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Original article

Disubstituted thiourea derivatives and their activity on CNS: Synthesis and biological evaluation

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ABSTRACT

A series of new thiourea derivatives of 1,2,4-triazole have been synthesized. The difference in structures of obtained compounds are directly connected with the kind of isothiocyanate (aryl/alkyl). The ^{1}H NMR, ^{13}C NMR, MS methods were used to confirm structures of obtained thiourea derivatives. The molecular structure of (**1**, **17**) was determined by an X-ray analysis. Two of the new compounds (**8** and **14**) were tested for their pharmacological activity on animal central nervous system (CNS) in behavioural animal tests. The results presented in this work indicate the possible involvement of the serotonergic system in the activity of **8** and **14**. In the case of **14** is also a possible link between its activity and the endogenous opioid system. All obtained compounds were tested for antibacterial activity against Gram-positive cocci, Gram-negative rods and antifungal activity. Compounds (**1**, **2**, **5**, **7**, **9**) showed significant inhibition against Gram-positive cocci. Microbiological evaluation was carried out over 20 standard strains and 30 hospital strains. Selected compounds (**1**–**13**) were examined for cytotoxicity, antitumor, and anti-HIV activity.

1. Introduction

Triazoles are an important group of compounds due to their wide range of applications as pharmaceutical agents. The therapeutic application of 1,2,4-triazole derivatives as widely used drugs is well documented; they show broad spectrum of activity, i.e.: antifungal [1–7], antiviral [8,9] antidepressant [10,11] and antimigrain [12].

Various new obtained derivatives of 1,2,4-triazole have been reported to possess interesting biological activity such as antimicrobial [13–17], antitumor [18–23], anti-inflamatory [24–27] antioxidant [28] and anticonvulsant [29]. Structural properties of triazoles, like moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions are main reasons for their superior pharmacological activities. Therefore triazoles are perspective scaffolds for designing new families of compounds with therapeutic importance.

In our previous study [30,31], we reported the synthesis and biological activity of thiourea derivatives.

In the recent literature, the thioureas are described as most useful class of agents with large number of activities including antiviral [32,33] antibacterial [34] and HDL-elevating properties [35].

Numerous compounds containing a thiourea group are selective ligands for 5-HT family of receptors, including 5-HT$_{2A}$, 5-HT$_{2B}$and 5-HT$_{2c}$ [36–39]. In addition, structural analyses of bioactive thioureas have shown that many of these compounds possessing two hydrophobic moieties and one central hydrophilic part form a butterfly-like conformation [40]. Anti-HIV agents also adapt this type of conformation (*e.g.* the nucleoside reverse transcriptase inhibitors (NNRTIs) [40].

Literature survey shows that only one connection between 1,2,4-triazole and thiourea was described. *N*-(1,2,4-triazol-3-yl)-*N*′arylthioureas act as effective uncouplers of oxidative phosphorylation in mitochondria [41].

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Prompted by these facts, we decided to design new compounds containing above-mentioned moieties *viz*. 1,2,4-triazole and thiourea, to explore their possible biological activities.

2. Result and discussion

2.1. Chemistry

The preparation of 18 thiourea derivatives is described, however 12 of them are new. 3-Amino-1,2,4-triazole was subjected to reaction with isothiocyanates in order to be transformed into the corresponding thiourea derivatives (Scheme 1 and Table 1). The chemical character of isothiocyanate substituent determined product of the reaction. When aryl derivative of isothiocyanate was used as reagent, the amino group of 3-amino-1,2,4-triazole was substituted. When substituent is bonded to isothiocyanate through the Csp^3 atom, the imine N2-atom of 1,2,4-triazole take part in the reaction and the products have unsubstituted amino group. Theoretical calculations [42] confirm this mechanism only for a hydrophilic solvent, like acetonitrile. In a hydrophobic solvent two forms of the product are expected.

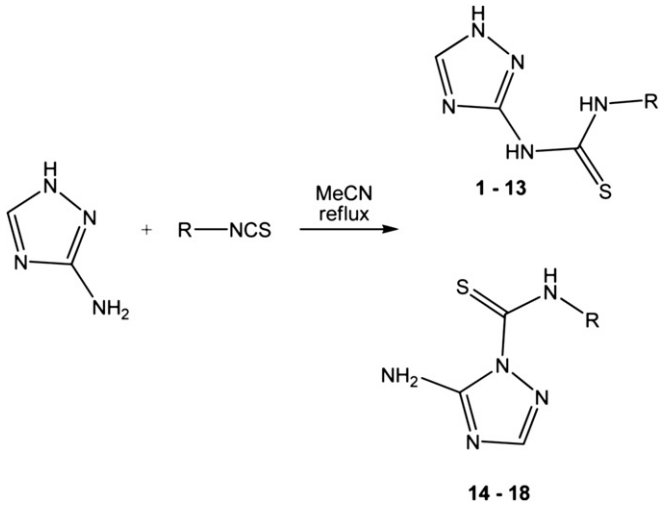
Obtained compounds were purified by flash chromatography. MS, ^{1}H NMR and ^{13}C NMR spectra confirmed the identity of the products. The molecular structure of **1** and **17** (Fig. 1) was determined by an X-ray crystal structure analysis.

2.2. Pharmacology

In this study we tested properties of two new thiourea derivatives, **8** and **14**. The connections between 1,2,4-triazole and thiourea have not been tested on central nervous system (CNS) yet. The first compound (**8**) possessed free thiourea system connected to 1,2,4-triazole whereas in a second (**14**) thiourea system was built-in 1,2,4-triazole. In this connection compounds **8** and **14** formed a good comparative group.

The spontaneous activity and amphetamine hyperactivity were evaluated. The effect on body temperature and behaviour of animals caused by administration of L-5-HTP, motor coordination as well as nociceptive and anticonvulsant activity was estimated. These allowed preliminary determination of the impact of the new substances on the CNS of experimental animals.

The tested compounds, used at a dose equivalent to 0.1 ED_{50}, did not statistically significantly change motor activity of animals and



Scheme 1. Synthesis of studied compounds.

Table 1
Structure and molar weight of the investigated compounds **1**–**18**.

Symbol	R	Mol. weight (g/mol)
1	Phenyl	219.06
2	4-Methoxyphenyl	249.07
3	4-Chlorophenyl	253.02
4	4-Methylphenyl	233.07
5	4-Fluorophenyl	237.05
6	2-Bromophenyl	296.97
7	4-Bromophenyl	296.97
8	3-Bromophenyl	269.97
9	4-Iodophenyl	344.95
10	3,4-Dichlorophenyl	286.98
11	Benzoyl	247.05
12	2-Fluorophenyl	237.05
13	3-Fluorophenyl	237.05
14	Benzyl	233.07
15	Cyclohexyl	225.10
16	Phenethyl	247.09
17	Methallyl	197.07
18	Ethyl formate	215.05

did not affect hyperactivity induced by administration of amphetamine, which seems to rule out the involvement of the catecholamine system in their action (Figs. 2 and 5).

