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One-pot synthesis of 5-phenylimino, 5-thieno or 5-oxo-1,2,3-dithiazoles and evaluation of their antimicrobial and antitumor activity

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ABSTRACT

We here report the synthesis and biological evaluation of rare 4-substituted-5-phenylimino, 5-thieno- and 5-oxo-1,2,3-dithiazoles. Dithiazoles were selectively obtained in moderate to high yields (25–73%) via a one-pot reaction from various ethanoneoximes with sulfur monochloride, pyridine in acetonitrile followed by treatment by corresponding nucleophiles (aniline, thioacetamide and formic acid). All the synthesized compounds were screened for their antibacterial (against bacteria *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus* and *Listeria inocua)*, antifungal (against pathogenic strains *Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Issatchenkia orientalis)* and antitumor (on human cell lines MCF-7 and MDA-MB-231) activity. 4-(2-Pyridinyl)-5*H*-1,2,3-dithiazole-5-thione and 4-ethylcarboxyl-5*H*-1,2,3-dithiazole-5-thione (**5d, 5h**) that are active against Gram-positive bacteria are significantly active against fungi. 4-(2-Benzofuranyl)-5-phenylimino-5*H*-1,2,3-dithiazole (**4e**) exerts antiproliferative activity.

Nitrogen-containing heterocycles with sulfur atom are an important class of compounds in medicinal chemistry. Owing to their growing use in compounds of therapeutic importance (antibacterial, anticancer drugs), the synthesis of *N*-heteroimino-1,2,3,-dithiazole derivatives has been actively pursued in the last past decade.[1] 1,2,3-Dithiazoles have attracted much attention among five-membered sulfur-nitrogen heterocycles because of their interesting physical and biological properties and versatile chemistry.[2] The abundance of monocyclic dithiazole chemistry has been derived from 4,5-dichloro-1,2,3-dithiazolium chloride **1** – Appel salt – which is readily prepared from chloroacetonitrile and sulfur monochloride.[3] The versatility springs from the susceptibility of **1** to attack by nucleophiles at S-1, S-2, C-4 and C-5 atoms.[4] A rank of 5-substituted 1,2,3-dithiazolium chlorides was obtained from monosubstituted acetonitriles and sulfur monochloride.[5] But these salts are not interesting from the synthetic point of view. The chlorine atom in C-4 position on iminodithiazoles **2** can not be displaced (Fig. 1). Stable 5-(arylimino)-4-chloro-5*H*-1,2,3-dithiazoles **2** are interesting because of their biological importance against some fungi, grasses and broad-leaved weeds.[6] In order to enhance potential antimicrobial activity of the dithiazole derivatives, various research teams varied the structures of the aryl groups with more complex aromatic moiety.[1]

In continuation of our work on bioactive 1,2,3-dithiazoles and search for more potent derivatives, we report in this paper the original synthesis of novel 4- and 5-substituted-1,2,3-dithiazoles **I, II, III** bearing at C-5 position an imino, a thioketone or an oxo functionality (Fig. 2). Expecting an enhancement of the antimicrobial potential, the antibacterial and antifungal activities were measured. We describe a systematic study of the reaction between substituted ethanoneoximes and sulfur monochloride according an easy one-pot protocol for the preparation of 5-phenylimino, 5-thione and 5-one-1,2,3-dithiazoles together with the scope and limitations of this method.

To obtain the never described class of compounds **I, II, III**, we decided to form an Appel salt intermediate bearing an alkyl, aryl functionality at C-4 position and to displace the chlorine atom at

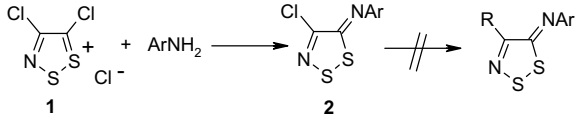

Figure 1.

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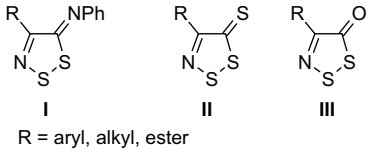


Figure 2.

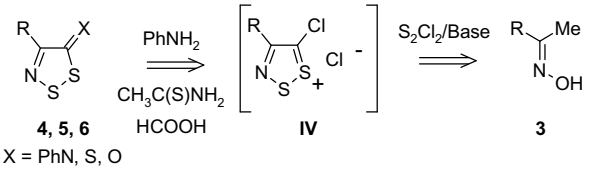
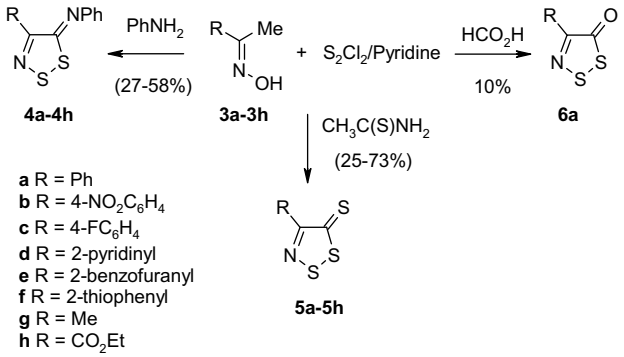


Figure 3.



