol) was added and the solution was stirred for 30 min. Then, the solvent was removed in vacuum, EtOAc was added and the organic layer was washed with water and brine, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (Hex/

EtOAc 4:6) to provide **11** as white solid (70% yield based on the recovered starting material). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.94 (s, 1H, H-3), 8.04 (d, 1H, J = 8.0 Hz, H-8), 8.02–7.98 (m, 5H, H-2′, H-3′ and H-5), 7.58 (t, 1H, J = 7.8 Hz, H-6), 7.44 (t, 1H, J = 7.8 Hz, H-7), 3.86 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 167.1 (C$OOCH_3$),146.4 (C-9a), 146.2 (C-2), 139.5 (C-4′), 132.8 (C-4a), 131.0 (C-2′), 130.6 (C-8a), 129.1 (C-1′), 127.9 (C-6), 126.6 (C-7), 126.2 (C-8), 125.7 (C-3′), 114.6 (C-5), 112.0 (C-3), 55.16 (COOCH_3). Anal. Calcd for $C_{17}H_{12}N_2O_2S$: C, 66.22; H, 3.92; N, 9.08. Found: C, 66.17; H, 3.87; N, 9.04; EI/MS m/z = 308.

4.2.10. Preparation of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzyl alcohol (12)

To a cooled at 0 °C solution of benzoic acid 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) methyl ester **11** (0.25 g, 0.82 mmol) in THF (29 mL), $LiAlH_4$ (0.021 g, 0.53 mmol) was added and the solution was stirred for 30 min at 0 °C. Then, EtOAc was added and the organic layer was washed with HCl 1 N, water and brine, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 4:6) to provide **12** as white solid (70% yield based on the recovered starting material). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.77 (s, 1H, H-3), 8.04 (d, 1H, J = 8.1 Hz, H-8), 7.99 (d, 1H, J = 8.1 Hz, H-5), 7.84 (d, 2H, J = 8.2 Hz, H-3′), 7.57 (t, 1H, J = 8.1 Hz, H-6), 7.43 (t, 1H, J = 8.1 Hz, H-7), 7.39 (d, 2H, J = 8.2 Hz, H-2′), 5.23 (t, 1H, J = 5.1 Hz, CH_2OH) 4.53 (d, 2H, J = 5.1 Hz, CH_2OH); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 147.9 (C-9a), 147.5 (C-2), 142.7 (C-1′), 133.5 (C-4′), 133.0 (C-4a), 130.3 (C-8a), 128.0 (C-2′), 127.8 (C-6), 126.2 (C-7), 126.1 (C-8), 125.6 (C-3′), 114.4 (C-5), 109.9 (C-3), 63.90 (CH_2OH). Anal. Calcd for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99. Found: C, 68.58; H, 4.35; N, 9.95; EI/MS m/z = 280.

4.2.11. Preparation of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzaldehyde (13)

To a solution of 4-(imidazo[2,1-*b*]benzothiazol-2′-yl) benzyl alcohol **12** (0.080 g, 0.28 mmol) in DCM (8 mL), Dess–Martin periodinane (0.16 g, 0.36 mmol) was added and the mixture was stirred for 20 min. The solvent was evaporated and the residue was purified by flash column chromatography (Hex/EtOAc 7:3) to provide **13** as white solid (98% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.0 (s, 1H, CHO), 8.98 (s, 1H, H-3), 8.01 (d, 2H, J = 8.3 Hz, H-3′), 8.00 (d, 1H, J = 8.0 Hz, H-8), 7.99 (d, 1H, J = 8.0 Hz, H-5), 7.96 (d, 2H, J = 8.3 Hz, H-2′), 7.59 (t, 1H, J = 8.0 Hz, H-6), 7.45 (t, 1H, J = 8.0 Hz, H-7); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 193.4 (CHO), 148.8 (C-9a), 146.2 (C-2), 140.7 (C-4′), 136.0 (C-1′), 132.8 (C-4a), 131.3 (C-2′), 130.5 (C-8a), 127.9 (C-6), 126.6 (C-7), 126.2 (C-8), 126.1 (C-3′), 114.6 (C-5), 112.4 (C-3). Anal. Calcd for $C_{16}H_{10}N_2OS$: C, 69.04; H, 3.62; N, 10.06. Found: C, 69.01; H, 3.67; N, 10.01; EI/MS m/z = 278.

