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European Journal of Medicinal Chemistry 41 (2006) 675–682
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Preliminary communication

Isothiazoles. Part XV. A mild and efficient synthesis of new antiproliferative 5-sulfanylsubstituted 3-alkylaminoisothiazole 1,1-dioxides

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Accepted 12 January 2006
Available online 15 March 2006

Abstract

5-Sulfanyl-3-alkylaminoisothiazole dioxide derivatives have been identified as a new class of potent inhibitors of rat aortic myocite proliferation. They were prepared by applying a simple methodology able to introduce a heteroatom on C-5 of the 3-alkylaminoisothiazole dioxide system. 3-Aminosubstituted-5-chloroisothiazole dioxides react smoothly not only with S-nucleophiles but also with N- and O-nucleophiles affording the corresponding 5-heterosubstituted isothiazole dioxides through an addition–elimination reaction. The behavior of 3-alkylamino-4-bromo-isothiazole 1,1-dioxide with S-, N- and O-nucleophiles affording the same products has also been described. On the contrary, the 3-amino-4,5-unsubstituted isothiazole dioxide system reacts easily only with sulfur nucleophiles affording the corresponding 4,5-dihydro-5-sulfanylderivatives through a simple Michael addition reaction.

Keywords: Farnesyl transferase; Smooth muscle cell; Isothiazoles; Nucleophilic addition

1. Introduction

We have previously shown that some 3-aminosubstituted isothiazole dioxide derivatives are effective inhibitors of *Tripanosoma brucei* protein farnesyltransferase (PFTase) in vitro and that by changing the substituents on C-5 it is possible to dramatically improve the inhibitory action of isothiazole dioxides versus mammalian PFTase compared to *T. brucei* PFTase [1,2]. In this previous study, compounds were tested on rat PFTase in vitro and in cultured cells, in relationship to rat smooth muscle cells (SMC) proliferation. The most effective compound on PFTase was shown to interfere with rat SMC proliferation by blocking cell cycle progression in G0/G1 phase. The concentration producing 50% inhibition of cell proliferation was 61.4 μM similar to those required to inhibit rat PFTase activity and [^{3}H]-farnesol incorporation into cellular proteins suggesting a direct interrelationship between these events. Depending on the substitution on C-5, compounds synthesized can be divided into two classes:

- compounds having an alkyl-, aryl or heteroarylsubstituent directly linked to the C-5 of the isothiazole nucleus (Fig. 1, class A);
- compounds having a sulfur atom acting as a linker between the isothiazole moiety and the substituents (Fig. 1, class B).

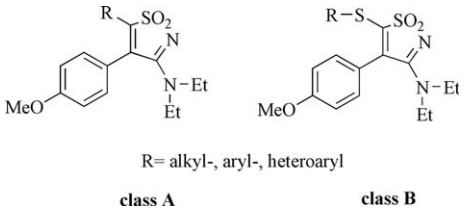

Fig. 1.

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doi:10.1016/j.ejmech.2006.01.009

Apparently, the substitution on C-5 with different substituents strongly alters the antagonist activity on PFTase. Interestingly, the presence of a sulfur atom as a linker between the isothiazole moiety and the substituents significantly increases the inhibitory activity on PFTase [2]. The scope of the present work was to obtain new compounds with high efficacy and specificity and try to better understand structure/activity relationships. With this aim we designed a series of derivatives:

- characterized by the presence of a secondary amino group on C-3 which could act not only as an acceptor but also as a donor in an hydrogen bond;
- characterized by the lack of the 4-methoxyphenyl group on C-4;
- moreover, to better clarify the importance of the planarity of the isothiazole ring, we planned the synthesis of either 4,5-dihydro-5-sulfanylisothiazole dioxides (class C, Fig. 2) or 5-sulfanylderivatives in which the C-4 C-5 double bond was conserved (class D, Fig. 2).

On these basis, we planned a synthetical pathway taking advantage of 3-alkylaminoisothiazole 1,1-dioxides **1** and 3-alkylamino-5-chloro-isothiazole 1,1-dioxide **4** as the key starting materials. It is worth noting that this method can be considered generally valid allowing the functionalization at C-5 with different heteroatoms (not only sulfur but also nitrogen and oxygen) producing several class of 5-heterosubstituted 3-alkylaminoisothiazole dioxides.

2. Chemistry

3-Alkylamino-isothiazole dioxides **1a**–**d** were reacted with an equimolecular amount of methylthiolate **2a** or of the mercaptans **2b**, **c** in presence of a catalytic amount of base (TEA) in acetonitrile as the solvent affording in satisfactory yield (40–92%) compounds **3a**–**i** whose structures were confirmed by analytical and spectroscopic data (Scheme 1). The regiochemistry of the reaction was confirmed by n.O.e. experiments. The regioselectivity of the reaction confirms the electrophilic character of C-5 evidencing that the system could be classified, from a formal point of view, as a cyclic unsaturated *N*-sulfonylamidine rather than a cyclic α,β-unsaturated sulfone. We tried also to generalize the reaction performing the nucleophilic addition with O- and N-nucleophiles but with unsatisfactory results. Alcohols do not react with **1a** even in presence of bases such as K_2CO_3 both catalytic and equimolecular. By using alkoxides, which are stronger bases, a mixture of unseparable products were obtained very likely through nucleophilic addition followed by ring opening. Starting from the 4,5-dihydro-5-sulfanylderivatives, attempts were made for their transformation into the corresponding 5-sulfanylderivatives containing the C-4–C-5 double bond. The use of the most common oxidants (e.g. DDQ, $KMnO_4$, NiO_2) failed in this process. This is not surprising taking into account that the oxidized sulfur atom provokes a lowering in the aromatic character of the isothiazole ring [3]. To overcome this problem, we considered the possibility to perform a nucleophilic addition–elimination reaction on 5-halogeno derivatives **4** [4]. In a typical reaction 5-chloroderivatives **4a**–**c** were reacted with equimolecular amount of **2a**–**d** in CH_3CN at room temperature, affording pure **5a**–**g** as the sole reaction products (Scheme 2). As an alternative approach to the 5-sulfanylderivatives, the alkylation of mercaptoisothiazole **6** was considered. By reacting **4a** with sodium hydrogen sulfide in MeOH as the solvent, compound **6** was successfully formed but it could be isolated in low yield owing to its rapid oxidation. Taking into consideration this problem, a one pot reaction was developed starting from the 5-chloroderivative **4a**, sodium hydrogen sulfide **7** and the appropriate alkylbromide **8** in methanol as the solvent. The validity of this method was proved by using benzylbromide as the alkylant affording compound **5h** in satisfactory yield (Scheme 3). This second approach appears to be very convenient when mercap-