The tested compounds, used at a dose equivalent to 0.1 ED_{50} and at half that dose, i.e. 0.05 ED_{50}, significantly decreased the number of writhing episodes (Fig. 6).

Their antinociceptive activity has been confirmed at the tested doses (Fig. 7).

The use of naloxone inhibited only the antinociceptive action of **14**, causing a repeated increase in the number of writhing episodes (from 6.7 ± 1.4 to 13.2 ± 0.99, respectively ($p < 0.05$)), which may indicate a link between this activity and the endogenous opioid system (Fig. 7).

The tested compounds, used at a dose of 0.1 ED_{50}, caused no coordination disorders, as they did not change the behaviour of mice in either test. The results obtained in all groups were similar to the values in the control group, indicating a lack of a depressive effect on the striated muscles and coordination (Figs. 3 and 4).

In the pentetrazol seizure test, none of the tested compounds clearly reduced the severity of clonic or tonic seizures, or protected the animals from dying.

Tests were also carried out to evaluate the effect of the new thiourea derivatives on the "head-twitch" response in mice caused by administration of a serotonin precursor, L-5-HTP, which may indicate the involvement of the serotonergic system in the observed effects (Fig. 8). The drug-elicited head twitch response (HTR) [43,44] is a selective behavioural model for 5-HT_2 agonist activity in rodents, and several previous studies have established that direct and indirect 5-HT agonists induce this effect [45–52]. Additionally, 5-HT_2 receptor antagonists selectively block HTR [52–54], and their potency is highly correlated with the antagonist's affinity for 5-HT_2 receptors [45,55].

Among the compounds tested, only **14** significantly ($p < 0.05$) reduced the number of the "head-twitch" episodes. The activity of **8** was also evident, but the result did not reach statistical significance. Since 5-HT_2 receptors mediate the occurrence of a typical "head-twitch" response caused by L-5-HTP, obtained data seem to suggest interactions with these receptors in the brain.

Tests of the effect of the new compounds on body temperature in normothermic mice showed that **8** and **14** only reduced it episodically at 90 min (Fig. 9).

The results presented in this work, based on literature data, indicate the possible involvement of the serotonergic system in the activity of the thiourea series compounds. 5-HT_2 and/or 5-HT_{1A} receptors are also probably involved, which seems to be confirmed

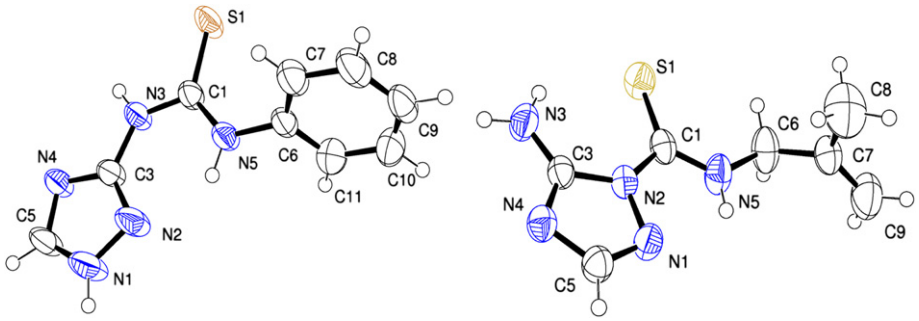


Fig. 1. Molecular structure of **1** (left) and **17** (right). Displacement ellipsoids drawn at 50% probability level.

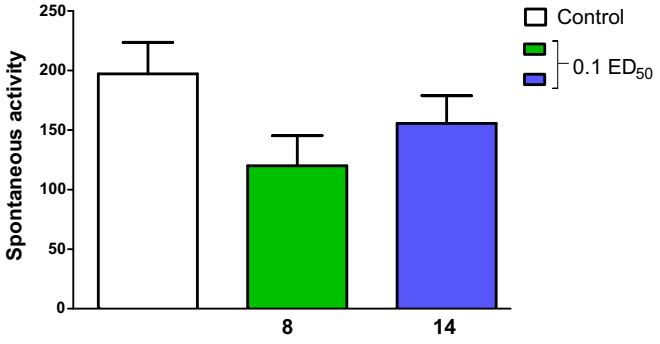


Fig. 2. The influence of **8** and **14** on spontaneous motor activity.

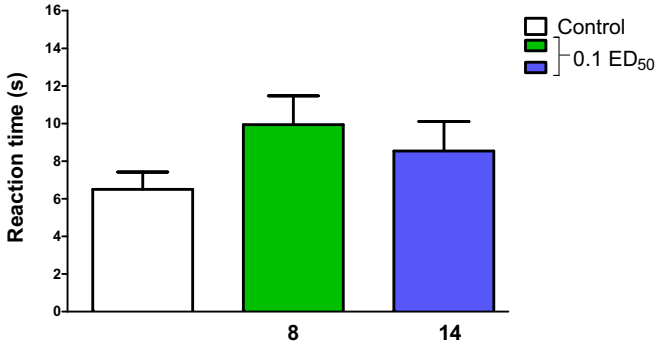


Fig. 4. The influence of **8** and **14** on motor coordination evaluated in chimney test.

by the inhibition of the "head twitch" response by **14**. In the case of **14**, one should also remember about the possible link between its activity and the endogenous opioid system, as the presence of free amino group should cause additional influence on the endogenous opioid system.