Scheme 1.

the C-5 position by a nucleophilic system. Our retrosynthetic route to targets compounds **4a**–**4h**, **5a**–**5h** and **6a** is shown in Fig. 3.

Surprisingly, other than Appel salt 4-substituted 1,2,3-dithiazolium chlorides **IV** are hardly available. 4-Phenyl- and 4-nitrophenyl-1,2,3-dithiazolium chlorides **IV** have been prepared from the reaction of acetophenone oxime **3a** and its 4-nitro derivative **3b** with sulfur monochloride.[7]

These salts **IV** were found to be unstable and were converted to stable 5-arylimino derivatives **4a, 4b** by the reaction with primary aromatic amines (Fig. 4). After optimization of the experimental conditions, 5-phenylimino-1,2,3-dithiazoles **4a**–**4h** and 5-thione-1,2,3-dithiazoles **5a**–**5h** were prepared from substituted ethanone-oximes **3a**–**3h** and sulfur monochloride following an easy one-pot protocol (Scheme 1 and Table 1).

4-Chloro-5-thiodithiazoles can be prepared by treatment of Appel salt **1** with hydrogen sulfide in acetonitrile[3] or with 2-cyanothioacetamide in dichloromethane.[5] But both methods are inconvenient because of toxicity of H_2S and high price of the second reagent. We decided to employ for this transformation thioacetamide which was successfully used by us in the preparation of 4,5-dichloro-3*H*-1,2-dithiole-3-thione from 3,4,5-trichlorodithiolium chloride.[9] We checked this possibility by replacing formic acid with thioacetamide solution in acetonitrile at the last step and found that thione **5a** was obtained in high yield (73%). This method was extended to other ethanoneoximes. 1,2,3-Dithiazol-5-thiones **5** were formed selectively in high to moderate yields (Table 1 and Scheme 1).

Replacing thioacetamide with formic acid at the last step of the reaction of ethanoneoximes with S_2Cl_2 led to imine **6a** selectively in poor yield. HCO_2H which was not used before for the synthesis of 1,2,3-dithiazol-5-ones gave the best yield of ketone **6a** and facilitates the work-up procedure.

5-Phenylimino, 5-thione and 5-one-1,2,3-dithiazoles **4, 5, 6** were tested for their in vitro antibacterial activity against a panel of Gram-positive and Gram-negative reference strains: all microbiological products were purchased from Biokar except Mueller Hinton Broth (Difco). Chemicals were of analytical grade from Sigma. The microorganisms used in this study were *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Candida albicans* ATCC10231 from the American Type Culture Collection, *Klebsiella pneumoniae* CIP53153, *Salmonella enterica* serovar Typhimurium CIP5858, *Enterococcus faecalis* CIP103214 from the French 'Collection de l'Institut Pasteur', *Candida glabrata* DSM6425, *Candida tropicalis* DSM1346, *Issatchenkia orientalis* DSM6128 from the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH', *Bacillus cereus* and *Listeria inocua* from our laboratory collection. The bacteria were grown for 24 h on nutritive agar medium (or tryptone soy agar medium for *E. faecalis* and *L. inocua*), at 37 °C (or 30 °C for *E. faecalis, L. inocua* and *B. cereus*). The yeasts were grown on yeast extract peptone dextrose medium at 30 °C for 24 h. The compounds were dissolved in DMSO 80% and 50 μg of each compound were tested for their antibacterial and antifungal activity with the agar well diffusion method. [10] For each experiment, DMSO 80% alone was used as a control. Determination of the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) or the minimum fungicidal concentration (MFC) of the molecules was made by macro-dilution broth method, according to Sham and Washington[11] for the bacteria or Shadomy and Pfaller[12] for the yeasts. The tested compounds were dissolved in DMSO. The maximum concentration of solvent used in liquid cultures (2.13%) did not affect the growth of the microorganisms.

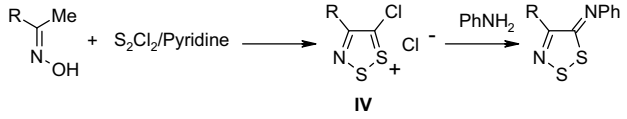


Figure 4.

Table 1
Preparation of the 4-substituted 5-phenyliminodithiazoles **4**, 4-substituted-5*H*-1,2,3-dithiazoles-5-thiones **5** and the 4-phenyl-5-phenylimino-5*H*-1,2,3-dithiazole **6**[8].