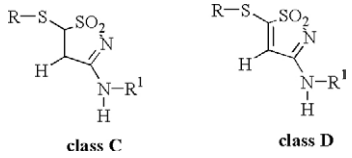
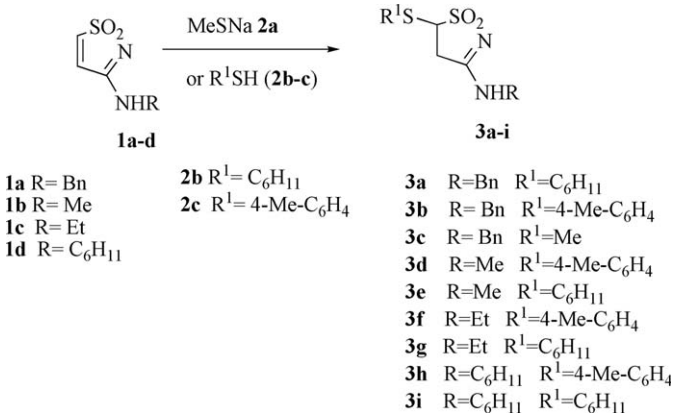
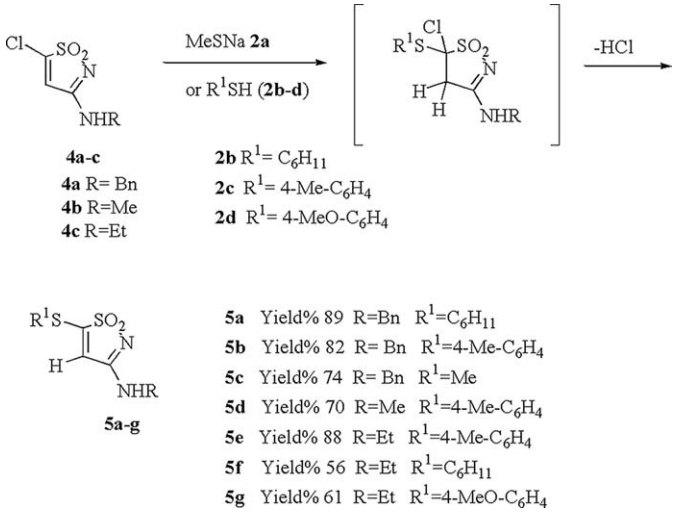


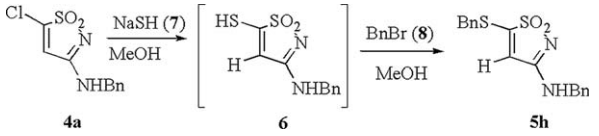
Fig. 2.



Scheme 1.



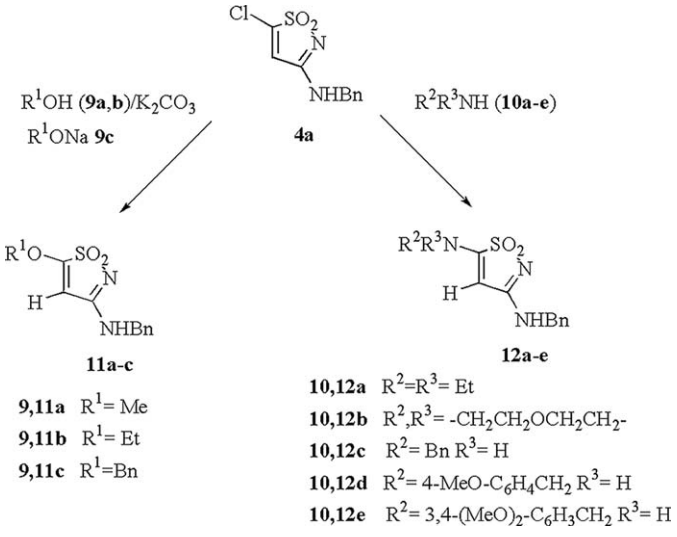
Scheme 2.



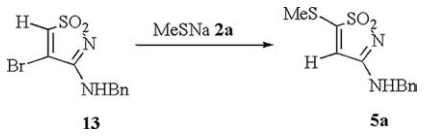
Scheme 3.

tan derivatives are not available or difficult to synthesize. Considering the positive results we thought it was worth evaluating other nucleophiles in order to generalize the reaction apart from the pharmacological interest of the products. The 5-chloroderivative **4a** was successfully reacted also with O- and N-nucleophiles affording the addition–elimination product in satisfactory yield (Scheme 4).

These results show that **4a** is a very reactive key intermediate due to the presence of the halogen on C-5. Basing on this consideration we verified the possibility to produce 4-substituted derivatives by applying the same addition–elimination procedure to the 4-bromoderivative **13**. The presence of a halogen on C-4 could in principle increase the electrophilic character of C-4 so favoring a nucleophilic attack. Compound **13** was reacted with **2a** in CH_3CN affording, as the sole reaction product, compound **5a** (Scheme 5). To confirm this result, **13** was reacted with other sulfur, oxygen and nitrogen nucleophiles showing always the same reaction pattern and the same regiochemistry. The reaction with sulfur and oxygen nucleophiles afforded the same regioisomers but in lower yield. The reaction with nitrogen nucleophiles afforded only a mixture of unseparable compounds. On the whole the reactivity toward nucleophiles of 4-bromoderivative **13** appeared to be less satisfactory in comparison with 5-chloroderivative. In order to explain the behavior of **13** and the total selectivity of the reaction we investigated on such reactions by way of ab initio, MP2 and DFT calculations. Computational results suggest a mechanism proceeding through an addition elimination nucleophilic reaction evolving through a [1,5] sigmatropic hydrogen shift [5].