Behavioural animal tests directed on central nervous system (CNS) were a part of larger project of research. Up to now six new compounds composed of urea and thiourea system were tested. Previously tested compounds formed a connection of urea or thiourea system and tricyclic imide (1-(,7,8,9,10-pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yl)-3-phenylurea and 1-(4-iodophenyl)-3-(1,7,8,9-tetramethyl-3,5-dioxo-4-azatricyclo [5.2.1.$0^{2,6}$]dec-8-en-4-yl)thiourea) [31,43] or thiourea system as a part of thiazepine ring (10-isopropyl-8-methyl-4-{2-[(*Z*)-4-methylphenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo [5.2.2.$0^{2,6}$] undec-8-ene-3,5-dione and 1-isopropyl-7-methyl-4-{2-[(*Z*)-3,4-dichlorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.$0^{2,6}$] undec-8-ene-3,5-dione) [30]. In the present work tricyclic imide connected to thiourea system is replaced by 1,2,4-triazole. Base on our previous research on central nervous system (CNS) for urea [56] or thiourea [31] and 1,3-thiazepine derivatives [30] of tricyclic imide and described in this paper connection of thiourea and 1,2,4-triazole, we could conclude that this group of compounds possesses significant influence on central nervous system (CNS) of laboratory animals. Comparison of urea and thiourea derivatives shows that urea has influence on "head twitch response" only. However thiourea and 1,3-thiazepine derivatives of tricyclic imides were active in "head twitch response" test similarly to urea derivatives and have influence on spontaneous motor activity and decrease body temperature of normothermic mice. Thiourea derivatives of 1,2,4-triazole are similar in influence on "head twitch response" and decrease body temperature of normothermic mice but significantly decreased the number of writing episodes and possessed antinoceptive activity. Based on performed research we could conclude that urea derivatives were less active than thiourea derivatives. Probably the urea or thiourea part only has influence on CNS activity. The replacement of imide moiety bound to thiourea with 1,2,4-triazole has no influence on the activity. As well as the difference in number of methyl groups connected to an imide has

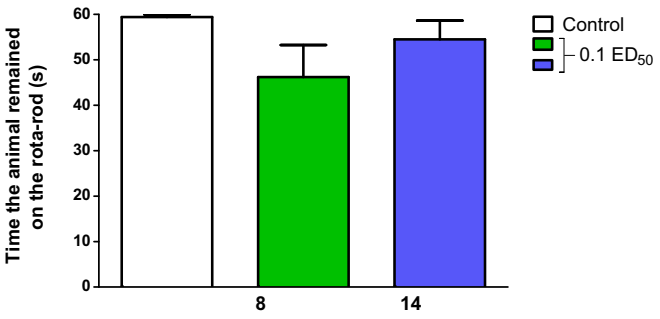


Fig. 3. The influence of **8** and **14** on motor coordination evaluated in rota-rod test.

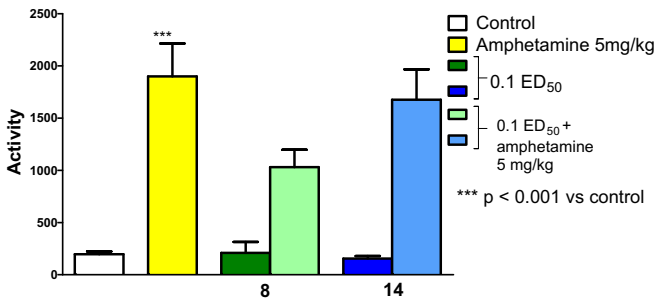


Fig. 5. The influence of **8** and **14** on amphetamine induced hyperactivity.

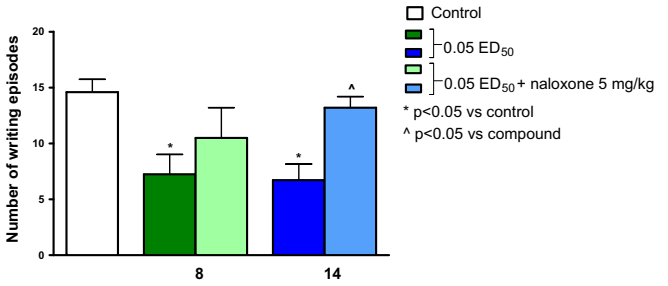


Fig. 6. The influence of naloxone, 5 mg/kg s.c. on antinociceptive activity of **8** and **14** evaluated in 'writhing' test. *Note*: the results are expressed as mean ± S.E.M. of a group of 8–10 mice.

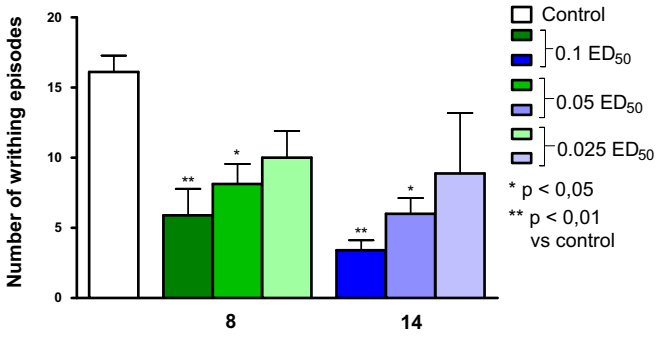


Fig. 7. The antinociceptive effects of **8** and **14** assessed in the 'writhing' test in mice. *Note*: the results are expressed as mean ± S.E.M. of a group of 8–10 mice.

not differentiated compounds on CNS activity. The molar masses of examinated compounds have fluctuated among 233 g mol^{-1} to 507 g mol^{-1} and this factor really has not influenced on pharmacological activity in this case. Even if the thiourea was closure in 1,3-thiazepine ring, the activity on CNS was comparable with the free thiourea. Only a free amino group connected to 1,2,4-triazole, which was part of thiourea, has an impact on the CNS activity. Comparison the activity on CNS of thiourea connected of tricyclic imides and 1,2,4-triazole derivatives could lead to the conclusion that generally thiourea compounds indicate the possible involvement of the serotonergic system but further 1,2,4-triazole with amino group added to thiourea should cause additional influence on the endogenous opioid system.

2.3. Microbiology

All obtained compounds were tested *in vitro* against a number of bacteria, including Gram-positive cocci, Gram-negative rods

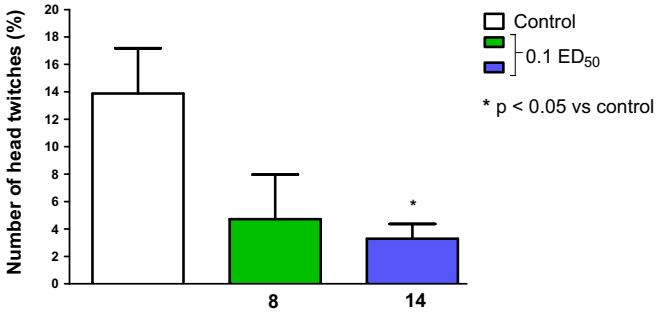


Fig. 8. The influence of **8** and **14** on 'head twitch' response evoked by 5-hydroxytryptophan (5-HTP). The results are expressed as mean ± SEM ($n = 10$).