Product	R	Yield %	mp (°C)	Formula
4a	Ph	55	76	$C_{14}H_{10}N_2S_2$
4b	4-$NO_2C_6H_4$	27	172	$C_{14}H_9N_3O_2S_2$
4c	4-FC_6H_4	46	68	$C_{14}H_9FN_2S_2$
4d	2-Pyridinyl	47	Oil	$C_{13}H_9N_3S_2$
4e	2-Furanyl	57	144	$C_{16}H_{10}N_2OS$
4f	2-Thiophenyl	35	106	$C_{12}H_8N_2S_3$
4g	CH_3	57	Oil	$C_9H_8N_2S_2$
4h	CO_2Et	58	Oil	$C_{11}H_{10}N_2O_2S_2$
5a	Ph	73	100	$C_8H_5NS_3$
5b	4-$NO_2C_6H_4$	56	188	$C_8H_4N_2O_2S_3$
5c	4-FC_6H_4	40	152	$C_8H_4FNS_3$
5d	2-Pyridinyl	50	100	$C_7H_4N_2S_3$
5e	2-Benzofuranyl	55	134	$C_{10}H_5NOS_3$
5f	2-Thiophenyl	25	92	$C_6H_3NS_4$
5g	CH_3	38	47	$C_3H_3NS_3$
5h	CO_2Et	59	Oil	$C_5H_5NO_2S_3$
6a	Ph	58	72	$C_8H_5NOS_2$

The in vitro antibacterial and antifungal activities of the compounds were evaluated against pathogenic or opportunistic pathogenic microorganisms from Gram-positive or Gram-negative bacteria and yeasts.

Antibacterial activity. The antibacterial assays were first performed by the agar diffusion method against Gram-negative bacteria *E. coli* ATCC25922, *S. enterica* serovar Typhimurium CIP5858, *K.*

pneumoniae CIP53153, *P. aeruginosa* ATCC27853, and Gram-positive bacteria *S. aureus* ATCC25923, *E. faecalis* CIP103214, *B. cereus* and *L. inocua*. When a product was active, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the macro-dilution broth method. MBC is defined as the lowest concentration of product that killed 99.99% of the inoculum. For the MIC, we did not test higher concentrations more than 48 μg/ml.

None of the tested iminodithiazoles **4a–4g** had any effect on the growth of the Gram-negative, Gram-positive bacteria and yeasts. In contrast compound **4h** bearing an ester group at C-4 position and thioketones **5a, 5d** and **5h** are moderately active against Gram-positive. The results showed that Gram-positive bacteria were more sensitive to different compounds than Gram-negative bacteria (Tables 2 and 3). Compound **5h** as well **6a** exhibited a weak activity against the Gram-negative bacteria *E. coli* (Table 3).

Antifungal activity. As for antibacterial activity, the antifungal activity was initially tested using the agar diffusion method. The assays were performed with the following pathogenic strains: *C. albicans* ATCC10231, *C. glabrata* DSM6425, *C. tropicalis* DSM1346 and *I. orientalis* DSM6128. No activity was found with the iminodithiazole substituted in C-4 position **4a–4g** except for the iminodithiazole **4h**. Tested yeasts *C. albicans*, *C. glabrata* and *C. tropicalis* were significantly inhibited by iminodithiazole **4h**, and the thioketones **5d, 5h** (Table 4). These first results allowed us to select the compounds and the fungi strains used for the determination of the MIC and the minimum fungicidal concentration (MFC). The ratio MFC/MIC was calculated in order to determine if the product had a fungistatic (MFC/MIC $\geqslant 4$) or fungicidal (MFC/MIC $\leqslant 4$) activity. The results are summarized in Table 5. Commercial antibiotics, Amphotericin B and fluconazole, were used as references for inhibitory activity against yeasts. The three products **4h, 5d** and **5h**, showed significant antifungal activity (Table 5). Amphotericin B and fluconazole have greater activity against pathogenic yeasts than the tested compounds, but amphotericin is nephrotoxic and fluconazole has only fungistatic activity.

Table 4
Antimicrobial activity of the compounds **4**, **5**, **6** detected by the agar diffusion method.

Compound (50 μg)	Yeasts			
	C. albicans	*C. glabrata*	*C. tropicalis*	*I. orientalis*
4a	–	–	–	–
4b	–	–	–	–
4c	–	–	–	–
4e	–	–	–	–
4f	–	–	–	–
4g	–	–	–	–
4h	+++	+++	++	–
5a	–	–	–	–
5b	–	–	–	–
5c	–	–	–	–
5d	+++	+++	++	–
5e	–	–	–	–
5f	–	–	–	–
5g	–	–	–	–
5h	+++	++	+	–
6a	–	–	–	–

Averaged diameter of the inhibition zone was measured in triplicate; –, inactive; +, inhibition zone 6–9 mm; ++, inhibition zone 9–12 mm; +++, inhibition zone >12 mm.

Table 2
Antimicrobial activity of the compounds **4**, **5**, **6** detected by the agar diffusion method.