Scheme 4.



Scheme 5.

3. Results and discussion

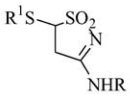
As said above, in the previous studies we observed that the most effective compounds on PFTase was shown to interfere with rat SMC proliferation at similar concentrations, suggesting a direct interrelationship between these events. To begin to test the potential activity of our compounds, we evaluated their antiproliferative effect on rat SMC. According to their chemical structures compounds synthesized and tested can be divided into five groups (series **1**, **3**, **4**, **5** and compound **13**) which, as expected, show different antiproliferative activity. To study the effect of sulfanylsubstituent on C-5, we directly compared compounds **1** (**1a**, **b** and **c**) and the corresponding compounds **5** (**5b**, **d**, **e** and **g**) which possess this substituent.

To evaluate the value of the planarity of the isothiazole ring, we tested compounds **3** (**3b**, **3d**, **3e**, **3f** and **3g**); moreover taking into account their availability, we decided to test compounds **4** (**4a**–**c**) and **13** too, in order to evaluate the relevance of the lipophilic halogens. Compounds tested and their activity are shown in Table 1. Compounds **3** (**3b**, **3d**, **3e**, **3f** and **3g**) did not show a very potent inhibitory activity on rat SMC proliferation, with compound **3b** and **3f** having, respectively, IC_{50} values equal to 62.7 and 70.5 μM, and compound **3d**, **3e** and **3g** that did not show any significant effect, even at 100 μM concentration (Table 1). These results confirm the statement that the saturation of the C-4 C-5 double bond has a detrimental effect on the antiproliferative activity of these compounds [2]. Infact, compound **1**, conserving the C-4 C-5 double bond (**1a**, **1b** and **1c**) elicits a significant antiproliferative effect with intermediate potency. Compounds **5** (**5b**, **d**, **e**, **g**) show the most potent inhibitory activity compared to the other series tested, with a reproducible dose dependent inhibitory effect (Fig. 3).

In particular compound **5d**, **5e** and **5g** inhibit rat SMC growth with IC_{50} values equal to 0.6, 0.58 and 0.21 μM, respectively. These compounds are characterized by the presence of the sulfur atom as linker between the planar isothiazole ring and the substituent confirming, also in this new class of inhibitors, the positive effect elicited by the linker. A direct comparison between compounds **5b** and **d**, **e**, **g** suggests that the presence of a sterically demanding group on C-3 is responsible for a significant reduction of the inhibitory activity on cell proliferation. Indeed, the substitution of the benzylamino group on C-3 with a smaller methylamino or ethylamino group ameliorates the inhibitory action on cell proliferation. Interestingly, compound **5g**, having substituted the phenylsulfanyl group with a more electrondonating group (MeO, compound **5g**) shows the most potent effect. Finally, compounds **4a**–**c** exert their antiproliferative action in a very small concentration

Table 1
Antiproliferative effect on rat SMC (IC_{50}) and % inhibition of rat PFTase and rat PGGTase-I by isothiazole dioxide

1



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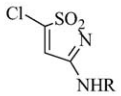

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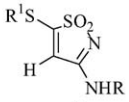

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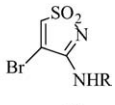

13

Compounds		**1a**	**1b**	**1c**	**3b**	**3d**	**3e**	**3f**	**3g**	**4a**	**4b**	**4c**	**5b**	**5d**	**5e**	**5g**	**13**
Proliferation	IC_{50} (μM)	12.3	9.0	8.4	62.7	N.A	N.A	70.5	N.A	2.5	N.D.	N.D.	2.9	0.6	0.58	0.21	1.28
PFTase	Percent inhibition (100 μM)	25	5.6	5.3	17.4	N.E.	N.E.	8.7	N.E.	46.2	N.E.	N.E.	0	8.4	42.7	11.2	50.2
PGGTase-I	Percent inhibition (100 μM)	35.8	50.1	26.1	50.2	N.E.	N.E.	32.8	N.E.	39.6	N.E.	N.E.	16.2	27.1	43.6	15.6	38.3

N.A. = not active; N.D. = not determinable; N.E. = not evaluated.

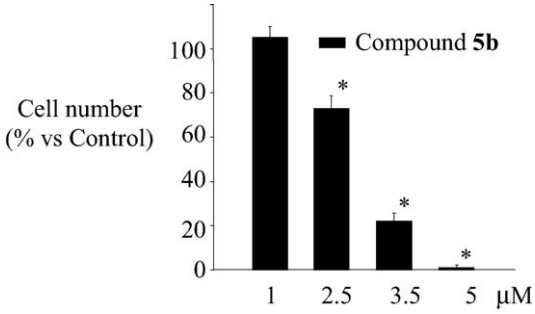

Fig. 3. Antiproliferative effect of compound **5b** on rat SMC.