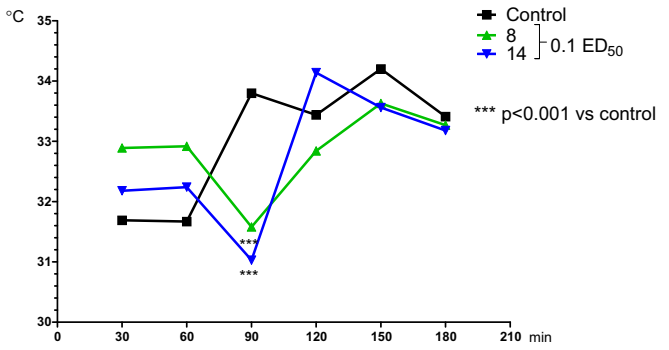


Fig. 9. The influence of **8** and **14** on the body temperature of mice. *Note*: each point represents the mean for a group of 10 mice.

and *Candida albicans*. Microorganisms used in this study have common application in the antimicrobial tests for many substances like antibiotics, disinfectants and antiseptic drugs or in research on new antimicrobial agents [57]. All the compounds were screened for their antimicrobial activity by disc diffusion method [58]. Compounds showing significant activity test were next examined for their minimal inhibitory concentration (MIC) [59]. The results of activity for compounds are summarized in Tables 2 and 3.

Preliminary test by disc-diffusion method showed antimicrobial activity against standard Gram-positive cocci, therefore the next step was evaluation of compounds' MIC values for standard hospital strains. The research was carried out over 20 standard strains, 20 hospital stains of *Staphylococcus aureus* and 10 hospital strains of *Staphylococcus epidermidis* used for routine antimicrobial media susceptibility testing. Hospital strains were isolated from different biological materials of patients hospitalized in Warsaw Medical University Hospitals.

Among *S. aureus* 10 strains showed methicilin susceptibility (MSSA) and 10 strains showed methicilin resistance (MRSA). MIC value of standard Gram-positive strain as in the range 400–12.5 μg/ml (Table 3).

MIC value of hospital *S. aureus* strain as in the range 50–12.5 μg/ml (Table 4).

Table 2
Antibacterial and antifungal *in vitro* activity expressed as diameter of growth inhibitory zone (GIZ, mm) for tested compounds (applied 400 μg per disc).

	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**13**
S. aureus NCTC 4163	–	23	–	14	11	17	–	12	12	16
S. aureus ATCC 25923	–	23	–	18	13	22	–	13	19	34
S. aureus ATCC 6538	–	15	–	14	12	16	–	12	12	15
S. aureus ATCC 29213	11	17	–	11	12	16	–	13	12	18
S. epidermidis ATCC 12228	12	20	–	16	12	16	–	–	11	22
B. subtilis ATCC 6633	16	22	13	22	17	30	16	18	20	32
B. cereus ATCC 11778	12	16	–	16	12	18	–	13	11	14
E. hirae ATCC 10541	–	23	–	11	13	25	–	13	–	16
M. luteus ATCC 9341	14	26	–	19	16	28	15	13	24	36
M. luteus ATCC 10240	12	30	12	23	15	29	14	16	38	40
E. coli ATCC 10538	–	–	–	–	–	–	–	–	–	–
E. coli ATCC 25922	–	–	–	–	–	–	–	–	–	–
E. coli NCTC 8196	–	–	–	–	–	–	–	–	–	–
P. vulgaris NCTC 4635	–	–	–	–	–	–	–	–	–	–
P. aeruginosa ATCC 15442	–	–	–	–	–	–	–	–	–	–
P. aeruginosa NCTC 6749	–	–	–	–	–	–	–	–	–	–
P. aeruginosa ATCC 27853	–	–	–	–	–	–	–	–	–	–
B. bronchiseptica ATCC 4617	–	–	–	–	–	–	–	–	–	–
C. albicans ATCC 10231	–	–	–	–	–	–	–	–	–	–
C. albicans ATCC 90028	–	–	–	–	–	–	–	–	–	–
C. parapsilosis ATCC 22019	–	–	–	–	–	–	–	–	–	–

–, Lack of the growth inhibition zone.

Table 3
Activities of obtained compounds against Gram-positive bacteria – minimal inhibitory concentations (MIC, μg/ml).

	1	**3**	**5**	**6**	**7**	**9**	**12**	**13**	Ciprofloxacin (5 μg per disc)
S. aureus NCTC 4163	100	25	50	50	50	25	200	50	0.5
S. aureus ATCC 25923	100	25	50	50	50	25	200	50	0.5
S. aureus ATCC 6538	100	25	50	50	50	25	200	100	0.5
S. aureus ATCC 29213	100	25	25	50	25	25	200	50	0.5
S. epidermidis ATCC 12228	50	25	25	25	25	25	200	50	0.5
B. subtilis ATCC 6633	50	12.5	12.5	12.5	12.5	12.5	100	50	<0.125
B. cereus ATCC 11778	100	12.5	6.25	25	12.5	12.5	100	50	0.5
E. hirae ATCC 10541	200	200	400	400	400	400	400	400	4
M. luteus ATCC 9341	50	25	6.25	6.25	25	12.5	100	50	2
M. luteus ATCC 10240	50	25	6.25	6.25	12.5	12.5	100	50	1

MRSA strains were more resistant to investigated compounds, the range of MIC value changed from 400 to 12.5 μg/ml (Table 5).

For hospital *S. epidermidis* (MRSA) the MIC value changed from 400 to 12.5 μg/ml and average value was 50 μg/ml (Table 6). The comparison between structure and biological activity is interesting. The activity depends only on an aryl substituent. Phenyl substituted fluorine, bromine, iodine atom and methoxy group is profitable for antimicrobial activity.

Due to the previously reported anti-HIV activities of thiourea derivatives [60,61], title compounds were tested in cell-based assay against the human immunodeficiency virus type-1 (HIV-1), using efavirenz as reference inhibitor. The cytotoxicity was evaluated in parallel with the antiviral activity.

None of test compounds showed selective antiviral activity against HIV-1. However, one of them, compound **14**, turned out cytotoxic for exponentially growing MT4 cells in the low micromolar range ($CC_{50} = 7.4 \pm 0.6$ μM) (Table 7). The antiproliferative activity against this CD4+ human T cell line derived from an haematological human tumor, prompted us to evaluate the antiproliferative activity of **14** also for a panel of other human haematological and solid tumours and for cell lines derived from normal human tissues. Interestingly, a CC_{50} (11 ± 0.7 μM) comparable to that obtained with MT-4 cells was found also for the cell line derived from a human prostate carcinoma (Tables 8 and 9).

3. Experimental protocol

3.1. Chemistry

The NMR spectra were recorded on a Bruker AVANCE DMX400 spectrometer, operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). The chemical shift values are expressed in ppm relative to TMS as an internal standard. Mass spectral ESI measurements were carried out on Waters ZQ Micro-mass instruments with quadrupol mass analyzer. The spectra were performed in the positive ion mode at a declustering potential of 40–60 V. The sample was previously separated on a UPLC column (C18) using UPLC ACQUITY™ system by Waters connected with DPA detector. Flash chromatography was performed on Merck silica gel 60 (200–400 mesh) using chloroform/methanol (19:1 vol) mixture as eluent. Analytical TLC was carried out on silica gel F_{254} (Merck) plates (0.25 mm thickness).