Compound (50 μg)	Gram-negative bacteria				Gram-positive bacteria			
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. Typhimurium*	*E. faecalis*	*S. aureus*	*B. cereus*	*L. inocua*
4a	–	–	–	–	–	+	–	–
4b	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–
4e	–	–	–	–	–	–	–	–
4f	–	–	–	–	–	–	–	–
4g	–	–	–	–	–	–	–	–
4h	–	–	–	–	–	+	+	–
5a	–	–	–	–	–	++	++	–
5b	–	–	–	–	–	+	–	–
5c	–	–	–	–	–	–	–	–
5d	+	–	–	–	+++	++	++	++
5e	–	–	–	–	–	–	–	–
5f	–	–	–	–	–	–	–	–
5g	–	–	–	–	–	+	–	–
5h	++	–	–	+	++	++	++	+
6a	+	–	–	–	–	–	–	–

Averaged diameter of the inhibition zone was measured in triplicate; –, inactive; +, inhibition zone 6–9 mm; ++, inhibition zone 9–12 mm; +++, inhibition zone >12 mm.

Table 3
Bactericidal activities, minimum inhibitory concentration and minimum bactericidal concentration (μg/ml)*.

Compound	Bacteria tested									
	E. coli		*E. faecalis*		*S. aureus*		*B. cereus*		*L. inocua*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4h	–	–	–	–	48	>48	48	>48	–	–
5a	–	–	–	–	32	>48	>48	>48	–	–
5d	>48	>48	48	>48	48	>48	>48	>48	16	>48
5g	–	–	–	–	48	>48	–	–	–	–
5h	>48	>48	>48	>48	48	>48	>48	>48	32	>48
6a	>48	>48	–	–	–	–	–	–	–	–

* Measured in triplicate.

Pharmacological assay on cancer cells. The preliminary antiproliferative activity of all the synthetized compounds **4, 5, 6** was tested in vitro on two cell lines.

Cell lines. The antiproliferative activity of dithiazoles was studied on MCF-7 and MDA-MB-231 human breast cancer cell lines (LGC Promochem). MCF-7 and MDA-MB-231 were grown in 1% penicillin–streptomycin (Dutscher).

Antiproliferative activity. Dithiazoles were dissolved in DMSO (Sigma–Aldrich) to obtain 10^{-3} M stock solutions from which further dilutions were made in the cell culture medium. A 50 μL aliquot of medium containing 2.10^{-5} M molecules was added to each well of 96-well plates. After equilibration at 37 °C, 50 μL of a 10^5 cell mL^{-1} suspension (5000 cells) were dispensed into all wells of the pre-equilibrated 96-well plate, diluting dithiazoles to a final concentration of 10^{-5} M. After 72 h growth, viable cells were quantified using the MTT cell proliferation assay. Briefly, 20 μL of MTT salt solution (5 g L^{-1} in PBS 100 mM, pH 7.4, sterile, protected from light) were added to each well, the plates were incubated for a further 4 h to allow MTT metabolism to formazan by the succinate-tetrazolium reductase system active only in viable cells. After incubation, the cell culture medium was removed, and cells were lysed with 100 μL DMSO. Plates were incubated for 10 min at 37 °C to allow solubilization of formazan crystals, and to remove bubbles from the wells. The optical densities were read on a plate reader (VERSAmax, Molecular Devices) at 550 nm. Data were then analyzed to calculate the % of growth inhibition through a comparison of samples with untreated cells (cell culture medium containing 1% DMSO, 0% growth inhibition) and lysed cells (cell culture medium containing 10% SDS, 100% growth inhibition). Data are presented as the mean percentage of growth inhibition ± SEM calculated from 24 measures from three independent experiments.

Table 5
Fungicidal activities, minimum inhibitory concentration and minimum fungicidal concentration (μg/ml)*.

Compound	Fungi tested*								
	C. albicans			*C. glabrata*			*C. tropicalis*		
	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC
4h	48	>48	–	32	48	1.5	32	>48	–
5d	32	>48	–	8	48	6	32	48	1.5
5h	48	>48	–	32	48	1.5	48	>48	–
Amphotericin B	0.25	0.5	2	2	2	2	1	2	2
Fluconazole	2	>128	>64	4	>128	>32	4	>128	>32

* Measured in triplicate.