range without a significant dose–response effect. It was therefore impossible to determine their IC_{50} values, the only exemption was the compound **4a** that inhibits rat SMC proliferation in a dose dependent manner with an IC_{50} equal to 2.5 μM. Compound **13**, which exhibits a bromine atom on C-4, elicits a significant and potent inhibitory action with an IC_{50} of 1.28 μM. Interestingly, compounds **4a**, **5e**, and **13** show a significant inhibitory effect on PFTase enzyme in vitro leading to 46.2, 42.7 and 50.2% inhibition of the enzymatic activity at 100 μM concentration (Table 1). Similar inhibitory effect was also observed on PGGTase-I at the same concentration (Table 1). All these compounds belong to the three groups that most potently inhibit rat SMC proliferation (Table 1). In addition, compounds **1** and **3**, which demonstrate a 10-fold lower antiproliferative effect than compound **4** and almost 100-fold than compound **5**, did not inhibit PFTase in vitro. Nevertheless, compounds **1** and **3** significantly affect PGGTase-I activity. In particular **1b** and **3b** reduce by 50.1, and 50.2%, respectively, the enzyme activity at 100 μM (Table 1). PGGTase inhibitors have been previously shown to block SMC growth in vitro [6,7], it is therefore conceivable that the observed antiproliferative effect shown by compounds **1** and **3** may be partially dependent from their action on PGGTase-I. However, some contradictory observations derive from compounds **5b**, **d**, and **g**, which did not have a significant inhibitory effect on PFTase and PGGTase-I but very potently affect cell proliferation.

4. Conclusions

In conclusion, a straightforward method to obtain 5-hetero-substituted 3-alkylaminoisothiazole 1,1-dioxides starting from 3-alkylamino-5-chloro-isothiazole 1,1-dioxides was developed. The corresponding 4,5-dihydroderivatives could be obtained in satisfactory yield only with sulfur nucleophiles. By this way a new class of 5-sulfanyl-3-alkylaminoisothiazole 1,1-dioxides is available which was tested for antiproliferative activity. These compounds are potent inhibitors of rat SMC proliferation, and pharmacological results confirm the hypothesis that the activity is affected by two principal factors such as the planarity of the isothiazole ring and the nature of the 5-position substituent. In vitro testing on mammalian PFTase and PGGTase-I activities demonstrated a significant, although not potent, inhibitory action of isothiazole dioxides on these enzymes. The lack of correlation between the antiproliferative action and the inhibitory effect on either PFTase or PGGTase-I in vitro, especially for compound **5**, led to conclusion that other molecular mechanisms may also account for the antiproliferative action of these classes of isothiazole dioxides.

5. Experimental

^{1}H-NMR spectra were obtained in $CDCl_3$ as the solvents (except when indicated) with Bruker AC 200, Bruker Avance 300 and Varian Gemini 200 instruments. Melting points were determined using a Büchi 510 (capillary) or an Electrothermal 9100 apparatus. IR spectra (Nujol mull) were recorded on a Jasco IR report 100 spectrophotometer. Mass spectra were obtained by electron impact ionization at 70 eV from a Finnigan MD 800 or INCOS 50 instruments using the direct exposure probe (DEP). Analyses were within ± 0.4% of the theoretical values. Physical and spectroscopic data for all new compounds are reported in Tables 2 and 3.

*5.1. General procedure for the preparation of compounds **3a–i***

Compounds **1a–d** [4] (0.5 mmol) was dissolved in MeCN (3 ml) and MeSNa (**2a**) (0.5 mmol) or the thiole **2b**, **c**

Table 2
Physical data for new compounds

Numbers	M.p. (°C)	Yield (%)	Anal (C,H,N)	Numbers	M.p. (°C)	Yield (%)	Anal (C,H,N)
3a	164 (Et_2O, white crystals)	80	$C_{16}H_{22}N_2O_2S_2$	**5d**	171 (iPr_2O, white powder)	70	$C_{11}H_{12}N_2O_2S_2$
3b	150–151(Et_2O, white crystals)	87	$C_{17}H_{18}N_2O_2S_2$	**5e**	149–152 (Et_2O, white powder)	88	$C_{12}H_{14}N_2O_2S_2$
3c	139 (Et_2O, white powder)	40	$C_{11}H_{14}N_2O_2S_2$	**5f**	130–133 (Et_2O, white powder)	56	$C_{11}H_{18}N_2O_2S_2$
3d	152–155 (iPr_2O, white crystals)	87	$C_{11}H_{14}N_2O_2S_2$	**5g**	150–153 (Et_2O, white powder)	61	$C_{12}H_{14}N_2O_3S_2$
3e	146–147 (iPr_2O, white powder)	78	$C_{10}H_{18}N_2O_2S_2$	**5h**	142 Dec. (iPr_2O, white powder)	60	$C_{17}H_{16}N_2O_2S_2$
3f	167–169 (iPr_2O, white powder)	92	$C_{12}H_{16}N_2O_2S_2$	**11a**	M.p. uncrystallizable oil	69	$C_{11}H_{12}N_2O_3S$
3g	155–156(iPr_2O, white powder)	84	$C_{11}H_{20}N_2O_2S_2$	**11b**	M.p. uncrystallizable oil	58	$C_{12}H_{14}N_2O_3S$
3h	194–196dec. (iPr_2O, white powder)	87	$C_{16}H_{22}N_2O_2S_2$	**11c**	184 (Et_2O, white powder)	30	$C_{17}H_{16}N_2O_3S$
3i	199–201 (iPr_2O, white powder)	91	$C_{15}H_{26}N_2O_2S_2$	**12a**	108 (Et_2O, white powder)	84	$C_{14}H_{19}N_3O_2S$
5a	173 (Et_2O, white powder)	89	$C_{16}H_{20}N_2O_2S_2$	**12b**	198 (Et_2O, white powder)	95	$C_{14}H_{17}N_3O_3S$
5b	184 Dec. (Et_2O, white powder)	82	$C_{17}H_{16}N_2O_2S_2$	**12c**	155 (Et_2O, white powder)	86	$C_{17}H_{17}N_3O_3S$
5c	149 (*i*PrOH, white powder)	74	$C_{11}H_{12}N_2O_2S_2$	**12d**	159 (Et_2O, white powder)	74	$C_{18}H_{19}N_3O_3S$
				12e	152 Dec. (Et_2O/iPr_2O, white powder)	93	$C_{19}H_{21}N_3O_4S$

(0.5 mmol) in presence of a catalytic amount of TEA was added under stirring at room temperature. At the end of the reaction (4–20 h; TLC acOEt/cyclohexane 1:1) the solvent was evaporated i.v. and the residue taken up with CH_2Cl_2, washed with diluted HCl (2 × 5 ml) and with water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compounds **3a**–**i** were obtained by direct crystallization of the residue with the solvent indicated or through purification by column chromatography (acOEt/cyclohexane 0:100 to 100:0) followed by crystallization.