The intensity measurements of diffraction reflections were carried out for **1** and **17** at 296 K with a KM4 diffractometer, using graphite monochromated CuKα radiation ($\lambda = 1.54178$ Å) and $\omega/2\theta$ scan mode. Crystal structures were solved by the SHELXS-97 program and refined by full-matrix least squares on F^2 using the SHELXL-97 program [62]. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were positioned geometrically and allowed to ride on their parent atoms, with $U_{iso}(H) = 1.2\ U_{eq}(C)$ and 1.5 for methyl groups.

3.1.1. General procedure

A solution of 1*H*-1,2,4-triazol-3-amine (0.0069 mol, 0.58 g) in acetonitrile (25 mL) was treated with appropriate isothiocyanate (0.0075 mol) and the mixture was refluxed for 8 h. Then solvent was removed on rotary evaporator. The residue was purified by

Table 4
Activity of compounds against hospital methicillin-susceptible strains of *S. aureus* (MSSA) – minimal inhibitory concentrations (MIC, μg/ml).

	1	**3**	**5**	**6**	**7**	**9**	**12**	**13**
256/08	50	25	50	50	25	25	>400	25
261/08	50	25	50	50	25	25	>400	25
267/08	50	25	50	50	25	25	>400	25
268/08	50	25	100	50	50	25	>400	25
269/08	50	25	50	50	25	25	>400	12.5
338/08	50	25	50	50	50	50	>400	24
339/09	50	25	50	50	50	50	>400	12.5
367/09	50	25	50	50	50	25	>400	12.5
369/09	50	25	50	50	50	50	>400	12.5
372/09	50	25	50	50	25	25	>400	25

Table 5
Activity of compounds against hospital methicillin-resistant strains of *S. aureus* (MRSA) – minimal inhibitory concentrations (MIC, μg/ml).

	1	**3**	**5**	**6**	**7**	**9**	**12**	**13**
275/08	100	25	100	100	100	50	>400	25
277/08	50	50	50	50	50	25	>400	12.5
306/08	50	25	50	50	50	50	>400	12.5
307/08	50	25	50	50	50	50	>400	12.5
309/08	50	25	50	50	50	25	>400	12.5
329/08	50	25	50	50	50	25	>400	12.5
325/09	50	25	50	50	50	25	>400	12.5
356/09	50	25	50	50	50	50	>400	12.5
357/09	50	50	50	50	50	50	>400	25
376/09	50	25	50	50	50	50	>400	25

Table 6
Activity of compounds against hospital methicillin-resistant strains of *S. epidermidis* (MRSA) – minimal inhibitory concentrations (MIC, μg/ml).

	1	**3**	**5**	**6**	**7**	**9**	**14**	**15**
311/08	100	25	50	100	50	50	>400	25
315/08	100	25	50	100	50	50	>400	12.5
316/08	100	25	100	100	50	50	>400	25
317/08	100	25	50	100	100	50	>400	25
318/08	50	25	50	50	25	12.5	>400	12.5
340/09	50	12.5	50	50	25	25	>400	12.5
341/09	100	12.5	100	100	50	50	>400	25
342/09	200	200	50	50	25	25	>400	25
343/09	50	25	50	50	25	50	>400	12.5
377/09	50	25	50	50	25	25	>400	25

Table 7
Cytotoxicity and anti-HIV-1 activity of **1**–**11**, **14** and **15**.

Compds	MT-4	HIV-1
	CC_{50}[a]	EC_{50}[b]
1	>100	>100
2	>100	>100
3	47	>47
4	59	>50
5	>100	>100
6	>100	>100
7	>100	>100
8	50	>50
9	46	>46
10	47	>47
11	>100	>100
14	7.4 ± 0.6	>7.4
15	>100	>100
EFV	37	0.002 ± 0.001

Data represent mean values for three independent determinations.
[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method.

Table 8
Antiproliferative activity of **14** against human leukaemia/lymphoma cell lines.

Compds	MT-4	CCRF-CEM	WIL-2NS	CCRF-SB
	CC_{50}[a]			
14	7.4 ± 0.6	39 ± 2	52 ± 1	42 ± 2
Camptotecin	0.004 ± 0.0004	0.003 ± 0.0005	0.005 ± 0.0007	0.004 ± 0.0005

Data represent mean values for three independent determinations.
[a] Compound concentration (μM) required to reduce cell proliferation by 50% under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication, as determined by the MTT method.

column chromatography (chloroform: methanol; 9.5:0.5 vol.). The compound was crystallized from acetonitrile.

*3.1.2. 1-Phenyl-3-(1H-1,2,4-triazol-3-yl)thiourea (**1**)*

1-Phenyl-3-(1H-1,2,4-triazol-3-yl)thiourea (**1**) has been synthesized as described previously [63].

Yield 83%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.22 (t, 1H, $CH_{arom.}$, J = 7.5 Hz); 7.39 (t, 2H, $CH_{arom.}$, J = 7.8 Hz); 7.62 (d, 2H, $CH_{arom.}$, J = 7.8 Hz); 8.5 (s, 1H, $CH_{arom.}$); 11.78 (s,1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 124.26 (2C), 127.96, 128.47 (2C), 138.72, 148.79, 157.43, 177.09. ESI MS: m/z = 218.2 $[M]^+$ (100%).

3.1.3. Crystal data

Crystal system triclinic, space group *P*-1, unit cell dimensions a = 8.690(2), b = 9.495(2), c = 14.226(3) Å, α = 72.05(3), β = 86.85(3), γ = 67.20(3)°, V = 1026.6(4) Å^3; Z = 4, d_c = 1.419 g cm^{-3}, μ = 2.591 mm^{-1}, $F(000)$ = 456. A crystal of dimensions 0.45 × 0.18 × 0.15 mm was used for intensity measurements. Within the θ range 3.27–80.26° [$-10 \le h \le 10$, $-11 \le k \le 11$, $-11 \le l \le 18$] 4353 reflections were collected. The 4216 unique reflections [R(int) = 0.0635] were used for the refinement of 272 parameters, including extinction coefficient [x = 0.027(2)]. Final R indices on F^2 for 2975 observed reflections [$I > 2\sigma(I)$] were: $R1$ = 0.0514, $wR2$ = 0.1361, goodness-of-fit 1.038, and largest difference peak/hole 0.52/−0.35 e Å^{-3}.