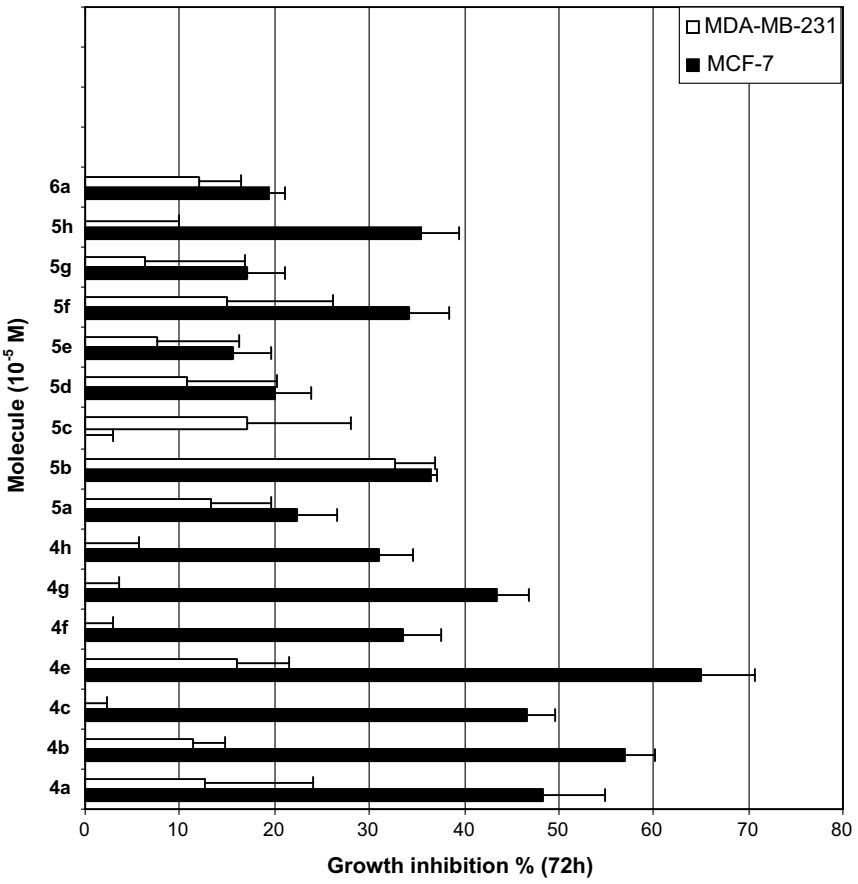


Figure 5. Antiproliferative activity of dithiazoles 10^{-5} M on MCF-7 and MDA-MB-231 breast cancer cell lines. Data are presented as the mean percentage of growth inhibition ± SEM calculated from 24 measures from three independent experiments.

Bioassays databanks (Bioinfobank Institute, http://cancer.bioinfo.pl/; Pubchem: http://pubchem.ncbi.nlm.nih.gov/) were screened using the compare methodology (http://dtp.nci.nih.gov/docs/compare/compare_methodology.html) to check the availability of antiproliferative data obtained with other dithiazoles, and specifically 1,2,3-dithiazoles.

Antiproliferative activity of dithiazoles is summarized in Figure 5. Most molecules exhibited a moderate to strong activity at 10^{-5} M. on MCF-7, with approximated GI50 equal to 10 μM for compounds **4a, 4b, 4c, 4e** and superior to 10 μM for compounds **4f, 4g, 4h, 5** and **6**. Dithiazoles also exerted a moderate activity on MDA-MB-231 with GI50 superior to 10 μM (0–30% growth inhibition at 10 μM). MDA-MB-231 were always more resistant than MCF-7 to the presence of dithiazoles in the culture medium, as previously observed with various antiproliferative compounds (thiazoloquinazolinones, thiazolocarbazoles).[13,14] Molecules active on MDA-MB-231 were then considered as good antiproliferative compounds, showing interest for further pharmacomodulation, as moreover, they were usually very active on MCF-7. Molecules active on MDA-MB-231 were then considered as good antiproliferative compounds, showing interest for further pharmacomodulation, as moreover, they were usually very active on MCF-7. Molecules active on MDA-MB-231 were then considered as good antiproliferative compounds, showing interest for further pharmacomodulation, as moreover, they were usually very active on MCF-7. The presence of an alkyl or benzyl substituent on the dithiazole ring was associated to a weak activity (**4a,4g,5a,5g,6a**). In the same way, substitution of the dithiazole ring by pyridine (**5d**), fluorobenzyle (**4c,5c**) did not increase the antiproliferative activity on MDA-MB-231. Molecules containing a thioketone substituent on the dithiazole ring (nitrobenzyl **5b**, ethylcarboxylate **5h**, benzofuranyl **5e**, thienyl **5f**) showed a better activity than the corresponding compounds containing a ketone or imine substituent (**4b,4h,4e,4f**, respectively). As a conclusion, dithiazoles substituted with a thioketone exerted the best antiproliferative activity, especially when benzofurane (**5e**), nitrobenzyle (**5b**), thiophene (**5f**) and ethylcarboxylate (**5h**) substituents were present in the structure. The benzofurane substituent could represent an interesting candidate for further pharmacomodulation as the benzofurane dithiazole containing an imine substituent (**4e**) was also very active on both cell lines. The pharmacological targets of these original heterocycles remain to be established.