5.2. General procedure for the preparation of compounds *5a–h*

Compound **4** (0.5 mmol) was dissolved in MeCN (3 ml) and MeSNa (**2a**) (0.5 mmol) or the thiole **2b**–**d** (0.5 mmol) in presence of a catalytic amount of TEA was added under stirring at room temperature. At the end of the reaction (12–48 h; TLC acOEt/cyclohexane 1:1) the solvent was evaporated i.v. and the residue taken up with CH_2Cl_2 and washed with water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compounds **5a**–**h** were obtained by direct crystallization of the residue with the solvent indicated or through purification by column chromatography (acOEt/cyclohexane 0:100 to 100:0) followed by crystallization.

5.3. One pot reaction of *4* and *13* to 3-benzylamino-5-benzylsulfanyl-isothiazole 1,1-dioxide (*5h*)

Compound **4** (70 mg, 0.27 mmol) was dissolved in MeOH (5 ml) and finely powdered NaSH (40 mg, 0.54 mmol) was added under vigorous stirring. After 20 min TLC evidences the disappearance of the starting material **4**. Pure benzyl bromide **8** (46.18 mg, 0.27 mmol) was added and stirring continued until the end of the reaction (24 h, TLC acOEt/cyclohexane 1:1). The solvent evaporated under reduced pressure and the residue taken up with CH_2Cl_2 and washed with water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compound **5h** was obtained after purification by column chromatography (acOEt/cyclohexane 0:100 to 100:0) followed by crystallization.

5.4. General procedure for the preparation of compounds *11a–b*

Compound **4** (50 mg, 0.20 mmol) was dissolved in MeOH (6 ml for **11a**) or EtOH (6 ml for **11b**) and finely powdered anhydrous K_2CO_3 (28 mg, 0.2 mmol) was added under vigorous stirring. At the end of the reaction (24 h, TLC acOEt/cyclohexane 1:1) the solvent was evaporated under reduced pressure and the residue taken up with CH_2Cl_2 and washed with diluted HCl (2 × 5 ml) and water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compounds **11a**, **b** were obtained after purification by column chromatography (acOEt/cyclohexane 0:100 to 100:0).

5.5. 3-Benzylamino-5-benzyloxy-isothiazole 1,1-dioxide (*11c*)

Compound **4** (50 mg, 0.20 mmol) was dissolved in THF (6 ml) and $PhCH_2ONa$ (0.20 mmol) was added under vigorous stirring. The solution turned deep red. At the end of the reaction (24 h, TLC acOEt/cyclohexane 1:1) the solvent was evaporated under reduced pressure and then residue taken up with CH_2Cl_2 and washed with diluted HCl (2 × 5 ml) and water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compounds **11c** was obtained after purification by column chromatography (acOEt/cyclohexane 0:100 to 100:0) followed by crystallization.

5.6. General procedure for the preparation of compounds *12a–e*

Compound **4** (0.27 mmol) was dissolved in MeCN (3 ml) and the opportune amine **10a**–**e** (0.54 mmol) was added under stirring at room temperature. At the end of the reaction (1–48 h; TLC acOEt/cyclohexane 1:1) the solvent was evaporated