*3.1.4. 1-(4-Methoxyphenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**2**)*

Yield 91%. Mp. 199–200 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.70 (s, 3H, OCH_3); 6.85 (d, 2H, CH_{arom}, J = 9.0 Hz); 7.32 (d, 2H, CH_{arom}, J = 9.0 Hz); 8.34 (s, 1H, CH═). ^{13}C NMR (DMSO-d_6) δ (ppm): 55.12, 113.92 (2C), 119.86 (2C), 124.25, 128.46, 132.88, 152.89, 154.28. ESI MS: m/z = 273.1 $[M + Na + H]^+$ (100%).

*3.1.5. 1-(4-Chlorophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**3**)*

Yield 87%. Mp. 206 – 200 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.44 (d, 2H, CH_{arom}, J = 8.7 Hz); 7.67 (d, 2H, CH_{arom}, J = 9.0 Hz); 8.55 (s, 1H CH═); 11.08 (s, 1H, NH); 11.26 (s, 1H, NH); 14.01 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 126.02 (2C), 128.41 (2C), 129.52, 137.76, 146.23, 153.11, 157.68. ESI MS: m/z = 276.2 $[M + Na]^+$ (100%).

*3.1.6. 1-(4-Methylphenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**4**)*

Yield 76%. Mp. 219–220 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3); 7.18 (d, 2H, CH_{arom}, J = 8.1 Hz); 7.47 (d, 2H, CH_{arom}, J = 8.4 Hz); 8.50 (s, 1H, CH═); 11.12 (s, 1H, NH); 11.71 (s, 1H, NH); 13.94 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 20.51, 124,31 (2C), 128.91 (2C), 134.72, 136.16, 157.12, 159.42, 177.08. ESI MS: m/z = 256.0 $[M + Na]^+$ (100%).

*3.1.7. 1-(4-Fluorophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**5**)*

Yield 81%. Mp. 211–212 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.18–7.24 (m, 2H, CH_{arom}); 7.57–7.62 (m, 2H, CH_{arom}); 8.54 (s, 1H, CH═); 11.24 (s, 1H, NH); 11.00 (s, 1H, NH); 14.00 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 114.99 (2C), 115.29 (2C), 126.89, 153.07, 158.02, 161.24, 177.63. ESI MS: m/z = 260.0 $[M + Na]^+$ (100%).

*3.1.8. 1-(2-Bromophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**6**)*

Yield 86%. Mp. 241–242 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.18–7.24 (m, 1H, CH_{arom}); 7.38–7.46 (m, 1H, CH_{arom}); 7.68–7.71 (m, 1H, CH_{arom}); 7.80–7.83 (m, 1H, CH_{arom}); 8.54 (s, 1H, CH═); 11.41 (s, 1H, NH); 11.68 (s, 1H, NH); 14.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 119.53, 127.57, 128.05, 129.69 (2C), 132.41, 133.24, 137.39, 178.05. ESI MS: m/z = 321.9 $[M + Na + H]^+$ (100%).

*3.1.9. 1-(4-Bromophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**7**)*

Yield 71%. Mp. 223–224 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.54 – 7.63 (m, 4H, CH_{arom}); 8.51 (s, 1H, CH═); 11.28 (s, 1H, NH); 11.79 (s, 1H, NH); 13.89 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 118.24, 126.97 (2C), 127.61 (2C), 132.75, 134.52, 137.22, 178.76. ESI MS: m/z = 321.9 $[M + Na + H]^+$ (100%).

*3.1.10. 1-(3-Bromophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**8**)*

Yield 88%. Mp. 203–204 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.31 – 7.42 (m, 2H, CH_{arom}); 7.53–7.57 (m, 1H, CH_{arom}); 8.04 (t, 1H, CH_{arom}, J = 1.8 Hz); 8.54 (s, 1H, CH═); 11.31 (s, 1H, NH); 11.86

Table 9
Antiproliferative activity of **14** against solid tumour and "normal" cell lines. Data represent mean values for three independent determinations.

Compds	SK-MEL-28	MCF7	SKMES-1	HepG2	DU145	MRC-5	CRL7065
	CC_{50}[a]						
14	72 ± 4	49 ± 1	85 ± 6	78 ± 5	11 ± 0.7	43 ± 3	47 ± 1
Camptotecin	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.004	0.04 ± 0.01	0.08 ± 0.0004	0.2 ± 0.1	0.3 ± 0.1

[a] Compound concentration (μM) required to reduce cell proliferation by 50% under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication, as determined by the MTT method.

(s, 1H, NH); 13.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 120.86, 126.50, 130.38 (2C), 134.85, 136.37, 139.07, 141.56, 179.44. ESI MS: m/z = 321.9 $[M + Na + H]^+$ (100%).

*3.1.11. 1-(4-Iodophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**9**)*

Yield 79%. Mp. 207–208 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.45–7.50 (m, 2H, CH_{arom}); 7.69–7.74 (m, 2H, CH_{arom}); 8.51 (s, 1H, CH=); 11.27 (s, 1H, NH); 11.79 (s, 1H, NH); 13.95 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 87.21, 125.68, 126.68 (2C), 137.07, 137.14 (2C), 138.63, 177.03. ESI MS: m/z = 367.9 $[M + Na]^+$ (100%).

*3.1.12. 1-(3,4-Dichlorophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**10**)*

Yield 93%. Mp. 128–129 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.38 (s, 1H, CH_{arom}); 7.55–7.59 (dd, 1H, CH_{arom}); 7.61–7.64 (d, 1H, CH_{arom}); 8.50 (s, 1H, CH=); 11.43 (s, 1H, NH); 11.81 (s, 1H, NH); 13.88 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 124.25, 125.47, 130.30 (2C), 130.52, 139.00, 143.29, 156.61, 177.39. ESI MS: m/z = 309.9 $[M + Na]^+$ (100%).

*3.1.13. 1-Benzoyl-3-(1H-1,2,4-triazol-3-yl)thiourea (**11**)*

1-Benzoyl-3-(1H-1,2,4-triazol-3-yl)thiourea (**11**) has been synthesized as described previously [63].

Yield 83%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.48–7.56 (m, 2H, $CH_{arom.}$); 7.60–7.65 (m, 1H, $CH_{arom.}$); 7.81 (s, 1H, CH=); 8.03–8.06 (d, 2H, CH_{arom}, J = 7.5 Hz); 11.94 (s, 2H, NH); 13.60 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 128.02 (2C), 128.49 (2C), 132.40, 133.84, 147.15, 154.24, 165.47, 178.65. ESI MS: m/z = 270.0 $[M + Na]^+$ (100%).