Databanks screening. Using the Bioinfobank Institute databank, antiproliferative data on NCI cancer cells lines were found for 33 molecules containing a thiazole ring (http://cancer.bioinfo.pl/drugs?search=thiazole) but no data were available for dithiazoles or bithiazoles. The Pubchem database (http://pubchem.ncbi.nlm.nih.gov/, search for the term 'dithiazole'), reports potential anticancer activity for various dithiazoles, some of them exerting a good antiproliferative activity on various NCI cell lines. However, the main conclusion of this databank screening is that the antiproliferative activity of 1,2,3-dithiazoles remains to be established for most molecules. Our study thus provides one of the first reports of in vitro antiproliferative activity of 1,2,3-dithiazoles on human breast cancer cell lines.

In conclusion, we described the antimicrobial and antitumor activity of according to us, never reported 1,2,3-dithiazoles functionalized at C-4 and C-5 positions. We reported an efficient one-step protocols to 1,2,3-dithiazole-5-phenylimines, 5-thiones and 5-ones from readily available substituted methyl ketone oximes. The thioketones **5d** and **5h** appear to be the most active of the series tested against Gram-positive microorganisms and yeasts. Detailed studies determining the mechanism of action of these compounds on the bacteria will be published later. As regards the antitumor interest of compound **4e**, biological testing on different cell lines should be in progress.

Acknowledgments

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N-[(5Z)-4-Phenyl-5H-1,2,3-dithiazol-5-ylidene]aniline, ***4a****:* yield 55%. Bright yellow crystals, mp 73–76 °C. Anal. Calcd for $C_{14}H_{10}N_2S_2$: C, 62.19; H, 3.73; N, 10.36. Found: C, 62.25; H, 3.68; N, 10.62. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19 (3H, m, Ph), 7.48 (5H, m, Ph), 8.22 (2H, m, Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 119.0, 125.6, 128.1, 129.0, 129.8, 130.3 (10 CH, Ar), 132.6, 153.1, 159.2, 165.4 (4 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 270 (M^+, 96).
N-[(5Z)-4-(4-Nitrophenyl)-5H-1,2,3-dithiazol-5-ylidene]-N-phenylamine, ***4b****:* yield 27%. Orange crystals, mp 171-172 °C. Anal. Calcd for $C_8H_4N_2O_2S_3$: C, 37.49; H, 1.57; N, 10.93. Found: C, 37.62; H, 1.73; N, 11.05. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.23 (3H, m, Ph), 7.48 (2H, m, Ph), 8.31 (2H, d, *J* 8.3, Ar), 8.49 (2H, d, *J* 8.3, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 124.2, 128.6, 135.3, 135.4 and 131.0 (9 CH, Ar), 143.5, 153.4, 157.9, 162.5, 164.0, 170.4 (6 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 315 (M^+, 13), 167 (54).
N-[(5Z)-4-(4-Fluorophenyl)-5H-1,2,3-dithiazol-5-ylidene]-N-phenylamine, ***4c****:* yield 46%. Bright yellow crystals, mp 65–68 °C. Anal. Calcd for $C_{14}H_9FN_2S_2$: C, 58.31; H, 3.15; F, 6.59; N, 9.71; S, 22.24. Found: C, 58.45; H, 3.26; N, 9.92. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.17 (5H, m, Ph), 7.47 (2H, m, Ar), 8.27 (2H, м, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 115.2, 118.9, 125.8, 128.2, 131.1 (9 CH, Ar), 152.9, 157.9, 157.9, 166.2 and 166.4 (5 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 288 (M^+, 34), 167((78).
N-[(5Z)-4-Pyridin-2-yl-5H-1,2,3-dithiazol-5-ylidene]aniline, ***4d*** yield 47%. Red oil. Anal. Calcd for $C_{13}H_9N_3S_2$: C, 39.60; H, 1.90; N, 13.19. Found: C, 39.72; H, 2.12; N, 13.31. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.16 (3H, m, Ar), 7.43 (3H, m, Ar), 7.82 (1H, m, Ar), 8.04 (1H, d, *J* 8.5, Py), 8.76 (1H, d, *J* 3.9, Py). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 119.0, 124.9, 125.6, 130.8, 136.2 150.2, 154.0 (9 CH, Ar), 148.2, 151.9, 154.1, 171.1 (4 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 271 (M^+, 46).
N-[(5Z)-4-(1-Benzofuran-2-yl)-5H-1,2,3-dithiazol-5-ylidene]-N-phenylamine, ***4e****:* yield 57%. Red crystals, mp 138–144 °C. Anal. Calcd for $C_{16}H_{10}N_2OS_2$: C, 61.91; H, 3.25; N, 9.02. Found: C, 61.82; H, 3.48; N, 8.79. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50 (9H, m, Ar), 8.21 (1H, s, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 110.0, 110.2, 111.7, 119.3, 122.9, 123.6, 126.1 and 126.8 (10 CH, Ar), 127.8, 130.3, 148.8, 150.1, 152.6 and 155.4 (6 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 310 (M^+, 89).
N-[(5Z)-4-Thien-2-yl-5H-1,2,3-dithiazol-5-ylidene]aniline, ***4f****,* yield 35%. Red crystals, mp 108 °C. Anal. Calcd for $C_{12}H_8N_2S_3$: C, 52.14; H, 2.92; N, 10.13. Found: C, 51.92; H, 2.88; N, 10.39. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.16 (1H, m, Ar), 7.29 (3H, m, Ar), 7.51 (4H, m, Ar), 8.34 (1H, d, *J* 4.9, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 119.6, 126.1, 127.2, 129.9, 130.0, 13.1 (8 CH, Ar); 135.0, 151.9, 154.3, 163.0 (4 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 276 (M^+, 42), 173 (–PhNC, 8), 167 (–ThCN, 53), 109(–PhNCSS, 55).
N-[(5Z)-4-Methyl-5H-1,2,3-dithiazol-5-ylidene]-N-phenylamine, ***4g****:* yield 57%. Red oil. Anal. Calcd for $C_9H_8N_2S_2$: C, 51.90; H, 3.87; N, 13.45. Found: C, 52.07;