Table 3
Spectral data for new compounds

Numbers	^{1}H-NMR (δ ppm, CD_3COCD_3 except when indicated)	IR (cm^{-1} NH)	^{13}C-NMR (δ ppm, CD_3COCD_3 except when indicated)
3a	($CDCl_3$) 1.35–1.77 (m, 10H, CH_2), 2.90 (dd, 1H, H-4, *J* 8, 17 Hz), 3.11–3.14 (m, 1H, CH), 3.64 (dd, 1H, H-4, *J* 8, 17 Hz), 4.52–4.61 (m, 3H, H-5 and CH_2Ph), 7.33–7.40 (m, 5H, arylH), 7.88 (bs, 1H, NH)	3306	($CDCl_3$) 25.81, 25.96, 26.02, 33.68, 33.76 (CH_2), 38.68 (C-4) 44.67 (CH), 46.67 (CH_2Ph), 58.75 (C-5), 127.90, 128.32, 128.93 (ArCH), 137.81 (ArC), 166.06 (C-3)
3b	2.33 (s, 3H, CH_3), 3.02 (dd, 1H, H-4, *J* 6.8, 17.1 Hz), 3.70 (dd, 1H, H-4′, *J* 8.8, 17.1 Hz), 4.50 (d, 2H, CH_2; *J* 5.9 Hz), 4.76 (dd, 1H, H-5, *J* 6.8, 8.8 Hz), 7.19–7.54 (m, 9H, ArylH), 7.87 (bs, 1H, NH)	3300	(DMSO-d_6) 21.40 (CH_3), 38.08 (C-4), 46.55 (CH_2Ph), 62.17 (C-5), 128.16, 128.43, 129.23, 130.65, 132.63 (ArCH), 129.07, 137.91, 138.55 (ArC), 166.21 (C-3).
3c	2.34 (s, 3H, CH_3), 2.98 (dd, 1H, H-4, *J* 7, 17.2 Hz), 3.65 (dd, 1H, H-4′, *J* 8.8, 17.2 Hz), 4.43 (dd, 1H, H-5, *J* 7, 8.8 Hz), 4.58 (d, 2H, CH_2Ph, *J* 5.9 Hz), 7.29–7.43 (m, 5H, ArylH), 7.93 (bs, 1H, NH)	3320	($CDCl_3$) 13.31 (CH_3), 27.85 ($NHCH_3$), 36.87 (C-4), 46.26 (CH_2Ph), 60.31 (C-5), 127.35, 127.76, 128.38 (ArCH), 137.24 (ArC), 165.54 (C-3).
3d	($CDCl_3$) 2.36 (s, 3H, $ArCH_3$), 2.88 (dd, 1H, H-4, *J* 7, 17.2 Hz), 2.96 (d, 3H, NCH_3, *J* 4.8 Hz), 3.45 (dd, 1H, H-4, *J* 8.8, 17.2 Hz), 4.58 (dd, 1H, H-5, *J* 7, 8.8 Hz), 5.85 (bs, 1H, NH), 7.17 (d, 2H, ArylH, AB syst., *J* 8 Hz), 7.52 (d, 2H, ArylH, AB syst., *J* 8 Hz)	3292	19.40 (CH_3), 27.85 ($NHCH_3$), 36.77 (C-4), 61.55 (C-5), 129.09, 132.01 (ArCH), 127.89, 137.64 (ArC),165.20 (C-3)
3e	($CDCl_3$) 1.33–2.14 (m, 10H, CH_2),2.85 (dd, 1H, H-4, *J* 7.5, 17 Hz),3.02 (d, 3H, CH_3, *J* 4.8), 3.12–3.14 (m, 1H, CH), 3.46 (dd, 1H, H-4, *J* 8.8, 17 Hz), 4.46 (dd, 1H, H-5, *J* 7.5, 8.8 Hz)	3289	24.32, 24.78, 24.83, 32.49, 32.56 (CH_2), 27.78 (CH_3), 37.34 (C-4), 43.23 (CH), 57.58 (C5),165.39 (C-3)
3f	($CDCl_3$) 1.20 (t, 3H, CH_3, *J* 7.3 Hz), 2.35 (s, 3H, CH3), 2.92 (dd, 1H, H-4, *J* 6.6, 16.8 Hz), 3.36–3.52 (m, 3H, CH_2 and H-4), 4.56 (dd, 1H, H-5, *J* 6.6, 8.4 Hz), 5.81 (bs, 1H, NH), 7.17 δ (d, 2H, ArylH, *J* 8 Hz), 7.53 (d, 2H, ArylH, *J* 8 Hz)	3131	12.42, 19.40 (CH_3), 36.70, 36.90 (CH_2), 61.25 (C-5), 129.07, 132.12 (ArCH), 127.77, 137.66 (ArC), 164.25 (C-3)
3g	($CDCl_3$) 1.25 (t, 3H, CH_3, *J* 7.3 Hz), 1.22–2.15 (m, 10H, CH_2), 2.85 (dd, 1H, H-4, *J* 7.5, 16.5 Hz), 3.11–3.18 (m, 1H, CH), 3.37–3.53 (m, 3H, CH_2 and H-5), 4.44 (dd, 1H, H-5, *J* 7.5, 8.8 Hz), 5.94 (bs, 1H, NH)	3360	12.46 (CH_3), 24.64, 4.78, 24.83, 32.51, 32.56, 36.64, 37.50 (CH_2), 43.23 (CH), 57.35 (C-5),160.40 (C-3)
3h	($CDCl_3$) 1.15–1.45, 1.45–1.80, 1.80–2.10 (3m, 10H, CH_2), 2.36 (s, 3H, CH_3), 2.90 (dd, 1H, H-4, *J* 6.6, 16.8 Hz), 3.42 (dd,1H, H-4, *J* 9, 16.8 Hz), 3.75–3.85 (m, 1H, CH), 4.55 (dd, 1H, H-5, *J* 6.6, 9 Hz), 5.31 (bs, 1H, NH), 7.19 δ (d, 2H, ArylH, AB syst., *J* 8.1 Hz), 7.55 (d, 2H, ArylH, AB syst., *J* 8.1 Hz)	3295	($CDCl_3$) 20.57 (CH_3), 24.76, 25.61, 32.15, 32.20 (CH_2), 38.19 (C-4), 52.24 (CH), 62.18 (C-5), 128.8, 138.9 (ArC), 130.23, 133.46 (ArCH), 164.5 (C-3).