*3.1.14. 1-(2-Fluorophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**12**)*

Yield 83%. Mp. 183–184 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.21–7.31 (m, 4H, CH_{arom}); 8.00–8.05 (t, 1H, CH=, J = 7.8 Hz); 8.52 (s, 1H, NH); 11.42 (s, 1H, NH); 11.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.38, 123.99, 126.53, 127.32, 127.42, 143.29, 153.84, 157.10, 177.98. ESI MS: m/z = 260.0 $[M + Na]^+$ (100%).

*3.1.15. 1-(3-Fluorophenyl)-3-(1H-1,2,4-triazol-3-yl)thiourea (**13**)*

Yield 89%. Mp. 179–180 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 6.88–7.06 (m, 1H, CH_{arom}); 7.24–7.54 (m, 3H, CH_{arom}); 7.78–7.82 (s, 1H, CH=); 8.29 (s, 1H, NH); 8.49 (s, 1H, NH); 11.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 110.63, 111.76, 119.72, 130.00, 140.44, 143.23, 160.07, 163.28, 177.12. ESI MS: m/z = 260.0 $[M + Na]^+$ (100%).

*3.1.16. 5-Amino-N-benzyl-1H-1,2,4-triazole-1-carbothioamide (**14**)*

5-Amino-*N*-benzyl-1H-1,2,4-triazole-1-carbothioamide (**14**) has been synthesized as described previously [64].

Yield 88%. Mp. 185–186 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 4.78–4.80 (d, 2H, CH_2, J = 6.0 Hz); 7.22–7.30 (m, 1H, CH_{arom}); 7.31–7.33 (m, 2H, CH_{arom}); 7.34–7.43 (m, 2H, CH_{arom}); 8.19 (s, 1H, CH=); 10.55 (s, 1H, NH_2). ^{13}C NMR (DMSO-d_6) δ (ppm): 46.80, 127.08, 127.51 (2C), 128.24 (2C), 137.17, 148.88, 157.08, 174.33. ESI MS: m/z = 256.0 $[M + Na]^+$ (100%).

*3.1.17. 5-Amino-N-cyclohexyl-1H-1,2,4-triazole-1-carbothioamide (**15**)*

5-Amino-*N*-cyclohexyl-1H-1,2,4-triazole-1-carbothioamide (**15**) has been synthesized as described previously [64]

Yield 86%. Mp. 169–170 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.05–1.18 (m, 1H, CH_2); 1.22–1.36 (m, 2H, CH_2); 1.44–1.62 (m, 3H, CH_2); 1.70–1.85 (m, 4H, CH_2); 4.10–4.20 (m, 1H, CH); 8.18 (s, 1H, CH=); 9.64 (d, 2H, NH_2, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 34.65 (2C), 34.84, 40.33 (2C), 63.38, 158.66, 167.07, 182.48. ESI MS: m/z = 248.1 $[M + Na]^+$ (100%).

*3.1.18. 5-Amino-N-(2-phenylethyl)-1H-1,2,3-triazole-1-carbothioamide (**16**)*

Yield 87%. Mp. 192–193 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.91 – 2.96 (t, 2H, CH_2, J = 7.8 Hz); 3.75–3.82 (q, 2H, CH_2, J = 7.1 Hz); 7.19–7.33 (m, 5H, CH_{arom}); 8.20 (s, 1H, CH=); 10.05–10.09 (t, 2H, NH, J = 5.5 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 33.15, 45.22, 126.31, 128.44 (2C), 128.56 (2C), 138.60, 148.80, 157.00, 173.78. ESI MS: m/z = 270.1 $[M + Na]^+$ (100%).

*3.1.19. 5-Amino-N-(2-methylallyl)-1H-1,2,4-triazole-1-carbothioamide (**17**)*

Yield 92%. Mp. 173–174 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.71(s, 3H, CH_3); 4.12–4.18 (m, 2H, CH_2); 4.73 (s, 1H, CH_2=); 4.82 (s, 1H, CH_2=); 8.19 (s, 1H, CH=); 9.86 (s, 1H, NH_2); 10.17 (s, 1H, NH_2). ^{13}C NMR (DMSO-d_6) δ (ppm): 30.34, 58.56, 120.44, 149.92, 158.82, 167.03, 184.41. ESI MS: m/z = 198.1 $[M + H]^+$ (100%).

3.1.20. Crystal data

Crystal system triclinic, space group *P*-1, unit cell dimensions $a = 6.181(1)$, $b = 8.287(2)$, $c = 10.239(2)$ Å, $\alpha = 88.65(1)$, $\beta = 83.16(1)$, γ = 68.19(2)°, V = 483.3(2) Å^3; Z = 2, d_c = 1.355 g cm^{-3}, μ = 2.678 mm^{-1}, $F(000)$ = 208. A crystal of dimensions 0.40 × 0.20 × 0.18 mm was used for intensity measurements. Within the θ range 4.35–67.64° [$-10 \le h \le 10$, $-11 \le k \le 11$, $-11 \le l \le 18$] 4353 reflections were collected. The 4216 unique reflections [R(int) = 0.0218] were used for the refinement of 120 parameters, including extinction coefficient [$x = 0.021(2)$]. Final R indices on F^2 for 1471 observed reflections [$I > 2\sigma(I)$] were: $R1$ = 0.0359, $wR2$ = 0.1016, goodness-of-fit 1.053, and largest difference peak/hole 0.21/−0.32 e Å^{-3}.

*3.1.21. 5-Amino-N-(2-ethoxycarbonyl)-1H-1,2,4-triazole-1-carbothioamide (**18**)*

5-Amino-*N*-(2-ethoxycarbonyl)-1H-1,2,4-triazole-1-carbothioamide (**18**) has been synthesized as described previously [65].

Yield 81%. Mp. 157–158 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.23–1.27 (t, 3H, CH_3, J = 7.05 Hz); 4.17–4.24 (q, 2H, CH_2, J = 7 Hz); 8.53 (s, 1H, CH =); 11.46 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ (ppm): 14.08, 63.88, 143.62, 149.93, 151.11, 171.89. ESI MS: m/z = 238.1 $[M + Na]^+$ (100%).

3.2. Biology

3.2.1. In vitro evaluation of antimicrobial activity

The antibacterial activity of compounds was tested against a series of Gram-positive bacteria: *S. aureus* ATCC 4163, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Enterococcus hirae* ATCC 10541, *Micrococcus luteus* ATCC 9341, *M. luteus* ATCC 10240 and Gram-negative rods: *Escherichia coli* ATCC 10538, *E. coli* ATCC 25922, *E. coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *P. aeruginosa* NCTC 6749, *P. aeruginosa* ATCC 27853, *Bordetella bronchiseptica* ATCC 4617. Antifungal activity was tested against yeasts: *C. albicans* ATCC 10231, *C. albicans* ATCC 90028, *Candida parapsilosis* ATCC 220191. Microorganisms used in this study were obtained from the collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.