H, 3.98; N, 13.70. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.54 (3H, s, CH_3), 7.22 (3H, m, Ph), 7.46 (2H, m, Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 18.6 (CH_3), 119.5, 125.9, 129.8 (5 CH, Ph), 152.4, 161.9, 166.3 (3 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 208 (M^+, 38), 167 (55).

Ethyl (5Z)-5-(phenylimino)-5H-1,2,3-dithiazole-4-carboxylate, ***4h****:* yield 58%. Red oil. Anal. Calcd for $C_{11}H_{10}N_2O_2S_2$: C, 49.61; H, 3.78; N, 10.52; O, 12.01; S, 24.08. Found: C, 49.88; H, 4.02; N, 10.42. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.43 (3H, t, *J* 7.34, CH_3), 4.38 (2H, q, *J* 7.34, CH_2), 7.16 (3H, m, Ph), 7,46 (2H, m, Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ:14.2 (CH_3), 63.0 (CH_2), 119.3 (2CH, Ph), 126.4 (CH), 129.8 (2CH, Ph), 139.4, 152.1, 153.3 and 160.4 (4 sp^2 tertiary C). MS (EI, 70 eV), *m/z* (%): 266 (M^+, 40), 167 (80).

General procedure for the synthesis of 5a–5h from ethanoneoximes: Pyridine (3 mmol) was added dropwise at −5 to 0 °C to a stirred solution of ethanoneoxime (1 mmol) and sulfur monochloride (2 mmol) in acetonitrile (10 ml) under an inert atmosphere of argon. The mixture was stirred at 0 °C for 15 min. Then thioacetamide (1.1 mmol) was added, the mixture was stirred at r.t. for 2 h, filtered and solvents were evaporated. The residue was separated by column chromatography (Silica gel Merck 60, light petroleum and then light petroleum–CH_2Cl_2 mixtures). Yields are given.

4-Phenyl-5H-1,2,3-dithiazole-5-thione, ***5a****:* yield 73%. Brown scales 95–100 °C. Anal. Calcd for $C_8H_5NS_3$: C, 45.47; H, 2.38; N, 6.63. Found: C, 45.55; H, 2.43; N, 6.70. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36 (3, m, Ph), 7.47 (2H, m, Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 128.0, 129.4 (4 CH, Ph); 130.5 (CH, Ph); 131.3, 179.3 (2 sp^2 tertiary C); 208.0 (C═S). MS (EI, 70 eV), *m/z* (%): 211 (M^+, 100), 135 (95), 103 (30).

4-(4-Nitrophenyl)-5H-1,2,3-dithiazole-5-thione, ***5b****:* yield 56%. Black-brown crystals, mp 182–190 °C. Anal. Calcd for $C_8H_4N_2O_2S_3$: C, 37.49; H, 1.57; N, 10.93. Found: C, 37.62; H, 1.73; N, 11.05. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.15 (2H, d, *J* 9.2, Ar), 8.31 (2H, d, *J* 9.2, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 122.5 and 123.5 (4 CH); 126.4, 130.1, 163.1 (3 sp^2 tertiary C), 207.3 (C═S). MS (EI, 70 eV), *m/z* (%): 256 (M^+, 38), 180 (100).

4-(4-Fluorophenyl)-5H-1,2,3-dithiazole-5-thione, ***5c****:* yield 40%. Brown crystals, mp 148–152 °C. Anal. Calcd for $C_8H_4FNS_3$: C, 41.90; H, 1.76; N, 6.11. Found: C, 41.83; H,1.89; N, 6.30. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.15 (2H, t, *J* 8.80, Ar), 7,98 (2H, m, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 115.4, 131.7 (4 CH, Ar), 130.2 (sp^2 tertiary C), 150.9, 140.6, 165.7 (3 sp^2 tertiary C), 208.0 (C═S). MS (EI, 70 eV), *m/z* (%): 229 (M^+, 78), 209 (10), 185(12), 153 (89).