3i	($CDCl_3$) 11.3–1.41, 1.61–1.78, 2.03–2.19 (3m, 20H, CH_2), 2.80 (dd, 1H, H-4, *J* 7.5, 16.8 Hz), 3.17 (bs, 1H, CH), 3.38 (dd, 1H, H-4, *J* 8.8, 16.85 Hz), 3.82 (bs, 1H, CH), 4.43 (d, 1H, H-5, *J* 7.5, 8.8 Hz), 5.50 (bs, 1H, NH)	3318	24.72, 25.36, 25.65, 25.81, 25.90, 32.27, 33.38, 33.44, 38.80 (CH_2), 44.78 (CH), 52.61 (CH), 58.45 (C-5), 164.05 (C-3)
5a	1.43–1.82 (m, 10H, CH_2), 3.59 (m, 1H, (CH), 4.67 (d, 2H, CH_2Ph, *J* 5.9 Hz), 6.54 (s, 1H, H-4), 7.30–7.46 (m, 5H, ArylH), 8.18 (bs, 1H, NH)	3253	($CDCl_3$) 25.55, 25.81, 32.89 (CH_2), 46.50 (CH), 46.83 (CH_2Ph), 112.96 (C-4), 128.10, 128.47, 129.03 (ArCH), 137.46 (ArC), 159.38, 161.97 (C-3, C-5)
5b	2.42 (s, 3H, CH_3), 4.63 (d, 2H, CH_2, *J* 5.9 Hz), 5.69 (s, 1H, H-4), 7.33–7.63 (m, 9H, ArylH), 8.09 (bs, 1H, NH)	3287	20.77(CH_3), 46.82 (CH_2Ph), 113.52 (C-4), 124.36, 137.26, 141.58 (ArC), 128.13, 128.45, 129.03, 131.44, 134.80 (ArCH), 168.42, 161.93 (C-3, C-5)
5c	2.676 (s, 3H, CH_3), 4.67 (d, 2H, CH_2; *J* 5.86 Hz), 6.42 (s, 1H, H-4), 7.42–7.39 (m, 5H, ArylH), 8.1–8.4 (bs, 1H, NH)	3350	14.86 (CH_3), 46.68 (CH_2Ph), 112.12 (C-4), 127.95, 128.3, 128.867 (ArCH), 137.32 (ArC), 161.49, 161.67 (C-5, C-3)
5d	($CDCl_3$) 2.41 (s, 3H, CH_3), 3.07 (d, 3H, $NHCH_3$, *J* 5.1 Hz), 5.65 (s+bs, 2H, H-4 and NH), 7.26–7.52 (m, 4H, ArylH)	3350	20.58 (CH_3), 28.91 ($NHCH_3$), 113.38 (C-4), 124.37, 141.35 (ArC), 131.23, 134.59 (ArCH), 161.41, 161.94 (C-5 and C-3)
5e	($CDCl_3$) 1.24 (t, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.49 (q, 2H, CH_2), 4.75 (bs, 1H, NH), 5.68 (s, 1H, H-4), 7.27 (d, 2H, ArylH, *J* 7.9 Hz), 7.50 (d, 2H, ArylH, *J* 7.9 Hz)	3295	13.45 (CH_2), 20.60 (CH_3), 37.81 (CH_2), 113.47 (C-4), 124.37, 141.38 (ArC), 131.26, 134.64 (ArCH), 160.93, 161.36 (C-3 and C-5)
5f	($CDCl_3$) 1.30 (t, 3H, CH_3, *J* 7.3 Hz), 1.26–2.17 (m, 10H, CH_2), 3.51–3.57 (m, 3H, CH and CH_2), 6.24 (s + bs, 2H, H-4 and NH);	3285	($CDCl_3$) 14.00 (CH_3), 25.45, 25.59, 32.85, 32.44 (CH_2), 47.15 (CH), 113.49 (C-4), 158.63 (C-5), 161.94 (C-3)
5g	($CDCl_3$) 1.26 (m, 3H, CH_3), 3.44–3.58 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 5.61 (s + bs, 2H, H-4 and NH), 6.98 (d, 2H, ArylH, *J* 9 Hz), 7.54 (d, 2H, ArylH, 9 Hz);	3277	($CDCl_3$) 14.13 (CH_3), 38.53 (CH_2), 55.77 (OCH_3), 113.61 (C-4), 116.08, 136.42 (ArCH), 117.24 (ArC), 161.30, 161.78, 161.87 (ArC and C-5 and C-3)
5h	($CDCl_3$) 4.36 (s, 2H, SCH_2), 4.60–4.63 (d, 2H, CH_2Ph, *J* 5.9 Hz), 6.1 (s, 1H, H-4), 6.05–6.2 (bs, 1H, NH), 7.33–7.37 (m, 10H, ArylH)	3204	($CDCl_3$) 37.50 (CH_2S), 47.42 (CH_2N), 113.03 (C-4), 128.32, 128.37, 128.45, 129.05, 129.13 (ArCH),133.99, 135.66 (ArC), 159.45, 161.65 (C-3, C-5)
11a	4.1 (s, 3H, CH_3), 4.66 (d, 2H, CH_2, *J* 6.6), 5.90 (s, 1H, H-4), 7.314–7.44 (m, 5H, ArylH)	3310	45.74($PhCH_2$),61.04(OCH_3),91.01(C-4), 127.84, 128.24, 128.8 (ArCH),137.49(ArC),162.35, 170.87 (C-3, C-5).
11b	1.45 (t, 3H, CH_3, *J* 7 Hz), 4.31 (q, 2H, CH_2, *J* 7 Hz), 5.86 (s, 1H, H-4), 7.38–7.41 (m, 5H, ArylH), 8.2 (bs, 1H, NH)	3316	12.60 (CH_3), 44.88 (CH_2Ph), 69.91 (CH_2), 89.88 (C-4), 126.88, 127.23, 127.83 (ArCH), 136.43 (ArC), 161.66, 168.8 (C-3, C-5)