3.2.1.1. Media, growth conditions and antimicrobial activity assays. Antimicrobial activity was examined by the disc diffusion and MIC method under standard conditions, using Mueller–Hinton II agar medium (Becton Dickinson) for bacteria and RPMI agar with 2% glucose (Sigma) for yeasts, according to CLSI (previously NCCLS) guidelines [58]. Solutions containing the tested agents were prepared

in methanol or DMSO. For the disc diffusion method, sterile paper discs (9 mm diameter, Whatman No. 3 chromatography filter paper) were dripped with the compound solutions tested to obtain 400 μg of substance per disc. Dry discs were placed on the surface of an appropriate agar medium. The results (diameter of the growth inhibition zone) were read after 18 h of incubation at 35 °C. Minimal Inhibitory Concentration (MIC) were examined by the twofold serial agar dilution technique [59]. Concentrations of the tested compounds in solid medium ranged from 3.125 to 400 μg/mL. The final inoculum of studied organisms was 10^4 CFU/mL (colony forming units per mL), except the final inoculum for *E. hirae* ATCC 10541, which was 10^5 CFU/mL. Minimal inhibitory concentrations were read off after 18 h s) of incubation at 35 °C.

3.2.2. Cell-based assays

Cell-based assays were performed at Dipartimento di Scienze e Tecnologie Biomediche, Università di Cagliari, Monserrato, Italy.

3.2.2.1. Test compounds. Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium.

3.2.2.2. Cells and viruses. Cell line and viruses were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell line supporting the multiplication of Human Immunodeficiency Virus type-1 (HIV-1) was the CD4+ human T-cells containing an integrated HTLV-1 genome (MT-4).

3.2.2.3. Cytotoxicity assays. Cytotoxicity assays were run in parallel with antiviral assays. Exponentially growing MT-4 cells were seeded at an initial density of 1×10^5 cells/ml in 96-well plates in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere, in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [66].

3.2.2.4. Antiproliferative assays. Cell lines derived from human haematological tumours [CD4+ human T-cells containing an integrated HTLV-1 genome (MT-4); CD4+ human acute T-lymphoblastic leukaemia (CCRF-CEM), human splenic B-lymphoblastoid cells (WIL-2NS), human acute B-lymphoblastic leukaemia (CCRF-SB)] were seeded at an initial density of 1×10^5 cells/ml in 96 well plates in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 100 units/ml penicillin G and 100 μg/ml streptomycin.

Cell lines derived from human solid tumours [skin melanoma (SK-28), breast adenocarcinoma (MCF-7), lung squamous carcinoma (SK-MES-1), hepatocellular carcinoma (HepG-2), prostate carcinoma (DU-145)] or normal tissues [foreskin fibroblasts (CRL-7065), lung fibroblasts (MRC-5)] were also seeded at 1×10^5 cells/ml in 96 well plates in specific media supplemented with 10% FCS and antibiotics, as above. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the MTT method [66].

3.2.2.5. Antiviral assays. Compound's activity against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cell acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 μL of RPMI containing 1×10^4 MT-4 cells were added to each well of flat-bottom microtitre trays, containing 50 μL of RPMI without or with serial dilutions of test compounds. Then, 20 μL of a HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation at 37 °C, cell viability was determined by the MTT method [66].

3.2.2.6. Linear regression analysis. The extent of cell growth/viability and viral multiplication, at each drug concentration tested, were expressed as percentage of untreated controls. Concentrations resulting in 50% inhibition (CC_{50} or EC_{50}) were determined by linear regression analysis.

3.2.3. Pharmacology

The experiments were carried out on male Albino Swiss mice (18–30 g). The animals were kept 8–10 to a cage under standard laboratory conditions (at a temperature of 20 ± 1 °C and a 12 h light/dark cycle) with free access to food (LSM, Motycz, Poland) and water. All experiments were performed between 9:00 a.m. and 4:00 p.m. The experiments were performed in accordance with the opinion of Local Ethics Committee for Animal Experimentation.

The substances investigated, marked as **8** and **14**, in all tests were administered intraperitoneally (i.p.), as a suspensions in aqueous solution of 0.5% methylcellulose (tylose) and were injected 60 min before the tests. All substances were administered in a volume of 10 ml/kg. The control animals received an equivalent volume of the solvent at the respective time before the test. All tests performed, suggested by Vogel and Vogel [67], are generally accepted as basic in investigation of the central activity by behavioural methods. The acute toxicity of the compound was assessed in mice acc. to Litchfield and Wilcoxon method [68], as the ED_{50} calculated as "the lost of righting reflex" within 48 h. The compounds were injected in doses equivalent to 0.1 LD_{50} (120.1 mg/kg for **8** and 18.06 mg/kg for **14**). In addition, the activity of compounds was assessed in the following tests:

- locomotor activity was measured for single mice in photoresistor actometers (circular cages, diameter 25 cm, two light beams; the number of crossed light beams by the mice was recorded) for 30 min as:
  a) spontaneous activity
  b) amphetamine-induced hyperactivity: mice received subcutaneously (s.c.) 5 mg/kg of amphetamine 30 min before the test;
- nociceptive reactions were studied in the acetic acid (0.6%)-induced 'writhing' test. The number of writhing episodes was measured for 10 min starting 5 min after i.p. administration of acid solution;
- motor coordination was evaluated in rota rod test [69] and chimney test;
- body temperature in normothermic mice was measured in the rectum by thermistor thermometer;
- pentylenetetrazole (110 mg/kg, s.c.)-induced convulsions were evaluated as the number of mice with clonic seizures, tonic convulsions and dead animals;
- 'head twitch' responses after 5-hydroxytryptophan (5-HTP), acc. to Corne et al. [43]. Mice received 5-HTP (180 mg/kg, i.p.) and the number of head twitches was recorded in 6 two-minute intervals (4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min).

3.2.4. Statistics

Obtained data were calculated by χ^2 test with Yates correction (pentylenetetrazole-induced seizures), and one-way ANOVA (other tests). Subsequent comparisons between treatment and control groups were carried out using a post hoc Dunnett's test, when $p < 0.05$.

Appendix A. Supplementary material

The experimental details and final atomic parameters for compounds **1** and **17** have been deposited with the Cambridge Crystallographic Data Centre as supplementary material (CCDC No. 853523 and 853524, respectively). Copies of the data can be obtained free of charge on request via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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