4-(2-Pyridinyl)-5H-1,2,3-dithiazole-5-thione, ***5d****:* yield 50%. Red crystals, mp 99–100 °C. Anal. Calcd for $C_7H_4N_2S_3$: C, 39.60; H, 1.90; N, 13.19. Found: C, 39.72; H, 2.12; N, 13.31. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.41 (1H, m, Py), 7.82 (1H, m, Py), 8.04 (1H, d, *J* 8.5, Py), 8.76 (1H, d, *J* 3.9, Py). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 124.9, 136.2, 150.2, 154.0 (4 CH, Py), 154.1, 171.1 (2 sp^2 tertiary C), 203.4 (C═S). MS (EI, 70 eV), *m/z* (%): 212 (M^+, 46), 168 (15), 136(77).

4-(1-Benzofuran-2-yl)-5H-1,2,3-dithiazole-5-thione, ***5e****:* yield 55%. Red crystals, mp 132–134 °C. Anal. Calcd for $C_{10}H_5NOS_3$: C 47.79; H, 2.01; N, 5.57; O, 6.37; S, 38.27. Found: C, 47.92; H, 2.23; N, 6.76. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.32 (1H, m, Bzf), 7.44 (1H, m, Bzf), 7.62 (1H, d, *J* 8.1, Bzf), 7.73 (1H, d, *J* 7.3, Bzf), 8.50 (1H, s, Bzf). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ:110.7, 111.4, 122.9, 123.6, 127.1 (5 CH, Bzf), 132.9, 140.8, 147.6 and 168.7 (4 sp^2 tertiary C), 205.2 (C═S). MS (EI, 70 eV), *m/z* (%): 251 (M^+, 23), 175 (59), 143 (58).

4-Thien-2-yl-5H-1,2,3-dithiazole-5-thione, ***5f****:* yield 25%. Red crystals, mp 89–92 °C. Anal. Calcd for $C_6H_3NS_4$: 216.9148. Found: 246.9151. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.14 (1H, m, Th), 7.53 (1H, d, *J* 5.1, Th), 8.36 (1H, d, *J* 4.4, Th). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 127.2, 130.7, 131.7 (3 CH, Th), 133.9, 164.0 (2 sp^2 tertiary C), 205.6 (C═S). MS (EI, 70 eV), *m/z* (%): 217 (M^+, 73), 141 (100), 109 (53).

4-Methyl-5H-1,2,3-dithiazole-5-thione, ***5g****:* yield 38%. Red crystals, mp 45–47 °C. Anal. Calcd for $C_3H_3CNS_3$: C, 24.14; H, 2.03; N, 9.38. Found: C, 23.68; H, 2.21; N, 8.83. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.56 (3H, s, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 18.5 (CH_3); 169.8 (sp^2 tertiary C); 206.6 (C═S). MS (EI, 70 eV), *m/z* (%): 149 (M^+, 58), 85 (24).

Ethyl 5-thioxo-5H-1,2,3-dithiazole-4-carboxylate, ***5h****:* yield 59%. Red oil. Anal. Calcd for $C_5H_5NO_2S_3$: C, 28.97; H, 2.43; N, 6.76. Found: C, 29.26; H, 2.68; N, 7.03. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42 (3H, t, *J* 7.3, CH_3), 4.43 (2H, q, *J* 7.3, CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ:14.1 (CH_3), 63.4 (CH_2), 160.1, 169.4 (2 sp^2 tertiary C), 204.6 (C═S). MS (EI, 70 eV), *m/z* (%): 207 (M^+, 53), 179 (6), 163 (20), 149 (65).

Synthesis of 6a from ethanoneoximes: Pyridine (3 mmol) was added dropwise at −5 to 0 °C to a stirred solution of ethanoneoxime (1 mmol) and sulfur monochloride (2 mmol) in acetonitrile (10 ml) under argon. The mixture was stirred at 0 °C for 15 min. Then formic acid (5 mmol) was added, the mixture was stirred at 0 °C for 30 min and refluxed for 1 h, filtered and solvents were evaporated. The residue was separated by column chromatography (Silica gel Merck 60, light petroleum and then light petroleum–CH_2Cl_2 mixtures). Yield is given.

4-Phenyl-5H-1,2,3-dithiazole-5-one, ***6a****:* yield 58%. Light yellow crystals, mp 70–72 °C. Anal. Calcd for $C_8H_5NOS_2$: C, 49.21; H, 2.58; N, 7.17. Found: C, 49.13; H, 2.73; N, 7.30. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.48 (3H, m, Ph), 8.15 (2H, m, Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 128.0, 128.8, 131.0 (5 CH, Ph); 130.9, 155.0 (2 sp^2 tertiary C); 189.4 (C═O). MS (EI, 70 eV), *m/z* (%): 195 (M^+, 20), 167 (30), 135 (7), 103 (37).

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