(continued on next page)

Table 3 *(continued)*

Numbers	^{1}H-NMR (δ ppm, CD_3COCD_3 except when indicated)	IR (cm^{-1} NH)	^{13}C-NMR (δ ppm, CD_3COCD_3 except when indicated)
11c	4.65 (d, 2H, CH_2N), 5.35 (s, 2H, CH_2O), 5.96 (s, 1H, H-4), 7.38–7.5 (m, 10H, ArylH), 8.25 (bs, 1H, NH)	3278	44.92(CH_2N), 75.02(CH_2O),90.81(C-4), 126.88, 127.29, 127.6–127.83, 127.93, 128.22 (ArCH), 133.5, 136.4 (ArC), 161.43, 138.54 (C-3, C-5)
12a	1.25 (t, 6H, CH_3), 3.53 (q, 4H, CH_2), 4.59 (d, 2H, CH_2), 6.12 (s, 1H, H-4), 7.37–7.4 (m, 5H, arylH), 7.42–7.67 (bs, 1H, NH)	3301	11.57 (CH_3), 44.26, 44.74 (CH_2CH_3, CH_2Ph), 80.5 (C-4), 126.6, 127.07, 127.71 (ArCH), 137.36 (ArC), 160.36, 164.21 (C-3, C-5)
12b	3.54 (t, 4H, CH_2, *J* 5 Hz), 3.77 (t, 4H, CH_2, *J* 5 Hz), 4.63 (d, 2H, CH_2Ph), 5.27 (s, 1H, H-4), 7.37–7.4 (m, 5H, ArylH), 7.7–7.9 (bs, 1H, NH)	3311	44.86, 47.1 (CH_2O, CH_2N), 64.96 (CH_2Ph), 82.77 (C-4), 126.68, 127.07, 127.77 (ArCH), 137.11 (ArC), 160.42, 163.76 (C-3, C-5)
12c	4.57 (d, 2H, CH_2), 4.61 (d, 2H, CH_2), 5.16 (s, 1H, H-4), 7.35–7.38 (m, 10H, ArylH), 7.55–7.8 (bs, 1H, NH)	3284, 3323	45.68–48.61 (CH_2N, CH_2N), 81.89 (C-4), 127.72, 127.88, 128.21, 129.04(ArCH), 137.78, 138.37 (ArC), 161.51, 165.36 (C-3, C-5)
12d	(DMSO-d_6), 3.73 (s, 3H, CH_3O), 4.24 (d, 2H, CH_2, *J* 3.29 Hz), 4.47 (d, 2H, CH_2, *J* 5.49 Hz), 5.02 (s, 1H, C-4), 7.19–7.39 (m, 9H, arylH), 8.68–8.83 (bs, 2H, NH)	3286, 3324	45.82 (CH_2), 48.30 (CH_2), 55.78 (OCH_3), 81.86 (C-4), 114.61 (ArCH), 128.00, 128.34, 129.17 (ArCH), 128.77, 138.55, 159.26, 160.54, 165.57 (C-5, C-3)
12e	3.80 (s, 6H, CH_3O), 4.41 (d, 2H, CH_2, *J* 5.9 Hz), 4.59 (d, 2H, CH_2, *J* 5.9 Hz), 5.1 (s, 1H, C-4), 6.92–7.03 (m, 3H, ArylH), 7.03–7.37 (m, 5H, ArylH), 7.54 (bs, 1H, NH), 7.72 (bs, 1H, NH)	3280, 3330	(DMSO-d_6) 45.82, 48.60 (CH_2N), 56.16, 56.25 (OCH_3), 82.05 (C-4), 112.07, 112.52, 120.07, 128.01, 128.33, 129.20 (ArCH), 130.16, 138.55 (ArC), 148.8, 149.44 (ArC), 160.57, 165.57 (C-3, C-5)

i.v. and the residue taken up with CH_2Cl_2 and washed with water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compounds **12a**–**e** were obtained by direct crystallization of the residue with the solvent indicated or through purification by column chromatography (acOEt/cyclohexane 0:100 to 100:0) followed by crystallization.

The same reaction conditions adopted for **4** were applied to 3-benzylamino-4-bromoisothiazole 1,1-dioxide **13**. As an example the reaction between **13** and **2a** are reported: Compound **13** (80 mg, 0.24 mmol) was dissolved in MeCN (5 ml) and equimolecular amount of MeSNa (**2a**) was added under stirring at room temperature. At the end of the reaction (8 h; TLC acOEt/cyclohexane 1:1) the solvent was evaporated i.v. and the residue taken up with CH_2Cl_2, washed with diluted HCl (2 × 5 ml) and with water (2 × 5 ml). The organic layer was separated, dried with Na_2SO_4 and the solvent evaporated to dryness. Pure compound **5a** were obtained crystallization with isopropanol.

5.7. Cell culture

SMC were cultured, from the intimal–medial layers of aorta of male Sprague–Dawley rats (200–250 g). Cells were grown in monolayers at 37 °C in a humidified atmosphere of 5% CO_2 in MEM supplemented with 10% (v/v) fetal calf serum (FCS), 100 U/ml penicillin, 0.1 mg/ml streptomycin, 20 mM tricine buffer and 1% (v/v) nonessential amino acid solution. The medium was changed every third day. Cells were used between the fourth and tenth passages.6 Eagle's MEM, trypsin ethylenediamine tetraacetate, penicillin (10,000 U/ml), streptomycin (10 mg/ml), tricine buffer (1 M, pH 7.4) and nonessential amino acid solution (100 ×) and FCS were purchased from Invitrogen (Carlsbad, CA, USA). Disposable culture flasks and Petri dishes are from Corning Glassworks (Corning, NY), and filters are from Millipore (Bedford, MA).

5.8. Cell proliferation assay

Cells were seeded at a density of 2×10^5 SMC per Petri dish (35 mm), and incubated with MEM supplemented with 10% FCS. Twenty-four hours later, the medium was changed to one containing 0.4% FCS to stop cell growth, and the cultures were incubated for 72 h. At this time (time 0), the medium was replaced with one containing 10% FCS in the presence or absence of known concentrations of the tested compounds, and the incubation was continued for a further 72 h at 37 °C. At time 0, just before the addition of the substances to be tested, three Petri dishes were used for cell counting. Cell proliferation was evaluated by cell count after trypsinization of the monolayers with use of a Coulter Counter model ZM [8]. The concentration of compounds required to inhibit 50% of cell proliferation (IC_{50}) was calculated by linear regression analysis of the logarithm of the concentration (in μmol/l) versus logit.

5.9. Assays for inhibition of PFTase and PGGTase-I

Recombinant rat PFTase and PGGTase-I were produced in Sf9 insect cells and purified as described in [9]. The standard reaction mixture for PFTase assay contains 0.75 μM (0.3 μCi) [^{3}H]-FPP and 5 μM Ras-CVIM, in a total volume of 20 μl containing 30 mM potassium phosphate, 1 mM $MgCl_2$, 20 μM $ZnCl_2$, pH 7.7. PGGT-I assay was carried out using 0.75 μM (0.3 μCi) [^{3}H]-GGPP and 5 μM H-Ras-CVLL as substrates. Stock solutions of the compounds were prepared in DMSO, and 1-μl aliquot was added to the reaction mixture (final concentration of DMSO was 5%). Reaction was initiated by adding PFTase or PGGTase-I. After incubation at 30 °C for 15 min, the reaction was stopped by adding 200 μl of 10% HCl in ethanol. The amount of the prenylated protein product was determined by the glass fiber filter method as previously described in [10].

Acknowledgements

We thank Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) for financial support (First).

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