4.2.12. Preparation of 2-(4″-methoxybiphenyl-4′-yl) imidazo[2,1-*b*]benzothiazole (14)

To a solution of 2-(4′-bromophenyl)imidazo[2,1-*b*]benzothiazole **3c** (0.050 g, 0.15 mmol) in THF (6 mL) under nitrogen, a solution of 4-methoxyphenylboronic acid (0.20 g, 1.4 mmol) in EtOH and a solution of Na_2CO_3 (0.20 g, 2.0 mmol) in H_2O were added. Then, $Pd(PPh_3)_4$ (cat. amount) was added and the mixture was put under reflux for 12 h. After the completion of the reaction EtOAc (10 mL) and H_2O (10 mL) were added and the mixture was filtered by Celite and washed with EtOAc. The organic layer was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 9:1) to provide **14** as yellow solid (18% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.11 (s, 1H, H-3), 7.95 (d, 1H, J = 8.2 Hz, H-5), 7.77 (d, 1H, J = 8.2 Hz, H-8), 7.62 (d, 2H, J = 8.2 Hz, H-3′), 7.58–7.54 (m, 4H, H-2′, PhH_o–OCH_3), 7.51–7.40 (m, 2H, H-6 and H-7), 7.02 (d, 2H, J = 8.8 Hz, PhH_m–OCH_3), 3.89 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 160.1 (PhC–OCH_3), 149.0 (C-9a), 147.5 (C-2), 141.5 (C1′ and C-4′), 134.4 (PhC-C1′), 132.5 (C-4a), 130.7 (C-8a), 128.6 (PhC_o–OCH_3), 127.7 (C-2′ and C-3′), 127.3 (C-6), 126.4 (C-7), 125.2 (C-8), 114.9 (PhC_m–OCH_3), 113.7 (C-5), 108.7 (C-3), 56.00 (OCH_3). Anal. Calcd for $C_{22}H_{16}N_2OS$: C, 74.13; H, 4.52; N, 7.86. Found: C, 74.18; H, 4.57; N, 7.80; EI/MS m/z = 356.

4.3. Cellular sensitivity to drugs

In ovarian carcinoma IGROV-1 cells, cellular sensitivity to drugs was evaluated by growth-inhibition assay after 72-h drug exposure. Cells in the logarithmic phase of growth were seeded in duplicate into 12-well plates. In the combined treatment with paclitaxel, cells were exposed to PFT 2 h before treatment with antimicrotubule agents. The highest final concentration of DMSO in culture medium was 0.5%. After treatment, adherent cells were trypsinized and counted by a cell counter (Beckam Coulter, Fullerton, CA). IC_{50} is defined as the drug concentration causing a 50% reduction of cell number compared with that of untreated control.

4.4. Determination of apoptosis

Apoptosis was determined in ovarian carcinoma IGROV-1 cells by TUNEL assay following 72 h-exposure to the selected compounds, PTX alone or in combination. Treated cells were fixed in 4% paraformaldehyde, for 60 min, at room temperature, washed, and resuspended in ice-cold PBS. The in situ cell death detection kit fluorescein (Roche, Mannheim, Germany) was used according to the manufacturers' instructions and the samples were analyzed by flow cytometry (Becton-Dickinson, Franklin Lakes, NJ).

4.5. Western blot analysis

Cells were treated as previously described for the indicated times with paclitaxel (PTX) or selected compounds alone or in combination at a cytotoxic concentration corresponding to IC_{50}. Cells were collected and lysed as previously described.[8]

4.6. Tumor models and evaluation of antitumor activity

The experiments were performed using female athymic Swiss nude mice. Mice were maintained in laminar flow rooms keeping temperature and humidity constant. Mice had free access to food and water. Experiments were approved by the Ethics Committee for Animal Experimentation of the Istituto Nazionale Tumori of Milan according to institutional guidelines.

Exponentially growing tumor cells (10^7 cells/mouse) were sc injected into the right flank of athymic nude mice. Tumor lines were achieved by serial sc passages of fragments (about $3 \times 3 \times 3$ mm) from growing tumors. Animals were treated iv at the indicated doses, every four days for 4 administrations starting when tumors were measurable (around 80 mg). Compounds were dissolved in DMSO and diluted in PBS containing 5% Cremophor to a final concentration of 10% DMSO.

Acknowledgments

This research has been developed under the umbrella of CM0602 COST Action 'Inhibitors of Angiogenesis: design, synthesis, and biological exploitation'. This study was supported by the Associazione Ricerche sul Cancro and Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) PRIN 2007—Program 'Sviluppo e caratterizzazione di nuovi inibitori di tirosine chinasi cellulari con attività antiproliferativa e antiangiogenica nei confronti di differenti tumori'.

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